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INVESTIGATION OF HYGIENE INDICATORS AT CONTROL POINTS OF PILOT-SELECTED BUTCHER SHOPS

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ABSTRACT

In current study it was aimed to investigate hygiene indicators at control points of pilot-selected butcher shops (n=10) by using the conventional swabbing method and ATP Bioluminescence swabbing method. Air sampling device (Newtry™ Air sampler HAS-100B) was used for the hygiene control of the microbiological load of air in cold storage rooms. The number of yeast-mold was measured for determination microbiological load of air. For the measurement of the microbial load in food handlers' hands, staphylococci, coliform bacteria counts were investigated. In order to measure the microbiological load of the surfaces coliform bacteria and total mesophile aerobe bacteria (TMAB) counts were investigated. Surface samples were collected from 3 different points which were determined as control points. These were: 1. cutting surfaces, 2. knife, 3. mincing machine. All the surfaces were mentioned as cleaned before the sampling. A scoring system was developed and good hygiene practice (GHP) status was defined for each butcher shops depending on this scoring system. The calculated scores showed us that, most of the selected butcher shops had satisfied level of GHP. The most important contamination point was found to be the cutting boards and mincing machines even they were cleaned. Air microbiological load in cold storage rooms were below the limits and hands of staffs were mostly clean in terms of coliform and *S. aureus*. We can conclude that; the scoring system can be used as a tool of hygiene status monitoring.

1.Introduction

Despite the developments in food science and technology, it is still difficult to control food hazards which may potentially risks for consumers (Duffy and Schaffner, 2002). Besides other pathways of contamination, food may be contaminated especially when biological, chemical or physical agents present in the processing environment during food production processes (Da Cruz et al., 2006). With this point of view, food related illnesses because of poor infrastructure and low level of food handlers' awareness are the most important issues for many countries (Scott, 2003). Among all food groups, the animal originated foods are more likely to be hazardous in terms of pathogen content (Barril et al., 2019; EFSA and ECDC, 2016). Especially, meat and meat products are routinely associated with food poisoning outbreaks whole over the world for several years. During production, processing and storage, these products are subjected to contamination by many pathogenic bacteria (Torso et al., 2015; Barril et al., 2019). Microbiological contamination of meat and

meat products induces meat spoilage, shortens shelf life and poses public health problems (Rao & Ramesh, 1992). The environment in which meat production is conducted plays crucial role in determining the safety and quality of the product (Moore & Griffith, 2002). As Barros et al., (2007) reported, the main contamination points in meat retail establishments are stainless steel boxes, beef tenderizers, grinders, knives, mixers, sausage stuffers, plastic boxes, floors and drains. Çetin et al., (2006) concluded that good hygiene practices and the implementation of sanitation standard procedures thorough cleaning of items such as cutting tables, knives and other utensils helps to reduce contamination by microbial agents in meat retails.

In general, there are many methods including microbiological, visual, physical, chemical and biochemical which can be used to assess the hygiene status of processing environments (Kymäläinen et al., 2009). Selection of the most appropriate method for microbiological analysis of surfaces can be done by the type of food produced. It is important to use the best method for isolating and detecting microorganisms (Salo et al., 2006). Control and hygiene monitoring of foods by microbiological methods offered a great deal in eliminating and/or reducing potential hazards and is viewed as essential part of quality control and food safety plans (Moore & Griffith, 2002; López-Campos et al., 2012). Microbiological methods, including hygiene swabbing and agar contact methods, are widely used for assessment of the cleanliness of food contact surfaces but require incubation periods of days (Vilar et al., 2008). ATP Bioluminescence methods are an attractive alternative because they provide fast and real-time assessment of surface cleanliness. Thus, ATP Bioluminescence method proved to be effective and reliable technique for monitoring sanitation and hygienic practices within the contexts of HACCP (hazard analysis critical control point) preventive systems (Chen, 2000; Ayçiçek et al., 2004; Vilar et al., 2008; Pérez-Rodríguez et al., 2008). Although this method does not identify the quantity of microorganisms or contaminant species, it can be used as a medium for monitoring hygiene and verifying cleanliness (Calvert et al., 2000). Consequently, to monitor the efficacy of disinfection procedures, some microbiological testing (using dipslides, contact plates, swaps) may be required. ATP Bioluminescence alone should not be interpreted as surrogate indicators for the presence of microbial pathogens (Shama & Malik, 2013). Thus, the combination of traditional culture based and luminescence methods could be applied as an integrated hygiene evaluation and monitoring strategy (Moore & Griffith, 2002; Lehto et al., 2011).

It is imperative to detect the sources of contamination and true critical points throughout the production process in order to provide food safety (Lehto et al., 2011). With this point of view, in current study it was aimed to investigate hygiene indicators at control points of pilot-selected butcher shops (n=10) by using the conventional swabbing method and ATP Bioluminescence swabbing method.

2.Materials and methods

2.1. Selecting the pilot butcher shops and planning the visits

During June-July when the highest weather temperature was the highest, 10 active working butcher shops were selected as pilot in Cyprus. In order to evaluate good hygiene practices (GHP) and good manufacturing practices (GMP), the control points where the current microbial load was expected high were determined. Pre-visits were conducted to the butchers to inform them about the visits and to define the scope of the work before the samples were collected. Selected pilot butcher shops were visited for sample collection during active work days for 4 times with 1-week interval.

2.2. Microbiological and ATP Bioluminescence analysis

Samples were taken from the control points which were determined during the preliminary visits. Air sampling device (Newtry™ Air sampler HAS-100B) was used for the hygiene control of the microbiological load of air in cold storage rooms. The number of

yeast-mold was measured for determination microbiological load of air. For the measurement of the microbial load in food handlers' hands, a sterile swab moistened with sterile physiological saline water, was used for sampling and staphylococci, coliform bacteria counts were investigated. In order to measure the microbiological load of the surfaces in contact with the food, sterile swab moistened with sterile physiological saline water was used. 10x10 cm^2 sized sterile plate templates were prepared in order to provide standard sampling. At the same time from the same surfaces sampling for ATP Bioluminescence measurement was performed in order to investigate organic pollution of the surfaces. Surface samples were collected from 3 different points which were determined as control points. These were: 1. cutting surfaces, 2. knife, 3. mincing machine. All the surfaces were mentioned as cleaned before the sampling. Total mesophile aerobe bacteria (TMAB) and coliform bacteria count were investigated for those surfaces. After inoculation, incubations were carried out in the conditions as indicated in Table 1. After incubation, 30-300 colony containing petri dishes were counted and the results were reported for surfaces, hands and air as CFU/100cm^2, CFU/hand and CFU/m^3, respectively.

Table 1. Media used, incubation conditions and references of methods

Micro-organisms	**Analytical reference method**	**Media name**	**Incubation conditions**		
			Incubation temperature	**Incubation period**	**O_2 requirement**
Aerobic colony count	ISO 4833	Plate Count Agar (LAB 149)	30°C ± 1 ºC	72 h ±3 h	Aerobic
Staphylococci *Staphylococcus aureus*	ISO 6888-1:1999 + A1:2003	Baird Parker Medium Agar (LAB 085) + Egg Yolk Tellurite Emulsion (X 085)	35 º- 37 ºC	24 h ± 2 h	Aerobic
		Brain Heart Infusion Broth (LAB 049)	Confirmation for *Staphylococcus aureus*		
		Rabbit Plasma (X086)			
Coliform bacteria	ISO 4832:2006	Violet Red Bile Glucose Agar (LAB 031)	30 ºC - 37 ºC	24 ± 2 h	Microaerophilic
		Brilliant Green Bile Broth (LAB051)	Confirmation		
Yeast and mould	ISO 6611: 2004	Yeast Glucose Chloramphenicol Agar (LAB 122)	25 ºC	5 days	Aerobic

For ATP Bioluminescence method, samples were collected from the defined surfaces with the help of special swabs designed for this method. 10x10 cm^2 sized sterile plate templates were prepared in order to provide standard sampling. The swabs were slightly moistened with sterile distilled water before the swabs were taken. After the samples were collected from the surfaces, they were placed in the ATP Bioluminescence device and the value was read. The results were given as RLU/100 cm^2 unit. All the results were evaluated according to the critical limits indicated in Table 2.

Table 2. Critical limits for microbiological and ATP Bioluminescence analyses' results

Sampling points	Microorganisms	Critical limits	Reference
Cold storage rooms' air	Mould and yeast	1 X 10^3 (3 $\log_{10}$) cfu/m^3	(Modified from) Luck and Gavron, 1990
Staffs' hands	Staphylococci	1 X 10^2 (2 $\log_{10}$) cfu/hand	(Modified from) Aksu and Kaya, 2000
	Coliform bacteria	1 X 10^2 (2 $\log_{10}$) cfu/hand	
Surfaces in contact with food	Aerobic colony count	1 X 10^2 (2 $\log_{10}$) cfu/100 cm^2	(Modified from) Aksu et al., 2017
	Coliform bacteria	0 (0 $\log_{10}$) cfu/100 cm^2	
ATP- bioluminescence	300 RLU/100 cm^2		(Modified from) Mulvey et al., 2011

2.3. Developing scoring system according to analysis results

A scoring system was developed and GHP status was defined for each butcher shops depending on this scoring system. For this purpose, a score value was assigned for each hygiene indicator analysis result. If the obtained results were equal or below the limits ten (10) points, if results were above the limits zero (0) point were assigned. All results were grouped under 3 headings. A percentile, based on the degree of impact on food safety, was assigned for points collected under each heading. Figure 1 demonstrates the calculating system and the analyses which were performed. Headings and percentages are as follows;

- Food Contact Surface 40%
- Food Handlers' Hands 40%
- Cold Storage Room20%

When all the results of a butcher were below the acceptable limits, the peak score would be 136. This point was defined as Top Point. GHP status of butchers was classified according to the points they collected. The classification was "Good" if >68 points, "Acceptable" if =68 and "Bad" if <68 points.

	Sampling point	Sampling parameter	# analysis results	Score values of results
Food contact surfaces			1	
			2	
			3	
		TMAB	4	
			1	
			2	
			3	
	CUTTING BOARD	C	4	
			1	
			2	
			3	
		TMAB	4	
			1	
			2	
			3	
	KNIFE	C	4	
			1	
			2	
			3	
		TMAB	4	
			1	
			2	
			3	
	MINCING MACHINE	C	4	
Total score calculated with the heading percantile (40%)				0
Food handlers			1	
			2	
			3	
		ST	4	
			1	
			2	
			3	
	FOOD HANDLERS' HANDS	C	4	
Total score calculated with the heading percantile (40%)				0
Air			1	
			2	
			3	
	COLD STORAGE ROOM	M	4	
Total score calculated with the heading percantile (10%)				0
TOTAL SCORE FOR ALL ANALYSIS				0

Figure 1. Calculation table scheme of GHP scoring system

3.Results and discussions

In current study, TMAB samples were collected from the clean surfaces of most commonly used equipment which are told to be clean. As Ghafir et al., (2008) mentioned the safety and quality of meat products can be estimated by investigating indicator microorganisms, such as aerobic mesophilic microorganisms and coliform bacteria. The number of TMAB provides an estimation for

overall population of microorganisms regarding the hygienic conditions. Coliforms are indicators of inadequate sanitary hygienic conditions and give the possible contamination and presence of enteric pathogenic microorganisms (da Silva et al., 2016). On the other hand, the increased application of Bioluminescence based methods may be directly linked to the adoption within the food industry of a concept for systematically putting in place measures to ensure the production of foods in which all forms of potential contaminants have been reduced to acceptable levels (Shama and Malik, 2013).

In our study, when the ATP Bioluminescence results of food contact surfaces were taken into consideration, it was determined that the surfaces where the organic pollution was the highest in all butcher shops' means, were the cutting boards in 3 of 4 visits. Only for 1 visit the value was the highest for mincing machine. The values for cutting boards ranged between min 11 RLU/100 cm^2 and max 48011 RLU/100 cm^2. The combination of traditional culture based and luminescence methods could be applied as an integrated hygiene evaluation and monitoring strategy (Moore & Griffith, 2002; Lehto et al., 2011). On the other hand, as Shama and Malik (2013) concluded ATP Bioluminescence alone should not be interpreted as surrogate indicators for the presence of microbial pathogens. The effectiveness of sanitation procedures has traditionally been evaluated using different methods such as visual inspection, swaps, dipslides and contact plates (Carrascosa et al., 2012; Tebbutt et al., 2007). In current study, parallel with ATP Bioluminescence results, when the TMAB counts considered as microbiological pollution indicators, the cutting boards had the highest TMAB value in all visits and ranged from min 1,6 $\log_{10}$ CFU/100 cm^2 to max 3,58 $\log_{10}$ CFU/100 cm^2. When the mean values of TMAB counts for all visits were evaluated according to the critical limits, all of them were over the limit (2 $\log_{10}$ CFU/100cm^2). Mean values of coliform counts for cutting boards were obtained between 0,6 $\log_{10}$ CFU/100 cm^2 and 1,56 $\log_{10}$ CFU/100 cm^2 (Table 3). These mean values of coliform counts for cutting boards did not meet the recommended critic limits (0 $\log_{10}$ CFU/100cm^2). Cutting boards may generally carry microbiological risk as reported in survey studies. Fidan and Ağaoğlu (2004) reported that they detected TMAB values as $6{,}1\times10^4$ (4,78 $\log_{10}$) CFU/cm^2 and coliform group bacterial levels as $4{,}1\times10^3$ MPN/25cm^2 in the samples taken from the restaurants' cutting surfaces in Ağrı. Those results were higher than that we obtained but the surfaces should have been completely clean as the owners told to be. In our study, mean TMAB counts of the knives were over the critical limits in 3 of 4 visits and were above the limits for meat mincing machines in all visits however they were all stated as clean. Depending on the results of coliform bacteria analyses, in 1 of 4 visits, the mean coliform counts were below the limits for knives but all were above for mincing machines. Coliform count means of mincing machines were ranged between 0,48 $\log_{10}$ CFU/100 cm^2 and 1,04 $\log_{10}$ CFU/100 cm^2. On the other hand, there were negative results for knives and reached max 1,65 $\log_{10}$ CFU/100 cm^2 in terms of coliform counts (Table 3). Similar to our results, several studies have demonstrated bacterial attachment onto stainless steel and other meat contact surface materials. Those results showed us that, mincing machines which may pose an important cross-contamination vehicle for meat, are not cleaned adequately. In the study of Garedew et al., (2016) approximately 72,2% of knives and other equipment in the butcher shops at Gondar town, Northwest Ethiopia were reported to be unsuitable in terms of hygiene. With the same study they observed that 7 (10,9%) knife swabs and 8 (12,5%) chopping (cutting) board swabs were positive for *Shigella* species. Depending on those findings they concluded that; lack of sanitary conditions is the most common cause of contamination of meat from different sources. Barril et al., (2019) determined hygienic risk of meat contact surfaces in butcher shops from Neuquén Province, Argentina. A total of 49 meat contact surfaces were sampled. According

to their study, pathogen microorganisms were found in 28,6% of environmental samples (*Salmonella* spp., 6,1%; non-O157 STEC, 2%; *L. monocytogenes*, 22,4%). As announced in Commission Decision 2001/471/EC and Commission Regulation (EC) No 1441/2007 on Microbiological Criteria for Foodstuffs, total viable counts and total *Enterobacteriaceae* on cleaned and disinfected surfaces in meat establishments should be ≤10 (1 $\log_{10}$) CFU/cm^2 and ≤1 (0 $\log_{10}$) CFU/cm^2, respectively. The critical limits that we defined to compare our results are similar to these criteria (Carrascosa et al., 2012). Mincing machines can be accepted as the main contamination points for meat. In this regard Papadopoulou et al., (2012) evaluated the transfer of pathogens population from pathogen (*Listeria monocytogenes*, *Salmonella enterica* ser. Typhimurium and *Escherichia coli* O157:H7) inoculated meat to non-inoculated beef fillets through meat mincing machine. Three different initial inoculum sizes (3, 5, or 7 log CFU/g) were tested and the inoculated beef fillets passed through meat mincing machine and then, six non-inoculated beef fillets passed in sequence through the same mincing machine without sanitation. As the result, all non-inoculated beef fillets were contaminated through mincing with all pathogens, regardless the inoculum levels used. This observation also showed us that mincing machines are important contamination points.

Table 3. Mean and min-max of ATP values (RLU/100 cm^2) and indicator bacteria counts (CFU/100 cm^2) for food contact surfaces in all butcher shops

Control Points		**Visit 1**		**Visit 2**		**Visit 3**		**Visit 4**	
	N	**Mean**	**Min-max**	**Mean**	**Min-max**	**Mean**	**Min-max**	**Mean**	**Min-max**
Cutting board ATP	10	9928	388-24334	14778	1272-48011	5679	11-19793	9668	331-46011
Cutting board TMAB	10	3	2,11-3,58	3,1	2,62-3,39	2,82	1,6-3,35	2,75	1,62-3,23
Cutting board C	10	0,85	0,3-1,6	0,6	0-1,48	1,56	0-1,6	0,78	0-1,7
Knife ATP	10	2496	17-9729	2816	13-18197	472	35-870	887	88-2562
Knife TMAB	10	2,1	0,3-2,58	2,75	1,38-3,4	2.31	1,2-2,95	1,86	1,15-2,18
Knife C	10	0	0-0,7	0,3	0-1,36	0.48	0,3-1,32	1,18	1,65-2
Mincing machine ATP	10	12606	64-63843	1334	28-9706	1155	16-2611	1674	10-12407
Mincing machine TMAB	10	2,35	1,28-2,96	2,62	1,65-3,14	2,51	1,79-2,97	2,02	1,56-2,41
Mincing machine C	10	1,04	0-1,95	0,48	0,7-1,4	0,48	0-1,34	1,04	0-2

It is stated that 60% of the staff working in the food establishments do not wash their hands correctly and 25-40% of the food-borne diseases originate from those working in food processing and food service (Temelli et al., 2005). In our study, microbiological samples from the hands of staffs working in the butcher shops, coliform bacteria were mostly negative and all were under the defined critical limits (Table 2). No measurements were detected negative for Staphylococci but no one was identified as *S. aureus*. However, many researchers detected *S. aureus* and should be accepted as a main risk for food handlers' hand hygiene. For instance, Lues and Van Tonder (2007) detected *S.aureus* as 88% and Ayçiçek (2004) 95%, Shojaei et al., (2006) 12,6%, Gorman et al., (2002) 4,5% on the hands of food handlers in different food production areas. Staphylococci counts on staffs' hands range from min 1,7 $\log_{10}$ CFU/hand to max 3,26 $\log_{10}$ CFU/hand (Table 4). Employee hygiene and particularly good hand hygiene, is crucial in reducing the contamination of food and minimizing the risk of food-borne

illnesses (Rediers et al., 2008). EC Commission Regulation 2073/2005 on microbiological criteria for foodstuffs is directed at food operators and provides food safety and process hygiene criteria (Lehto et al., 2011). As Barker et al., (2004) mentioned; fingers contaminated by an environmental surface can transfer viruses to up to seven clean surfaces. In the study of Temelli et al., (2005), the hygienic status of personnel hands working in meat cutting units was evaluated. TMAB, coliform bacteria, *E. coli*, *Enterobacteriaceae*, staphylococci, coagulase positive staphylococci and yeast and mold were enumerated. Average coliform bacteria counts were found as 10^3 (3 $\log_{10}$) CFU/ml on the personnel hands working in butcher shops and *E. coli* was found in the hands of personnel working in butcher shops as 37,5%. While mean staphylococci counts (CFU/ml) of personnel hands were found as 10^4 (4 $\log_{10}$), coagulase positive staphylococci were detected as 40% in butcher shops.

In food plants ambient air contains many substances, which are present in free form or connected to the substances called bioaerosol. There are many sources for air-borne microorganisms such as employees, ventilation, air conditioning, packaging material, entering air from outside and etc. Microbial factors include bacteria, yeasts, molds and viruses. Airborne microorganisms can be found on solid particles such as dust or in aerosol droplets, besides in the form of single organisms resulting from evaporation of water droplets or can occur with the development of certain types of molds (Özer and Kesenkaş, 2015). According to the air microbiological load measurement results; none of the butcher shops' cold storage rooms exceed the defined limit (3 $\log_{10}$ CFU/m^3) for mold and yeast counts. These cold storage rooms were the places where naked carcasses and un-wrapped meat products were kept. Because of that, obtaining low level of mold and yeast loads is satisfying result in terms of food safety and hygienic storage conditions. Asefa et al., (2010) investigated the patterns of fungal growth on dry-cured meat products, identified the important sources and factors of contamination. They collected 642 samples from the meat, production materials, room installations and indoor and outdoor air of the production facility. Standard mycological isolation and identification procedures were followed. Totally, 901 fungal isolates were obtained; of which 57% were molds while 43% were yeast. In our study we haven't detected mold and yeast load over the limits but such studies like Asefa et al., performed should be conducted in order to make risk evaluation for air microbiological load.

Table 4. Mean and min-max of indicator bacteria counts (CFU/ hand) for food handlers' hands and air of cold storage rooms (CFU/m^3) in all butcher shops

Control Points	N	Visit 1		Visit 2		Visit 3		Visit 4	
		Mean	Min-max	Mean	Min-max	Mean	Min-max	Mean	Min-max
Hands ST	10	2,76	2,28-3,23	2,76	2,26-3,15	2,8	2,18-3,26	2,3	1,7-2,57
Hands C	10	0	0-0,6	Nd	Nd	0,3	0,95-1	0,3	0-1,2
Storage rooms'air MY	10	1,566	1,41-1,76	1,61	1,43-1,89	1,88	1,28-2,14	1,72	1,45-2

ST: Staphylococci; C: Coliform bacteria; MY: Molds and yeasts; TMAB: Total mesophile aerobe bacteria; Nd: not detected. Microbiological results presented as $\log_{10}$ values

Table 5. Calculated score and the GHP status of all butcher shops depending on scoring system

Pilot butcher shops	*Calculated score	Hygiene status
Bs 1	74	Good
Bs 2	80	Good
Bs 3	74	Good
Bs 4	52	Bad
Bs 5	72	Good
Bs 6	60	Bad
Bs 7	68	Acceptable
Bs 8	64	Bad
Bs 9	76	Good
Bs10	100	Good

Table 5 presents the calculated scores of each butcher shops depending on the developed scoring system and the GHP status according to the scores. 6 of 10 (60%) butcher shops had the score over 68 which means "good" hygienic status. 3 butcher shops were defined as to apply "bad" hygiene practice and 1 of them was obtained to be "acceptable". Those results and also our evaluations on visual inspection that we have made during the visits showed us that, most of the selected butcher shops had satisfied level of GHP. The visual inspection is a method is still widely used to assess the level of cleanliness (Carrascosa et al., 2012; Tebbutt et al., 2007). Parallel to the goal in our study, Barril et al., (2019) collected the samples to investigate indicator microorganisms from the meat contact surfaces and characterized a checklist for the hygienic-sanitary risk of the butcher shops based on the quantitative results. They concluded that, risk quantification was useful to identify failures in different areas of the butcher shops. Leotta et al., (2016) performed a comprehensive evaluation of risk (before training and after training actions) in butcher shops of Buenos Aires, Argentina under a pilot program called "Healthy Butcher Shops". During this study, risk was quantified on a 1-100 scale as high-risk (1-40), moderate-risk (41-70) or low-risk (71-100). A total of 172 raw ground beef and 672 environmental samples were collected from 86 butcher shops during the evaluation (2010-2011) and verification (2013) stages of the study. Risk quantification resulted in 43 (50,0%) high-risk, 34 (39,5%) moderate-risk, and nine (10,5%) low-risk butcher shops before the training and 19 (22,1%) high-risk, 42 (48,8%) moderate-risk and 25 (29,1%) low-risk butcher shops after the training programs. As the conclusion of the program, they reported that risk quantification was useful to identify the hazards in butcher shops and the reduction of microbiological load in ground beef and the environment was possible.

4.Conclusions

As a result; although it is stated that it has been cleaned, it was seen that microbiological load is high, especially when cutting boards are not cleaned well, organic pollution and indicator microorganisms as high as that can create a risk for public health, and that was similar for meat mincing machines. It has been determined air mycological load of cold storage rooms is acceptable but monitoring and air disinfection should be periodically carried out in the cold air stores where the non-packaged products are stored. As a result of risk scoring system, most of the (60%) butcher shops were observed to be in good position in terms GHP.

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EFFECT OF HEATED ONION EXTRACT ON WHITE BUTTON MUSHROOM (*AGARICUS BISPORUS*) POLYPHENOL OXIDASE

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ABSTRACT

Heated onion extract exhibited a more potent inhibitory effect towards the browning of button mushroom (*Agaricus bisporus*) compared with the fresh onion. The inhibitions were 65.10% and 25.33% for fresh onion extracts for pyrocatechol and 4-methylcatechol, respectively. The percentage of inhibition increased to 68.51% for pyrocatechol and 42.33% for 4-methylcatechol when added with the heated onion extracts. Onion extracts inhibited the white button mushroom PPO non-competitively. The inhibitory efficiency of the onion extracts increased with increasing heating temperature and time. The percentage of inhibition for the non-heated onion extracts declined drastically from 89% to 50% for pyrocatechol and 77% to 31% for 4-methylcatechol after 9 days of storage at 4°C. Meanwhile, percentage of inhibition declined from 93% to 64% and 83% to 36% for pyrocatechol and 4-methylcatechol for heated onion extracts. Onion extract could be considered as a potential natural inhibitor for preventing browning of fruits and vegetables.

1. Introduction

Browning usually occurs in vegetables and fruits during handling, processing and storage. Food browning is normally undesirable due to a reduction in nutritional value, change in sensory perceptions, and decreased consumer acceptance of food (Lim *et al.* 2020). The enzyme that is responsible for the enzymatic browning of fruits and vegetables is known as polyphenol oxidase (PPO). PPO (EC 1.14.18.1) catalyzes hydroxylation of monophenols to *o*-diphenols, followed by oxidation of *o*-diphenols to *o*-quinones in the presence of oxygen, which lead to the formation of brown pigments (Ercili-Cura *et al.* 2015).

White button mushrooms (*Agaricus bisporus*), also known as champignon mushrooms and common mushrooms, which belongs to family Agaricaceae (Rachappa *et al.* 2020). It is one of the most popular edible fungi in the world. White button mushrooms are a good source of protein, minerals and vitamins such as vitamin C, B complexes and D. Besides the nutritional value, these edible mushrooms are also famous for their medicinal properties (Rachappa *et al.* 2020; Sinha *et al.* 2021). However, white button mushrooms have very short shelf life of between 3 to 5 days which is mainly due to the post-harvest enzymatic browning (Gholami *et al*. 2017). Therefore, it is important to study and control enzymatic browning of mushrooms in order to extend their commercial shelf life while preserving their nutritional value.

Chemicals anti-browning agents such as L-cysteine, sodium metabisulfite, have been the most studied for the used in processed foods. However, consumers are concern about sulphite

containing browning agents could cause problem to human health. Thus, this present research was to look for alternative ways to prevent and delay enzymatic browning of white button mushrooms. Onion was used as a natural inhibitor to investigate its inhibition towards white button mushroom PPO.

2. Materials and methods

2.1. Plant materials and chemicals

Fresh white button mushrooms (*Agaricus bisporus*) were bought from a local market in Kuala Lumpur. They were supplied by Champ Fungi Sdn. Bhd. in Telok Gong, Port Klang, Selangor, Malaysia. Red onions were bought from local market (Giant Hypermarket, Taman Connaught, Cheras, Kuala Lumpur). All chemicals used were analytical grade and were used as obtained.

2.2. Enzyme extraction

The extraction method was adopted from Wong and Lee (2014) with slight modification. White button mushrooms (60g) were washed and sliced. The samples were then homogenized with 600 mL of 0.05M sodium phosphate buffer (4°C, pH6.8) and 1% (w/v) of polyvinylpyrrolidone using an LB-8011ES industrial blender (Waring Laboratory, Torrington, CA, USA) at maximum speed (22,000 rpm) for 3 minutes. The homogenates were then subjected to centrifugation at 8000 rpm for 15 min at 4°C using a Universal 320R centrifuge (Hettich, Tuttlingen, Germany). The supernatant containing PPO was filtered under vacuum by Buchner filter (WP6211560 Vacuum pressure pump, Millipore Sigma, Burlington, MA, USA). The filtrate obtained was pipetted drop by drop into 600 mL of cold acetone (-20°C). The precipitates obtained were centrifuged at 8000 rpm for 15 min at 4°C. The white resultant powder was dried overnight at room temperature and stored at 4°C. In order to obtain the enzyme extracts, 1 g of acetone powder was suspended in 10 mL of pre-chilled 0.05M sodium phosphate buffer (pH 6.8) and stirred until all the powders were dissolved. The suspension was then centrifuged at 8000 rpm for 15 min at 4°C. The supernatant was used as the crude PPO extract.

2.3. Onion extract preparation

Red onions were used as natural inhibitors and the preparation of red onions was slightly modified from Lim *et al.* (2019). Red onions (100 g) were rinsed and sliced into small pieces. All the pieces of red onions were then homogenized with 100 mL of 0.05M sodium phosphate buffer (pH 6.8) at 22, 000 rpm for 3 min. Homogenized onion was centrifuged at 8000 rpm for 15 min. After centrifugation, the supernatant was then filtered and the filtrate was used as the fresh onion extract. Heated onion extract was prepared by incubating the fresh onion extract at 100°C for 10 minutes.

2.4. Influence of heat treatment temperature for onion extract on the inhibitory effect of white button mushroom PPO

Onion extracts were immersed in a water bath (Memmert Lab Companion, Jeio Tech, Selangor, Malaysia) in a temperature range of 30-100°C for 10 min prior to the PPO assay.

2.5. Influence of heat treatment time for onion extract on the inhibitory effect of white button mushroom PPO

This assay was done by preparing the heated onion extracts by immersing the onion extract into a water bath (Memmert Lab Companion, Jeio Tech, Selangor, Malaysia) at 100°C. An aliquot of onion extract was removed from the water bath at every 2 min interval until 14th min. The onion extract was then immediately cooled down to room temperature prior to the addition into the reaction mixture for PPO assay.

2.6. Influence of storage time of fresh and heated onion extracts on the inhibitory effect of white button mushroom PPO

Fresh onion and heated onion extracts prepared were kept in the refrigerator for 9 days. Aliquots of the onion extracts were taken on day 1, 3, 5, 7, 9 to be added into the reaction mixture. The efficacy of the inhibition was determined by measuring the absorbance by using a

spectrophotometer (Secoman, Champigny-sur-Marne, France).

2.7. Assay of PPO activity

PPO activity was determined via measurement of an increase in absorbance at 400 nm for pyrocatechol and 410 nm for 4-methylcatechol, respectively by using a PRIM Light spectrophotometer (Secoman, Champigny-sur-Marne, France) at 15 seconds interval. The reaction mixture contained 0.1 mL of enzyme solution, 0.9 mL of 0.05M sodium phosphate buffer (pH 6.8), 1 mL of onion extract (1 g/mL) as inhibitor and 1 mL of substrate. A control contained of 0.1 mL of enzyme solution, 1.9 mL of 0.05M sodium phosphate buffer (pH 6.8) and 1 mL of substrate. The initial velocity was calculated from the slope of the absorbance *vs.* time curve, where a single unit of PPO activity was defined as the amount of enzyme that caused a 0.001 absorbance change per min (Lim *et al.* 2019).

2.8. Statistical analysis

All the experimental data was performed by using Microsoft Office Excel. The data were presented as mean ± standard deviation (SD) (n=3) and also as percent relative activity.

3. Results and discussions

3.1. Inhibitory effect of onion extract on white button mushroom PPO

Table 1 shows the inhibitory effect of onion extract on white button mushroom PPO activity. Regardless of the substrate used, the heated onion extract at 100°C for 10 min exhibited a stronger inhibitory effect on white button mushroom PPO than did the fresh onion extract. These results were in agreement with those reported by Lim *et al.* (2019), Lim and Wong (2018), Wong and Lee (2014) and Kim et al. (2005) when sweet potato PPO, ginger PPO, cassava leaves PPO and pear PPO, were treated with heated onion extracts respectively.

However, a higher inhibition percentage of white button mushroom PPO by heated onion extract was found from this study (68.51%) than that of sweet potato (41.47%) (Lim *et al.* 2019) as well as ginger (33.11%) as reported by Lim and Wong (2018). Maillard reaction products produced during heating of onion extract probably would increase the inhibitory effect on browning of white button mushroom (Kim et al. 2005). It was also reported that the thiol compounds contained in onion might be the active components responsible for the inhibition of browning (Phisut and Jiraporn 2013; Akhtar 2015).

Table 1. The effect of natural inhibitors on white button mushroom PPO

Substrate	Inhibitor	Inhibition (%)	K_m (mM)	V_{max} (EU/min/ml)	Type of inhibition
Pyrocatechol	Fresh onion extract	64.74 ± 3.76	21.22	1414.43	Non-competitive
	Heated onion extract	68.51 ± 2.19	21.93	1096.49	Non-competitive
	Control	-	21.05	3508.77	-
4-methylcatechol	Fresh onion extract	25.33 ± 0.65	24.92	778.82	Non-competitive
	Heated onion extract	42.33 ± 1.77	25.58	639.39	Non-competitive
	Control	-	25.25	1010.10	-

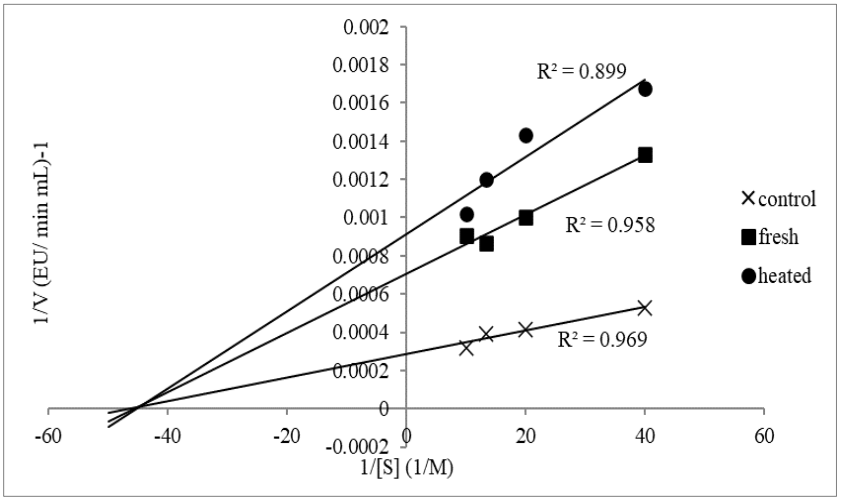

Figure 1. Lineweaver-Burk plot of fresh onion extract and heated onion extract on white button mushroom PPO using pyrocatechol.

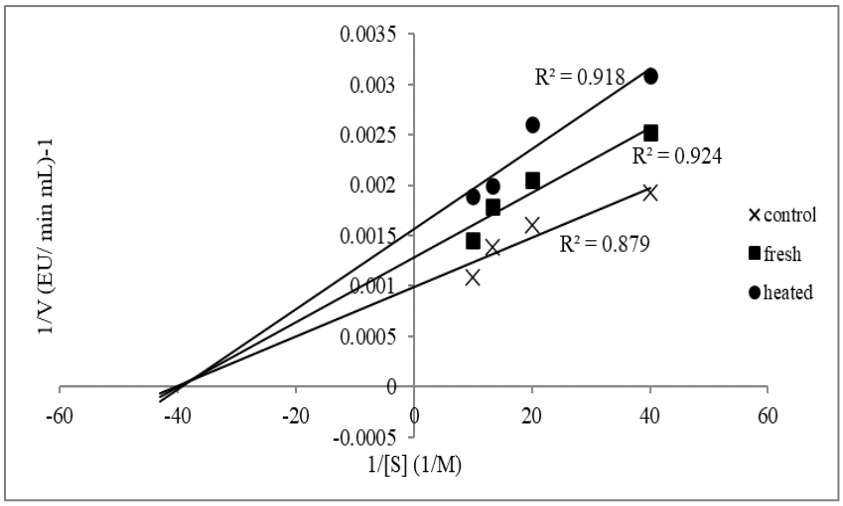

Figure 2. Lineweaver-Burk plot of fresh onion extract and heated onion extract on white button mushroom PPO using 4-methylcatechol.

Figures 1 and 2 show the Lineweaver-Burk plots of white button mushroom PPO in the presence of fresh onion extract and heated onion extract using pyrocatechol (Figure 1) and 4-methylcatechol (Figure 2), respectively. It can be seen that both the fresh and heated onion extracts inhibited the white button mushroom PPO non-competitively as the K_m of the enzyme was similar; while the V_{max} values decreases with the addition of both fresh and heated onion extracts. Similar type of inhibition has been found by Wong and Lee (2014) for cassava leaves PPO, which was non-competitive. According to Mohan *et al.* (2013), a non-competitive inhibitor binds to the enzyme other than active site and does not affect the affinity of the enzyme for the substrate. However, different type of inhibitions were found when onions were used to inhibit yam and ginger PPO (Yapi *et al.* 2015; Lim and Wong 2018).

The V_{max} values dropped drastically from an initial of 3508.77 EU/min ml to 1414.43 EU/min mL and 1096.49 EU/min mL, respectively when fresh and heated onion extracts were added with pyrocatechol as the substrate. The V_{max} decreased to 778.82 EU/min ml and 639.39 EU/min ml respectively from 1010.10 EU/min ml for 4-methylcatechol, when fresh and heated onion extracts were added (Table 1). 35% and 31% of the PPO activities retained when the

fresh and heated onion extracts for pyrocatechol and 75% and 58% of the PPO activities retained when the fresh and heated onion extracts for 4-methylcatechol (Table 1). These results show that onion extracts posed a higher inhibition power when pyrocatechol was used as substrate as higher percentages of PPO activities were inhibited.

3.2. Influence of heat treatment temperature for onion extract on the inhibitory effect of white button mushroom PPO

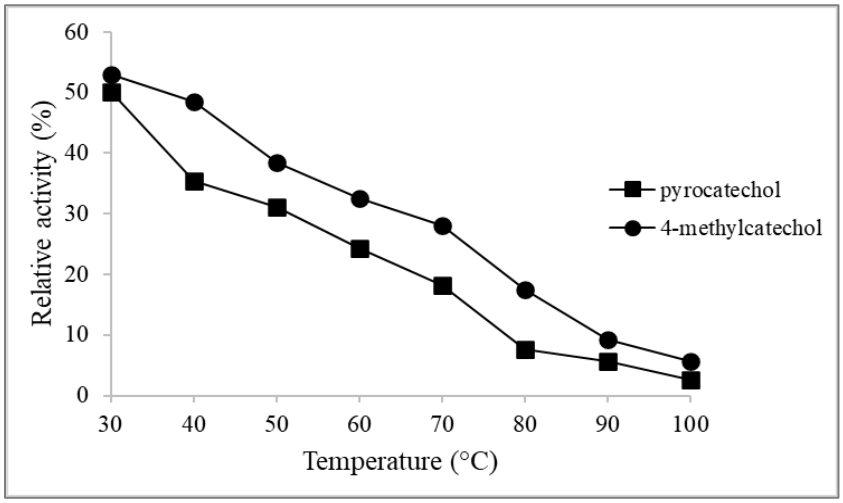

Figure 3. Effect of heating temperature of onion extract on the inhibitory effect of white button mushroom PPO

Figure 3 exhibits the inhibitory effect of onion extract after heating at various temperatures (30-100°C) for 10 min using pyrocatechol and 4-methylcatechol, respectively. As shown in Figure 3, onion extracts treated at a higher temperature exhibited a stronger inhibition towards white button mushroom PPO. This phenomenon was probably due to the inhibitory effect of Maillard reaction products increased with increasing treatment temperature (Lim *et al.* 2019).

The percentage of inhibition for the heated onion extracts increased from 47% to 94% and 50% to 97% with the increased of incubation temperatures from 30 to 100°C using 4-methylcatechol and pyrocatechol, respectively in this study. Similar results were obtained by Lee (2007) whereby the banana PPO activity markedly inhibited (22% to 65%) when the onion extracts were added after heating from 50 to 100°C.

3.3. Influence of heat treatment time for onion extract on the inhibitory effect of white button mushroom PPO

As shown in Figure 4, the longer the heating time on the onion extracts, the higher the decrease in residual PPO activity. Only 3% and 12% of relative PPO activities were found for 4-methylcatechol and pyrocatechol, respectively after 14 minutes heating of the onion extracts. These could be caused by the prolonged heating times may produce an increasing amount of inhibitory compounds, already formed at shorter heating times and or generate additional inhibitory compounds. The findings from this study was in agreement with those reported by Lee et al. (2007), whereby inhibition of taro PPO was increased with increasing of heating time for onion extracts.

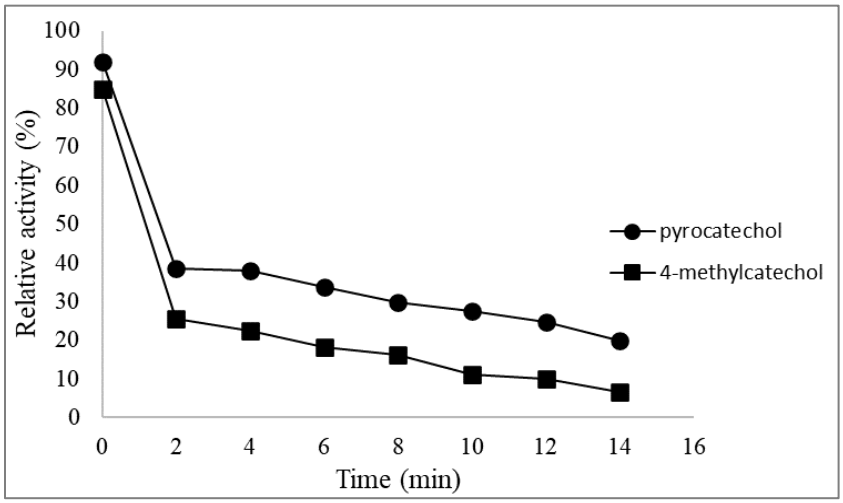

Figure 4. Effect of heating time of onion extract on the inhibitory effect of white button mushroom PPO

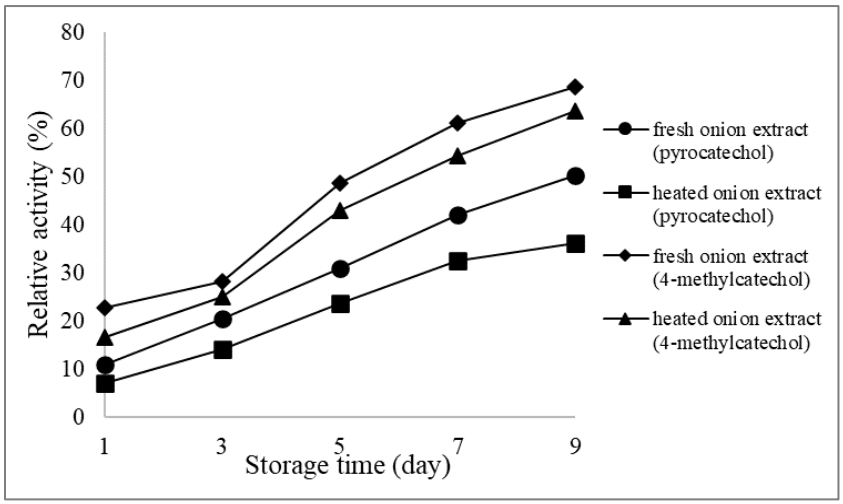

Figure 5. Effect of storage time of fresh and heated onion extracts on the inhibitory effect of white button mushroom PPO

3.4. Influence of storage time of fresh and heated onion extracts on the inhibitory effect of white button mushroom PPO

According to Figure 5, the relative PPO activities increased with the increasing of storage time, which indicated that the inhibitory effect of the onion extracts decreased with increased of storage time. It was noted that the relative PPO activity of white button mushroom increased from 11% to 50% in the presence of non-heated onion extracts, whereas 36% of the PPO activity was retained at day 9 of storage for the heated onion extracts when pyrocatechol was used. The fresh onion extracts showed nearly 77% inhibition when 4-methylcatechol was employed in the reaction mixture. However, the inhibitory effect drastically dropped to 31% after 9 days of storage. Similar pattern were observed when heated onion extracts were used, whereby a lower relative activity was obtained with 4-methylcatechol. The explanation for this could be due to the decreased in antioxidant activity of onion extracts with the increased of storage time (Lanzotti 2006).

4. Conclusions

This concluded that onion extract can be used as a natural inhibitor to prevent browning of white button mushroom. It can be potentially used to replace sulphite-containing anti-

browning agents and other chemical inhibitors which would possibly cause undesirable side effects. Inhibitory effect markedly increased with increase in temperature for a definite time and with heating time at a fixed temperature, while inhibitory effect decreased with storage time.

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EFFECTS OF PEPTIDES DERIVED FROM THE ANTARCTIC SCALLOP ADAMUSSIUM COLBECKI ON OBESE RATS' ADIPOSE TISSUE HISTOPHYSIOLOGY

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ABSTRACT

The researchers have currently investigated the possibilities of organisms from extreme living conditions as sources to obtain active biomolecules for therapy of different metabolic diseases including obesity. The purpose of the present study was to evaluated the effect of functionally active peptides derived from the tissues of Antarctic scallop (PAS) Adamussium colbecki on rat`s visceral white adipose tissue (WAT) state during high-calorie diet-induced obesity development. It has been found that after daily oral administration of PAS at a dose of 5 mg•kg-1 of body weight for 6 weeks, obese rat`s WAT histophysiology was improved, which was manifested in the decline in chronic inflammation due to decrease in the relative visceral fat weight, the size of white adipocytes, the fibrosis level in WAT and the crown-like structure presence in comparison with the rats, which were on a high-calorie diet (HCD). In addition, the body weight gain and the mast cell number in WAT of the rats with PAS showed an intermediate value, as they did not differ from both control and HCD groups. These data allow suggesting that oral administration of PAS to obese rats affects WAT inflammatory state and, in particular, ameliorates complications after HCD intakes.

1.Introduction

The bivalve Antarctic scallop (Adamussium colbecki) is recognized as an endemic to Antarctic waters (Cerrano et al., 2006; Cronin et al., 2020). Nowadays, the psychrophiles – the organisms that live in extremely low temperatures (usually -20°C – +10°C), have been actively investigated to obtain new active biomolecules for different aims (biotechnology industry, pharmacology etc.) (Bhatia et al., 2021). Marine organisms are considered a valuable source of bioactive compounds with high nutraceutical and pharmaceutical potentials. The biodiversity of the marine environment constitutes a practically unlimited resource to obtain biologically active substances. In recent years, special attention is focused on the hydrobionts of the Antarctic region which are poorly explored. To adapt to very exigent and aggressive surroundings these organisms produce plenty of molecules that may differ structurally from the terrestrial analogs. It is widely known that proteins of marine organisms have good functionality, high nutritional value, and therefore they could serve as a source to produce low-molecular-weight fragments or/and peptides (Baty et al., 2007).

Adamussium colbecki may be a novel source of cold-adapted proteolytic enzymes, antioxidants, and probiotic substrate. In our previous studies (Raksha et al., 2016; Raksha et al., 2020a), we found highly active enzymes (fibrino(geno)lytic and trypsin-like) in the crude extract of this hydrobiont. In addition, Adamussium colbecki exhibits higher activities of catalase, glutathione reductase, and glutathione peroxidases compared with the Mediterranean species (Benedetti et al., 2017). Also, many species of mollusks are included as an important compound in the Mediterranean diet (Tuttolomondo et al., 2019), usage of which demonstrated some efficiency in decreasing adiposity and improving cardiovascular risk factors in overweight/obese adult humans with metabolic syndrome and/or diabetes (Salas-Salvadó et al., 2019).

The aim of this study was to investigate the influence of functionally active peptides derived from the tissues of Antarctic scallop (PAS) Adamussium colbecki on the rats' visceral WAT (relative visceral fat weight, white adipocytes size, crown-like structure, tissue fibrosis level and presence of mast cells) in high-calorie diet-induced obesity.

2. Materials and methods

2.1. Isolation of functionally active proteins from the tissues of Antarctic scallop (PAS) Adamussium colbecki.

The soft tissues of the hydrobiont were hydrolyzed to produce the peptides. According to the necessity to obtain a fraction of peptides that would fully maintain the biological activity, the use of acid or alkaline hydrolysis, despite their high efficiency, is inappropriate. Therefore, enzymatic hydrolysis was chosen. Hydrolysis was performed using trypsin (3000 U·g-1 protein) at 37°C and pH 8.0 for 24 h. The degree of hydrolysis was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis in 10% polyacrylamide gel. The hydrolysis was stopped by boiling the sample to inactivate the enzyme. The hydrolysate was then centrifuged at 10 000 g for 15 min. The peptide fraction was prepared by microfiltration using a membrane with a molecular weight cut-off (MWCO) of 5 kDa. The obtained peptide fraction was further lyophilized. After dissolving the peptide lyophilizate in 0.9% NaCl, it was used for animal studies as the fraction of peptides derived from the tissues of marine hydrobiont.

2.2. Animals and Experimental design of high calorie diet-induced obesity (HCD).

Sixty albino nonlinear male rats with an initial body mass of 110 ± 10 g were used in this study. All experiments on animals were performed in compliance with the international principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Article 26 of the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV, dated February 21, 2006), as well as all norms of bioethics, biological safety, and the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (September, 2001). All manipulations were approved by the Ethical Committee of Educational and Scientific Centre "Institute of Biology and Medicine" Taras Shevchenko National University of Kyiv.

The experiments started after 7 days of animal acclimation in the animal facility of Taras Shevchenko National University of Kyiv, maintained under constant conditions of temperature (22 ± 3 °C), humidity (60 ± 5%), and light (12 h light/12 h dark cycle). Standard rodent food and water were provided ad libitum. For the first week, all rats had ad libitum access to standardized food "Purina rodent chow" and water. On the 8th day, animals were randomly divided into two groups (20 and 40 animals). Rats of the first group were fed with a standard ration (3.81 kcal/g), whereas those of the second group received a

high-calorie diet (HCD) (5.35 kcal/g). The high-calorie chow included 60% standard food composition, 10% pork fat, 10% hen eggs, 9% sucrose, 5% peanut, 5% dry milk, and 1% vegetable oil (Halenova et al., 2018). To confirm the development of obesity the animals were weighed one time a week until the average body gain reached a significant difference of at least 30% between the two groups. After that, HCD group was divided into two subgroups (20 animals in each subgroup), so in sum we have 3 groups:

1) rats without treatment fed with a standard ration (Control group);

2) rats fed with a HCD (HCD group);

3) rats fed with a HCD and receive administration of PAS (HCD+PAS group).

PAS was administered by intragastric (i.g.) at a dose of 5 mg•kg^{-1} of body weight every other day for the next 6 weeks.

Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week. Body weight gain, relative daily food (kcal/day/g body weight) and relative daily water consumption (ml/day/per rat) was determined for each rat. In the last day of the experiment the epididymal, retroperitoneal, perirenal fat pads were dissected and immediately weighed and relative visceral fat weight was calculated.

2.3. Histology examination.

On the last day of the experiment, the animals were removed from the experiment by decapitation by guillotine, and visceral white adipose tissues (WAT) from perirenal localization were taken out. WAT were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 72 h, dehydrated and embedded into paraffin wax. From the paraffin blocks, 5 µm sections were prepared and stained with Hematoxylin and Eosin (H&E).

2.4. Histochemical detection of tissue collagen content (fibrosis level) in WAT.

Detection of collagen fibers was carried out using histochemical picrofucsin by Van Gieson trichrome staining with addition of hematoxylin to visualize nuclei (Mishra et al., 2015). For quantitative determination of tissue collagen content, microphotographs were obtained using an objective with 4 × magnification; for each subsequent microphotography, the field of view was moved to the right, retaining 20% of the previous field until the entire cut was taken. To create one large composite panoramic image, the cross-linking software Adobe Photoshop CS6 (Adobe Systems, USA) was used. From each image, the area of the red-stained region (red - collagen fibers) was calculated and expressed as a percentage of the total area.

2.5. Histochemical detection of mast cells in WAT.

Mast cells were detected by staining in a 0.1% aqueous solution of toluidine blue (Altintas et al., 2011).

Subsequent research was performed using a light microscope Olympus BX41 (Japan). Microphotographs were obtained using the Olympus DP20 (Japan) digital camera and the QuickPHOTO MICRO software (Promicra, Czech Republic). The cross-sectional area of white adipocytes, the number of mast cells and crown-like structure (CLS), tissue fibrosis (the area occupied by collagen fibers) were measured using ImageJ software (National Institutes of Health, USA).

2.6. Statistical analysis.

SPSS 16.0 software was employed for data analysis. The normality of data distribution was determined by the Kolmogorov-Smirnov test. Significance of the observed changes was assessed by parametric (one-way ANOVA with Tukey's post hoc multiple comparison tests) and nonparametric (Kruskal–Wallis test for independent samples) methods. $P < 0.05$ indicated a statistically significant difference.

The results obtained are presented as mean ± standard error of the mean (SEM).

3.Results and discussions

3.1.Body weight changes, relative WAT mass changes, food and water intake.

The body weight gain in the HCD rats increased by 40% in comparison with the control group (Table 1). The value of body weight gain in the rats of HCD+PAS group was at an intermediate state: it did not differ from either the control or the HCD group. Nevertheless, the relative visceral fat weight in HCD+PAS group demonstrated significant difference between HCD group: its value decreased by 30%; and also, between the control group: its value increased only by 17%. In contrast, the relative weight of visceral fat in the HCD group increased by 65% in comparison to the control. It should be emphasized that the change in the relative weight of visceral fat in the HCD + PAS group was not correlated with the decrease in food intake, since the relative daily food intake in the HCD + PAS group was as high as in the HCD group (the values are increased by 32%, and 36%, respectively, compared with the control). The relative daily water intake was decreased in the HCD and HCD+PAS groups by 17% and 24%, respectively, compared with the control. Obtained data indicate that there is no effect of PAS on the relative daily intake of food and water.

Table 1. Body weight gain, relative daily water and food consumption, visceral fat weight of experimental groups of animals.

Parameters	Control group	HCD group	HCD + PAS group
Body weight gain (%)	195±23	271±17 *	235±23
Relative visceral fat weight (%)	1.78±0.03	2.93±0.31 *	2.08±0.03 * #
Relative daily food consumption (kcal/day/g body weight)	0.251±0.002	0.344±0.002 *	0.332±0.006 *
Relative daily water consumption (ml/day per rat)	38.9±0.7	32.2±0.4 *	29.5±1.5 *

Data are presented as the M ± SEM;
* $p < 0.05$ compared with the control value,
# $p < 0.05$ compared with the HCD group

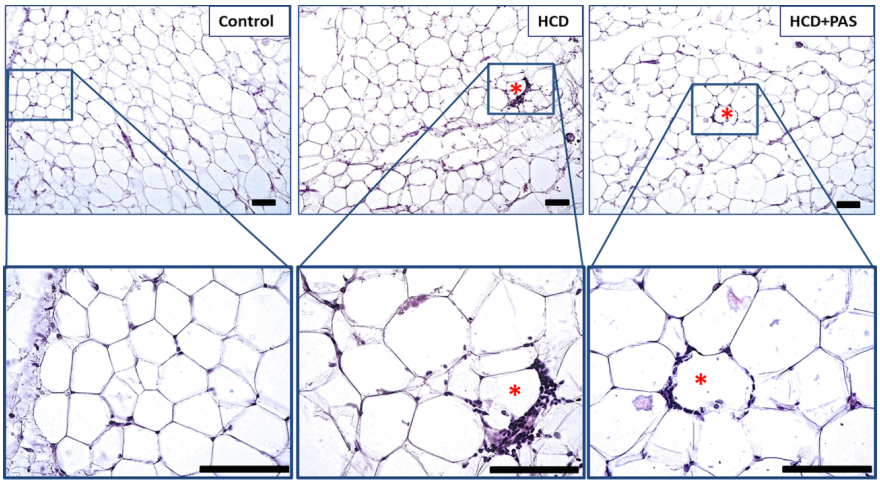

Figure 1. Microscopic appearance of the rats' WAT sections of Control, HCD, HCD+PAS group: H&E staining; scale bar 100 µm. Note, asterisk – crown-like structure

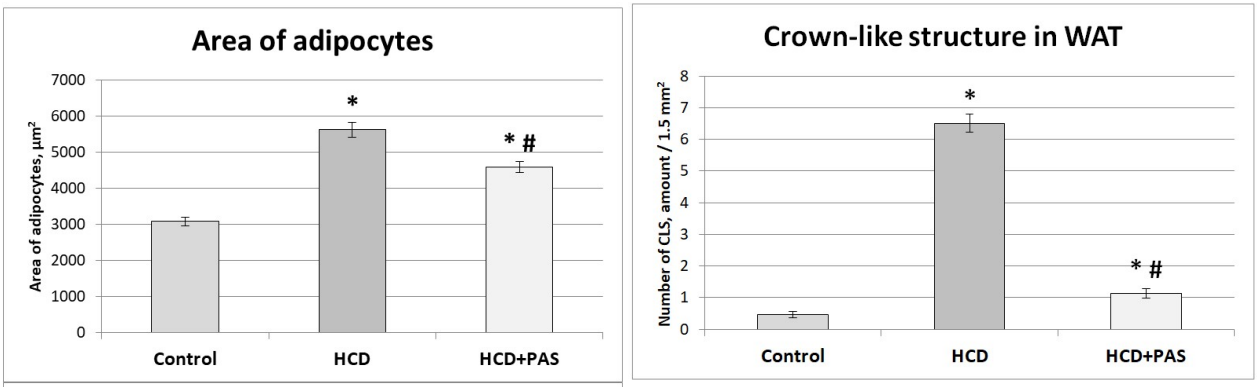

Figure 2. Results of WAT morphometric analysis: CLS number and cross-section area of white adipocytes. Data are presented as the M ± SEM; * - $p < 0.05$ compared with control value, # - $p < 0.05$ compared with HCD.

3.2. Histology examination of WAT.

As shown in Figure 1 (asterisk), the crown-like structures (CLS) appear in WAT of obese rats – dead adipocytes, which are surrounded by leukocytes. CLS is the one of chronic inflammation markers in WAT. In the rats that received PAS (HCD+PAS) remain to appear CLS, however with a lower number of leukocytes. Morphometric assay (Figure 2) of WAT reveals an increase in CLS in the HCD group by 14-fold compared with the control. The administration of PAS (group HCD+PAS) leads to a decrease in CLS by 5.7-fold compared with the HCD group, but this value is increased by 2.5-fold compared with the control. The adipocytes area was increased in the HCD group by 85% in comparison with the control, while in the HCD+PAS group only by 50% (that is smaller by 18% in comparison with the HCD group).

3.3. Fibrosis level in WAT.

During developing obesity, the inflammation state of WAT changes, which manifests in chronic mild status (Figure 3). These connect with leukocyte infiltration (Figure 3, arrow head) and collagen fibers deposition rising in WAT of HCD group. Markedly, collagen fiber deposition increased not only in the connective tissue septa between lobules of adipose tissue, however collagen fibers localized around each individual adipocyte. The relative area occupied by collagen fibers (Figure 4) in the HCD group increased by 3.5-fold compared to the control. Fibrosis level after application of PAS (group HCD+PAS) decreased by 2.4-fold compared to the HCD group, but still increased by 1.5-fold in comparison to the control.

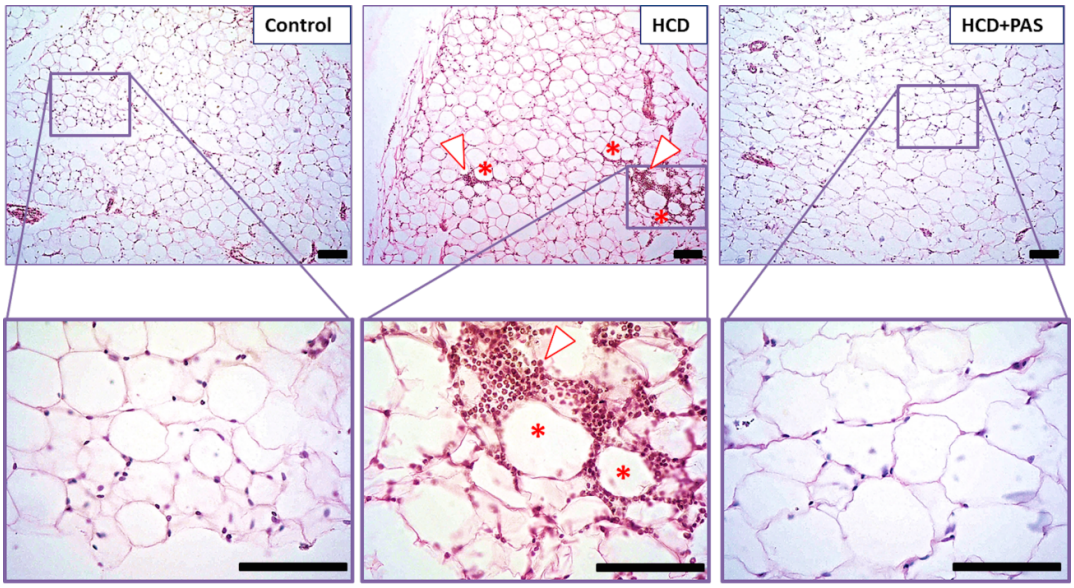

Figure 3. Microscopic appearance of the trichrome histochemical staining for fibrosis level detection in WAT sections of Control, HCD, HCD+PAS group: Van Gieson staining; scale bar 100 µm. Note, asterisk – crown-like structure, arrow head – leukocytes infiltration.

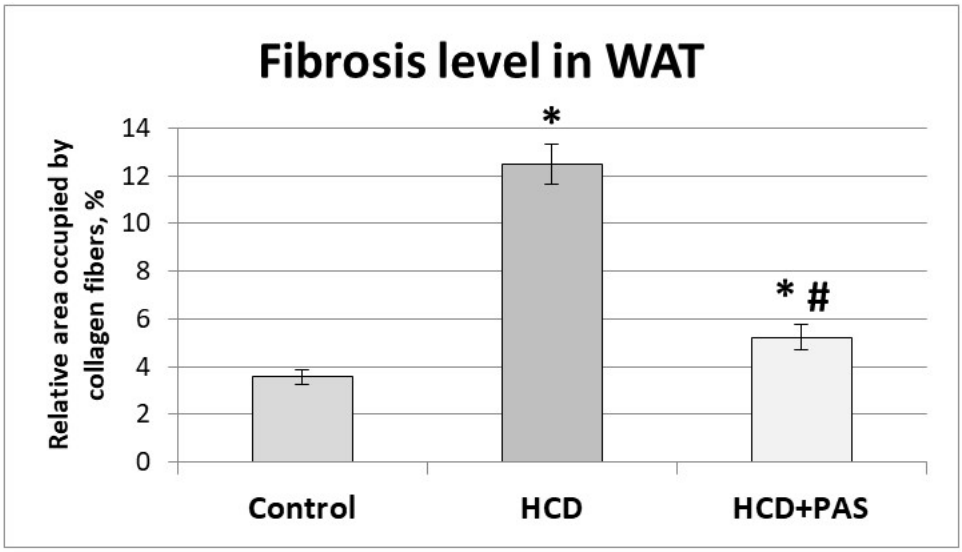

Figure 4. Fibrosis level in WAT. Data are presented as the M ± SEM; * - $p < 0.05$ compared with control value, # - $p < 0.05$ compared with HCD.

3.4. Mast cells analysis in WAT.

On WAT histophysiology also influences the mast cells presence. In control group mast cells localized near capillary and along connective tissue septa (Figure 5). In HCD group mast cells recruiting in adipose tissue lobules between septa, moreover they mainly were in degranulated state. In some cases, mast cells take part in the formation of CLS together with leukocytes. After oral administration of PAS (HCD+PAS group) the presence of mast cells in adipose tissue lobules fell, and they did not make CLS. The number of mast cells in WAT (Figure 6) in the HCD group increased by 55% in comparison to the control. In HCD+PAS group the value of this parameter takes

intermediate meaning – it`s not significantly differ from control and HCD group both.

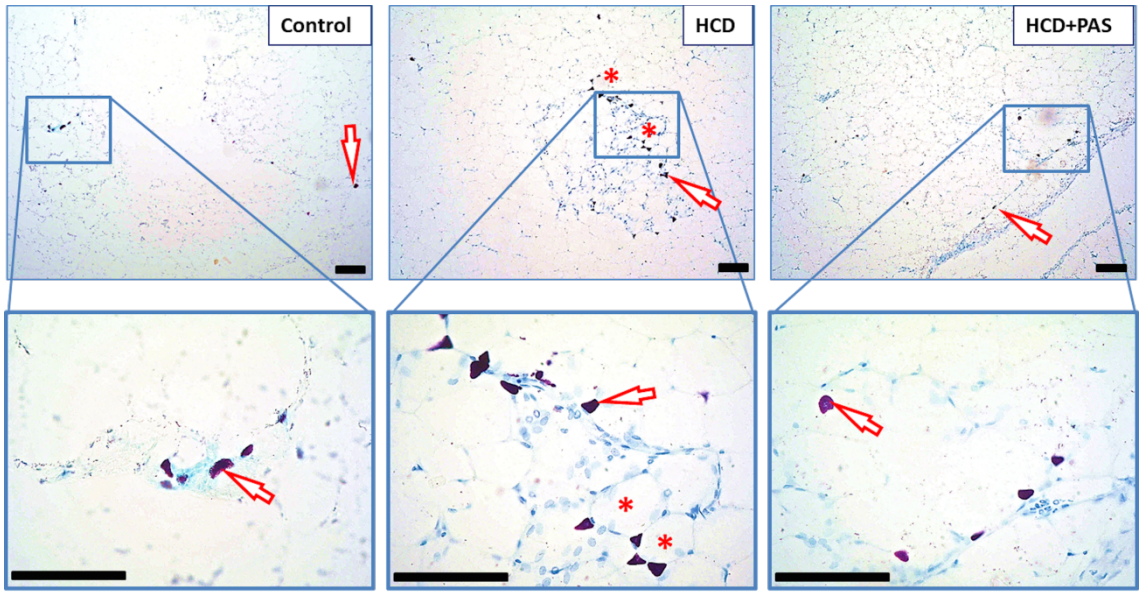

Figure 5. Microscopic appearance of the histochemical staining for mast cells detection in WAT sections of Control, HCD, HCD+PAS group: toluidine blue staining; scale bar 100 µm.
Note, asterisk – crown-like structure, arrow – mast cells.

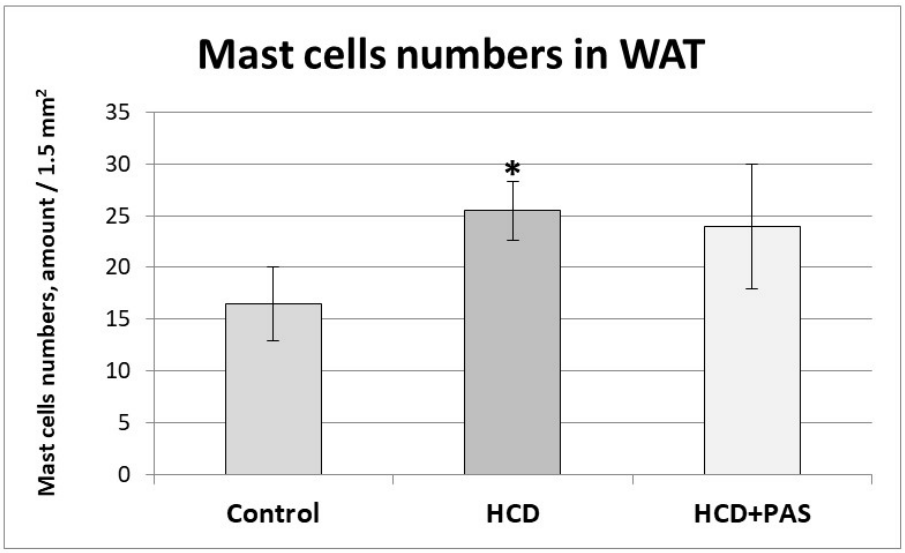

Figure 6. Mast cells detection in WAT. Data are presented as the M ± SEM; * - $p < 0.05$ compared with control value, # - $p < 0.05$ compared with the HCD group.

3.5. Discussions

The results of this study clearly show that oral administration of functionally active peptides derived from the tissues of *Adamussium colbecki* significantly reduced the relative weight of visceral fat, the size of white adipocytes, the appearance of CLS, and the level of fibrosis in WAT of obese animals.

Given our previous data (Raksha et al., 2018; Raksha et al., 2019), we suggest that the beneficial effect of PAS may be partly realized through the influence on oxidative status,

namely, the activity of key antioxidant enzymes and the level of lipid peroxidation products.

One of the explanations of the obtained data may be the anti-inflammatory and immunomodulatory properties of extracts derived from marine mollusks (Anmad et al., 2018). This coincides with our previous results - we observed the decrease in the concentration of the proinflammatory cytokine in obese animals, which received the peptide fraction. In a mouse model of lipopolysaccharide-induced acute lung inflammation, an application of hypobranchial gland extract from the Muricidae mollusk *Dicathais orbita* (the active compound is probably 6-bromoisatin) decreased the level of tumor necrosis factor α and interleukin-1 β in bronchoalveolar lavage fluid (Ahmad et al., 2017). In addition, some mollusk peptides possess strong/moderate antitumor cytotoxicity and immune modulatory properties, which is provided by various terminal alkynyl-containing cyclic peptides - Dolastatin 17, Onchidin, Coololid-1, Kulokinalid-1 (explored mainly from marine mollusk *Dolebella auricularia*, marine pulmonate mollusk *Onchidium sp*., marine mollusk *Philinopsis speciosa Pease*, Marine cephalaspidean mollusk *Philinopsis speciosa*, respectively) (Chai et al., 2016). Some members of Bivalvia (*Perna canaliculus* and *Anadara kagoshimensis*) shown similar in vivo and in vitro anti-inflammatory effects, that manifested in inhibition of leukotriene production; inhibition of lipoxygenase products in arachidonic acid pathway; inhibition of cholesterol, TNF-α, IL-1, IL-2, IL-6 biosynthesis; antioxidant potency; antiplatelet aggregation potency; fibrinolytic potency; reduction in neutrophil superoxide burst activity (Khan, et al., 2019). In all, marine natural product pharmacology for finding molecules with immune-modulatory properties is steadily developing and in the period of 2016-2017, it counted 313 marine compounds with novel pharmacological characteristics (Mayer et al., 2021).

Another explanation for the anti-obesity effect of PAS may be related to the gut microbiota (Ballini et al., 2020). PAS can be a probiotic substrate and influence the production of secondary metabolites by gut microorganisms. Mollusks have been shown to be an essential ingredient in the Mediterranean diet, which, if consumed for 12 months, stimulates the changes in microbiota - an increase in *Roseburia* and *Oscillospira*, a decrease in *Prevotella,* which result in an increase in insulin sensitivity in obese people (Haro et al., 2016). Many cohort studies between Mediterranean and non-Mediterranean diets demonstrated influence on the risk of development metabolic syndrome, obesity, type 2 diabetes mellitus, cancer, and neurodegenerative diseases (Lăcătușu et al., 2019).

Only a few studies have demonstrated the influence of bioactive peptides derived from marine hydrobionts on obesity development (Jin et al., 2018). The investigation of di- and tripeptides from marine hydrobionts on human white pre-adipocytes showed a final (after differentiation) decrease in lipid content, downregulation of both PPARγ and C/EBPα expression (key regulators of adipogenesis) (Henda et al., 2015). Other studies showed effect of bioactive peptides derived from marine organisms on management of type 2 diabetes mellitus through a few mechanisms: protecting pancreatic β-cells, enhancement of glucose-stimulated insulin secretion, regulation of glucose uptake and lipid accumulation, regulation of the insulin-signaling pathways (for example, natural peptides display upregulation insulin receptors, phosphoinositide-3-kinase, translocation of glucose transporter protein GLUT4 and downregulation phosphatase and tensin homologue (PTEN)) (Xia et al., 2016). Also, the mechanisms of action on decrease hyperglycemia in case of type 2 diabetes mellitus proposed that natural peptides with α-amylase and α-glucosidase inhibitory activity (Siow et al., 2017; Mojica et al., 2017).

However, further detailed studies are needed to integrate the applied methodology, dose, routes of administration, and mechanisms of action of mollusk-derived active peptides to the therapeutic treatment of obesity (Hu et al., 2016) and metabolic syndrome.

4. Conclusions

The extract of functionally active peptides from the tissues of Antarctic scallop (PAS) *Adamussium colbecki* administrated to the obese rats have potential to stimulate partial recovery of white adipose tissue histophysiology and thereby promote therapeutic activity in obesity treatment.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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DEVELOPMENT AND OPTIMIZATION OF AN OZONE FOOD PRESERVATION SYSTEM USING RESPONSE SURFACE MODELLING (RSM)

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ABSTRACT

This paper is aimed to design and implement an ozone food preservation system to increase the shelf life of food products. An experimental procedure based on response surface modeling has been proposed in order to optimize the geometrical dimensions of a planar surface dielectric barrier discharge ozone generator, with minimum energy consumption. The experiments were conducted in an ozone-treated cold room using green pepper, strawberries and sardines. A comparative analysis using a similar untreated control room has shown that such a system improves significantly the shelf life of the products for long-term storage.

1.Introduction

Ozone has been harnessed for beneficial purposes such as purification of drinking water, deep treatment of waste water, bleaching, deodorization, aquaculture, medicine and so on because of its strong oxidative potential (Rakovsky, et al., 2009; Nogales et al., 2008; Ramdani et al., 2016; Nemmich, et *al*., 2015). It is capable of inactivating bacteria, bacterial spores, molds, yeasts, protozoan cysts and viruses (Giese and Christenser, 1954; Scott and Lesher, 1963; Kim, 1998). Ozone has been tested on nearly every type of food during storage and processing to improve the safety and to extend the shelf-life of these products (Jbilou, et al ,2018; Nehari, et al., 2019). Ozone not only inactivates microbial contaminants, but is also potentially useful in decreasing the level of pesticides, such as azinphosmethyl, captan, formethanate-HC1 and ethylenethiourea, on fresh produce (Ong et al., 1996).

The food industry is interested considerably in using ozone to enhance the shelf-life and safety of food products and in exploring new applications of the sanitizer. Ozone is currently used in many countries and its use in food processing has been approved recently in the United States (Federal Register, 2001). Additionally, ozone-treated produce has just been introduced in the United States market.

The use of ozone in the food industry has been applied to shelf-life extension of commodities during storage. Recently, there has been a renewed interest in ozone and its application in food processing. Application of ozone for washing fruits and vegetables with ozone is gradually gaining acceptance.

Ozone can be produced by electric discharge, photochemical, chemical, thermal, chemonuclear, and electrolytic methods (Nassour et al., 2018; Horvath et al., 1985). The dielectric barrier discharge (DBD) method is commonly used to produce large amounts of

ozone. The DBD produces ozone when a high voltage alternating current is applied across a discharge gap in the presence of oxygen or air (Kim et *al*., 2003; Moon et al., 2007; Boonduang et al., 2012).

Ozone generators have been widely studied for optimizing the output generation rate of ozone, according to several parameters such as the high voltage level, the oxygen rate, the frequency of the voltage and other factors. However, no experimental study was performed to identify the optimal geometrical sizes of an ozone generator, i.e. the dimensions of the inter-electrodes gap and the effective length of the generator, giving the highest ozone concentration level with the smallest power consumption.

The aim of the present work is to propose an experimental procedure based on response surface modeling (RSM) for the identification of the optimal values of a surface DBD ozone generator used in food storage cold rooms, based on ozone generation output and power consumption (Taguchi 1987).

2. Materials and methods

2.1. Ozone generator

The most efficient method for generating ozone is to pass oxygen (O_2) through DBD-plasma, which converts it into ozone, a molecule with 3 oxygen atoms (O_3).

A planar surface DBD reactor was developed, comprising a dielectric barrier made of Bakelite of dimensions 190x180x2 mm^3 (Figure1). The electrodes are made of aluminum adhesive strip placed on both sides of the Bakelite plate.

The high voltage electrode consists of several strips of dimensions 170x2 mm^2 while the ground electrode is an aluminum strip bonded to the other face of dimensions 170x160 mm^2.

Several active high-voltage electrodes have been made on the top side so that their number could vary from 2 to 14 and the distance "d" between adjacent electrodes could take 5 different values (2, 4, 6, 8 and 10 mm).

The experimental laboratory setup used for the experiments is described in figure 2. It consists of a cold chamber with adjustable temperature from 2 to 10 °C (refrigerator) of a volume equal to 0.5 m3.

The surface DBD ozone generator is fixed on the upper wall of the enclosure, powered by a high-voltage high-frequency power supply (5 kV, 100 mA, 22 kHz) placed outside the enclosure. A portable ozone analyzer (O_3 Technologies) was used to measure the ozone concentration produced in the chamber in ppm.

The applied voltage U was measured with a digital scope (GWINSTEK GDS-3154) using a high voltage probe (Tektonix P6015A), while the current I was measured by a current probe. Moreover, the consumed power of the ozone generator was estimated by calculating the average value of the product U*I over 25000 points.

A list of values of voltage U and current I is provided by the scope and then the power is estimated by calculating the average value of the product U*I.

For the experiments of food storage, three different food products were tested: strawberry, green pepper and sardine. Notice that one product at once was placed in the storage room and analyzed separately, in both treated and untreated enclosures.

All the experiments were performed under stable conditions of temperature (5 °C) and humidity (85%) inside the storage room.

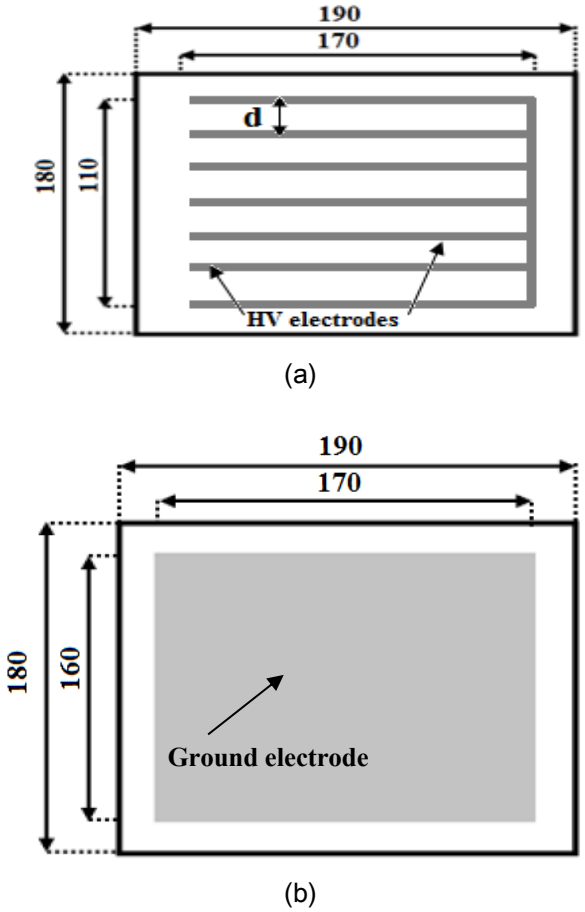


Figure 1. Surface dielectric barrier discharge ozone generator used in this study. Top (a) and bottom (b) view of the flat ozone generator (all dimensions in mm)

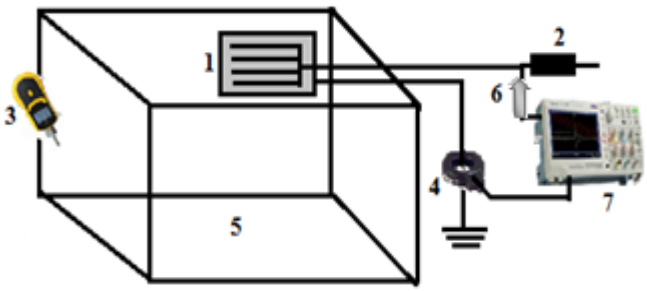


Figure 2. Descriptive schematic of the ozone air treatment system
1. Ozone Generator, 2. High-voltage transformer, 3. Ozone monitor, 4. Current probe, 5. Cold room (fridge), 6. High voltage probe,7. Digital oscilloscope.

2.2. Experimental Designs Methodology

Methodology of the experimental designs makes it possible to determine the number of experiments to be achieved according to a well-defined objective, to study several factors simultaneously, to reduce dispersion related to measurements, to appreciate the effects of coupling between factors and finally to evaluate the respective influence of the factors and their interactions (Frigon, 1996; Taguchi, 1987; Eriksson, 2000).

Before starting the experiments, it is necessary to set the best and suitable design which can model the process with the most possible precision. In this paper, the Composite Centred Faces design (CCF), which gives quadratic models, was adopted. It is possible to determine a quadratic dependence between the output function to optimize (response) and the input variables u_i ($i = 1, \ldots\ldots, k$) (factors):

$$y = f(u_i) = c_0 + \sum c_i u_i + \sum c_{ij} u_i u_j + \sum c_{ii} {u_i}^2 \quad (1)$$

Knowing that $\Delta u_i$and u_{i0} are respectively the step of variation and the central value of factor i, reduced centred values of input factors may be defined by the following relation:

$$x_i = (u_i - u_{i0})/\Delta u_i \quad (2)$$

With these new variables, the output function becomes:

$$y = f(x_i) = a_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} {x_i}^2 \quad (3)$$

The coefficients can be calculated or estimated by a data-processing program, in such a way to have a minimum variance between the predictive mathematical model and the experimental results.

MODDE 5.0 software (Umetrics AB, Umea, Sweden) was used, which is a Windows program for the creation and the evaluation of experimental designs [MODDE 5.0, 1999]. The program assists the user for interpretation of the results and prediction of the responses. It calculates the coefficients of the mathematical model and identifies best adjustments of the factors for optimizing the process. Moreover, the program calculates two significant statistical criteria which make it possible to validate or not the mathematical model, symbolized by $R^2$and Q^2. The former is called the goodness of fit, and is a measure of how well the model can be made to fit the raw data; it varies between 0 and 1, where 1 indicates a perfect model and 0 no model at all. The latter is called goodness of prediction, and estimates the predictive power of the model. LikeR^2, Q^2 has the upper bound 1, but its lower limit is minus infinity. For a model to pass the diagnostic test, both parameters should be high, and preferably not separated by more than 0.2 - 0.3.

3. Results and discussions

The experimental study was carried out by considering the two most important factors of a surface DBD ozone generator, which are the number N of high voltage electrode-wires and the distance between wires d(in mm).

Design of experiments methodology is useful for screening and optimization. Screening experiments are designed here to identify the domain of variation of two factors, classical "one-factor-at-a-time" experiments. The optimization stage of an experimental procedure should enable the determination of factor values for which the consumed power is a minimum. In addition, several studies have shown that typical ozone concentrations used for food processing in cold rooms should be in the range between 2 and 7 ppm (particles per million) (Rice, Rip G., et *al*, 1997; Hampson, Brian C., et *al* 1997).

Therefore, we estimated the operating time Δt of the ozone generator to ensure an ozone concentration between 2 and 7 ppm and also the power consumed by the generator according to factors d and N.

3.1. Screening experiments

The variation limits of number of wires N and distance dare defined by following "one-factor-at-a-time" experiments.

- Experiment 1: Variable number of wires N (4–14), with a constant value of wires distance d= 10 mm.
- Experiment 2: Variable distance d (2–10 mm) with a constant value of the number of wires N = 6.

Obtained results of above experiments are given in figures 3and 4. The operating time Δt to

achieve an ozone concentration range2-7 ppm and the consumed power were considered as significant for the evaluation of the process and represented as function of the two-control factor. The domain of variation of N and d were then defined based on results obtain in the previous section. The variation domain for each factor was determined as follows:

- Number of wires: N_{min} = 8; N_{max} = 12;
- Distance between wires: d_{min} = 6 mm; d_{max} = 10 mm.

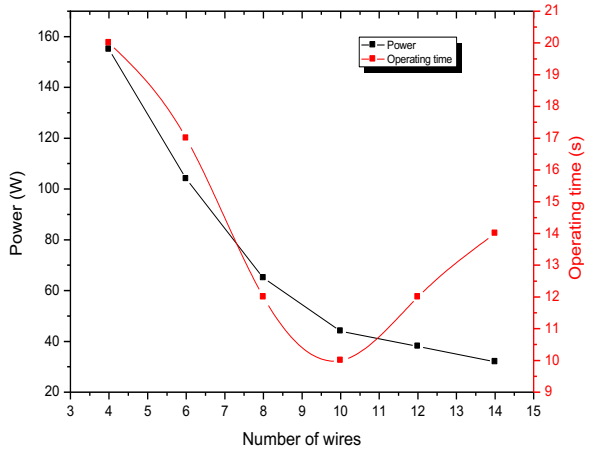


Figure 3. Variation of time Δt and power P as function of the number of wires (d=10 mm)

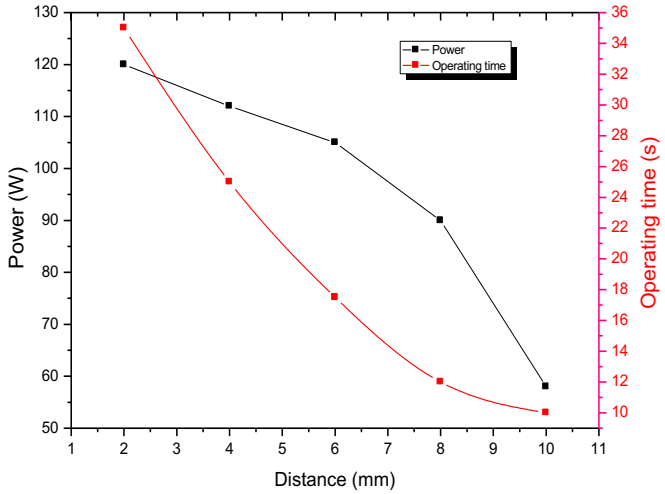


Figure 4. Variation of the operating time Δt and the power P as function of the distance d between wires (N= 6)

3.2. Set Point Identification

The identification of the optimal values of both factors were determined by using a central CCF design; the two levels "max" and "min" are the limits established in previous section for each of the two variables, (N_{min}, N_{max}) and (d_{min}, d_{max}). The central point (N_c and d_c) being calculated as follows:

$$Nc = (N_{min} + N_{max})/2 = 10 \quad (4)$$

$$D_c = (d_{min} + d_{max})/2 = 8\,mm \quad (5)$$

The experimental results of the CCF design are given in Table I. Besides the operating time Δt, the power P (W), was also considered as a significant response and reported in the table 1.

Table 1. Results of the CCF design

Exp.	Number of wires N	Distance d(mm)	Operating time Δt (s)	Power P (w)
1	8	6	12	65
2	12	6	12	38
3	8	10	12	65,6
4	12	10	12	45,3
5	8	8	15	91,5
6	12	8	15	58,4
7	10	6	10	44
8	10	10	10	44,8
9	10	8	13	67,1
10	10	8	12,5	67,1
11	10	8	12	67,1

The mathematical models of time operating Δt and Power P were obtained with MODDE 5.0 and plotted as shown in figures 5 and 6. Since the statistical criteria R^2 and Q^2 were close to the unit, as noted in figures 5 and 6, both models were validated and have been used for prediction and optimization analysis.

According to the results, we found that the effects of both factors are significant for the power and the operating temperature. Concerning the power P, the coefficient of the number of wires N is negative. On the other hand, the distance does not have an important effect on the power in the studied interval, however the parameter N^2seems to have an important influence on the power.

Concerning the operating time ΔT the most significant parameters are N^{*2} and d^{*2}. Moreover, the results obtained using the mathematical models do not indicate any significant interaction between the geometric factors N and d, their effect not being significant for the two responses.

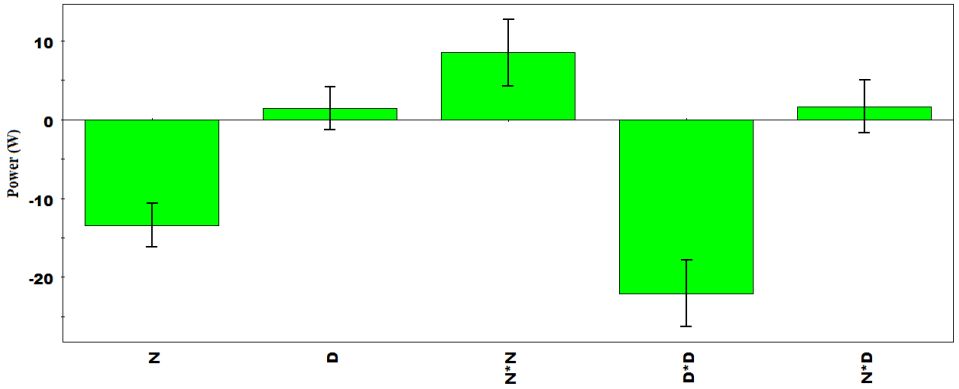


Figure 5. Plotted coefficients of the obtained models for Power (W) (R^2 = 98%; Q^2 =86%)

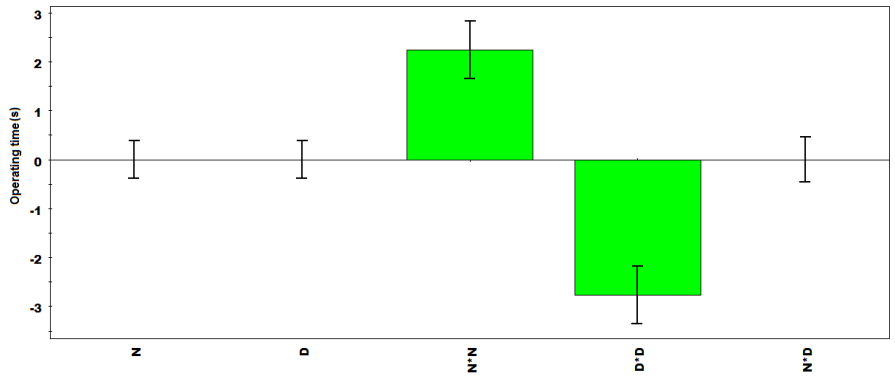


Figure 6. Plotted coefficients of the obtained models for the operating time (s) **(R^2 = 98%; Q^2 =93%)**

Furthermore, MODDE 5.0 includes an optimization routine that can simultaneously process multiple responses assigned by different weights. The software offers the possibility to identify the optimal values of the two factors that should minimize the power consumed and the duration of operation (Figure.7).

According to this model, the process optimum (ie, minimize power and operating temperature with an ozone concentration of 7ppm) should be obtained for N ≈ 11 and d= 6mm. In figure 8 are represented the iso-response contours obtained with the present model.

	Response	Criteria	Weight	Min	Target	Max
1	Operating time	Minimize	1		9.5921	10.0921
2	Power	Minimize	1		34.1576	39.3556

Iteration: 5001 Iteration slider:

	1	2	3	4	5	6
	Number of wires	Distance	Operating time	Power	iter	log(D)
1	10.6489	6	10.0775	39.3402	5000	-0.014
2	10.6327	10	10.066	43.3775	5001	0.3058
3	10.6626	6	10.0876	39.2749	5000	-0.0107
4	10.6323	10	10.0657	43.3789	5000	0.3058
5	10.6237	10	10.0596	43.4064	5000	0.3054
6	10.6626	6	10.0876	39.2749	5000	-0.0107
7	10.592	6	10.0381	39.6188	5000	-0.0224
8	10.6302	10	10.0642	43.3857	5000	0.3057

Figure 7. Results of the optimization routine of MODDE 5.0 for minimization of the power and operating time

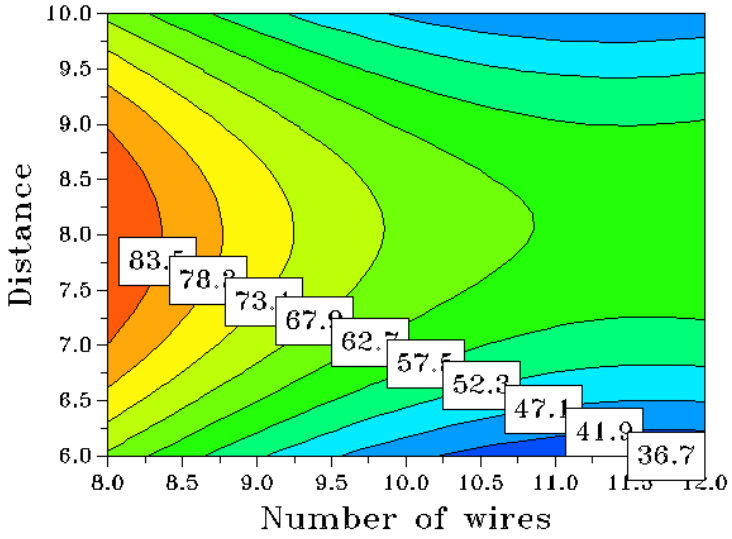


Figure 8. Response contour plots of power P

3.3. Food storage application

The typical ozone concentrations typically used for the treatment in cold food storage rooms should be in the range 2-7 ppm (Graham, 1998). Consequently, a time control system has been developed using Arduino card programming to control the On /Off time periods of the ozone generator to ensure a continuous ozone concentration ranging between 2 and 7 ppm. To achieve these concentrations, preliminary tests were carried out to determine the suitable operation periods of the ozone generator.

Once the ozone generator is set On, an ozone concentration C_{O3}equal to7 ppm is quickly achieved after an operating time of 10 s. After this period of 10 s, the ozone generator is set Off so that the concentration will not exceed 7 ppm. In figure 9, is plotted the decline of $C_{O3}$with respect to time. Obtained results show that the duration during which the concentration decreases from 7 to 2 ppm, in the conditions of humidity and temperature of the storage room, is equal to 25minutes. Based on these results, the time control system of the ozone generator that has been set to maintain an ozone concentration between 2 and 7 ppm, is an operating time of 10 seconds and shutdown intervals of 25 minutes.

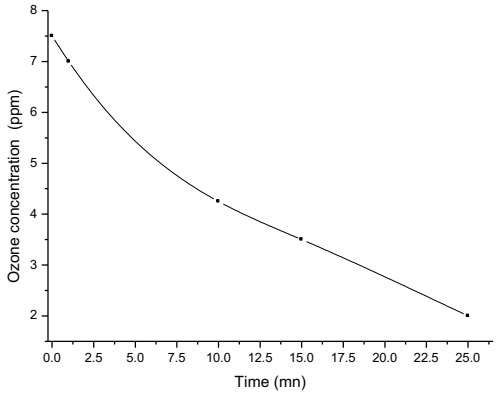
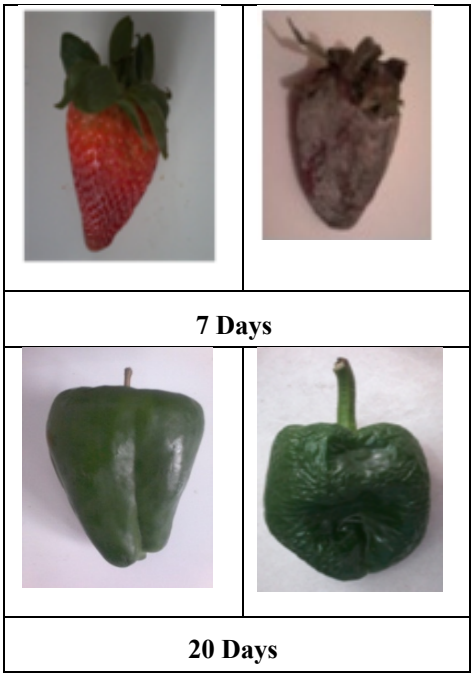


Figure 9. Decline of the ozone concentration with respect to time during the shutdown of the ozone generator

The evolution of the food products was analyzed by visual control after several days of storage in the treated and untreated rooms. As shown in figure.10, Photographs were taken after7 days for Strawberry, 20 days for green pepper and 5 days for Sardine.

These results show clearly that foods stored in the enclosure treated with ozone are much more resistant to contamination compared to similar products placed in the untreated enclosure. Indeed, the ozone is a powerful oxidizer that has been recommended by several researchers (Liew et al, 1994; Pandiselvam et al, 2019) to reduce the decomposition of the product and prolong the storage period, by eliminating bacteria and stopping their development. Ozone also reacts with ethylene, the gas responsible for the ripening of fruits and vegetables because it causes damage and increases decomposition (Skog et al, 2001; Aziz et al, 2018). In addition, it is mentioned in several studies (Smilanick et al, 2003; Tuffi et al, 2012; Montesano et al, 2004) that the exposure of fruits and vegetables to ozone can slow the sporulation of fungi harmful to citrus fruits, especially Geotrichum and Pennicilium. Moreover, the application of ozone for sardine storage leads to significantly lower counts of aerobic mesophiles, psychrotrophic bacteria, anaerobes, coliforms, and both lipolytic and proteolytic microorganisms in sardine muscle, and of surface counts of mesophiles and psychrotrophic bacteria in sardine skin. Furthermore, a more interesting feature that was observed which consists in a less amount of mass loss for the food products stored in the ozone treated enclosure. As shown in figure.11, a mass loss up to 50% and 60% was observed for strawberry and green pepper respectively.



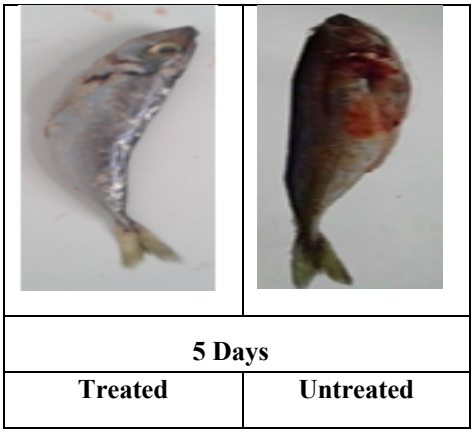


Figure 10. Comparison between the states of ozone-treated and untreated food products after several storage days

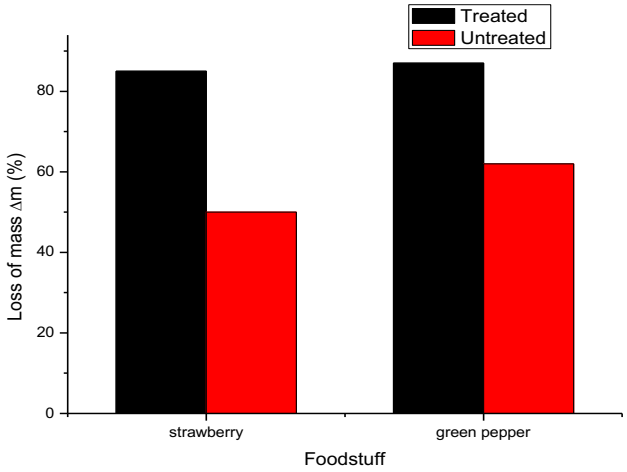


Figure 11. Mass loss of the food products after storage

4. Conclusions

An experimental procedure based on RSM technique was proposed for the determination of the optimal factors of the ozone generator, regarding the effects of the ozone concentration and the consumed power responses. Moreover, an ozone disinfection system for food storage was developed and analyzed for storage of Strawberry, green pepper and sardine. It was shown that food products stored in the enclosure treated with ozone are much more resistant to contamination compared to similar products placed in the untreated enclosure. Furthermore, it was observed that the weight of the ozone-treated product is greater than products stored in the untreated storage room.

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MATHEMATICAL MODELING AND OPTIMIZATION OF LOW-TEMPERATURE VACUUM DRYING FOR BANANA

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ABSTRACT

Bananas are one of the most common energy yielding fruits, and also a nutritional source for human health. In this study, low-temperature vacuum drying was applied to preserve banana because this method offers a low nutrient loss, a decrease in drying time leading to low energy cost, and the low moisture content preventing products from microbial spoilage. Four mathematical models were built, and a multi-objective optimization problem was established for the drying process. The restricted area method with R*(Z) optimal combination criterion was used to optimize for the drying mode of banana including temperature of 52.76 ^{o}C, pressure of 0.006 mmHg and drying time of 13.94 hours. Experimental results showed that the energy consumption was 3.96 kWh/kg, the residual water content was 3.64 %, the vitamin C loss was 3.27 % and the maximum rehydration capacity was 95.17 %, which convinced that dried bananas had achieved a minimum cost, the best quality, and a long-term storage.

1. Introduction

Bananas (*Musa* spp., Musaceae) are one of the highly appreciated fruits over the world. Banana plants are usually found in tropical and subtropical regions because of their fast-growing ability and good suitability with various types of fertilizer (Qamar et al., 2018). There are more than 300 banana varieties with planting area of approximately 5.2 million hectares (Qamar et al., 2018), (FAOSTAT, 2019). Most of varieties is widely distributed in Indo-Malaysian, Asian, Caribbean, Latin American, African and Australia. As regards to the banana production yield, there were nearly 117 million tons of banana produced in 2019 (FAOSTAT, 2019). Ecuador, Costa Rica, and Philippines are countries that achieved the highest banana production and export yield whereas America, Belgium and Russia were the biggest banana importers 2017 (FAO, 2020).

Bananas are rich in biologically active compounds such as phenolics, vitamin C, minerals, biogenic amines and phytosterols that beneficial for human health as well as effective in protecting the body against numerous oxidative stresses (Voora et al., 2020; Singh et al., 2016). Moreover, vitamin C is one of the most important substances against free radicals in human body. It also contributes to the growth of tissues, health of bones and teeth, the recovery of the wounds, the regeneration of collagen, the activation of hormones, the hydroxylation of proline and nitrosamine formation (Ranjha et al., 2020). Reducing the reactions relating to severe allergies, preventing human from being infected and positive moderating for the immune system are some of the advantages that vitamin C can also provide (Iqbal et al., 2004). 100 g fresh pulp of banana provides about 68 g of water, 121.8 g of carbohydrate, 30-60 mg of phenolic compounds, 1.1 g of protein, 11.7 mg of vitamin C, 1.0 mg of sodium, 8.0 mg of calcium, 385.0 mg of potassium and other components (Qamar et al.,

2018). This nutrition fact could explain why the consumption of bananas gradually increases in several potential markets such as Europe and North America (WTO and International Trade Center, 2019).

However, bananas are prone to be perishable due to their high moisture content (Simal et al., 1997). There are about 1.6 million of bananas being spoiled and thrown away every day in developing countries. For instance, although the number of fresh bananas in Vietnam increased dramatically (approximately 1.4-1.9 million tons), banana exported yield only accounted for a minority in 2019 (FAO, 2020; (WTO and International Trade Center, 2019). Consequently, finding the best way to preserve this highly appreciated fruit as well as diversifying products made from banana are necessary.

There have been some methods for the long-term preservation and production of bananas including freezing, frying, and drying. Although freezing has been successfully employed to preserve food, the formation of ice crystals during freezing process could cause damage to the microstructure of food materials, leading to a decline in the preference of customers (Dzung N.T., 2016). Furthermore, the low temperature ranges used in freezing will cause a significant rise in energy cost (Dzung N.T., 2016). Another method applied for banana production is frying. This technique is beneficial since it forms the attractive appearance, adds flavor, and creates crispy texture for products. However, substantial odor enhancement via autoxidation, decomposition and fat hydrolysis may cause quality deterioration in fried products (Perkins E. I., 2007). A considerable increase of nutrition loss could occur inside products during frying (Mihaela et al., 2010).

On the other hand, drying is a popular method which has been applied for preserving fruits and vegetables since ancient times. Trends of drying are either to find an appropriate preservative technique for prolonging food shelf life or create ready-to-eat products retaining beneficial values for human health. In accordance with this advantage, drying is also a prevalent technique to preserve the original characteristics of bananas from structure, color, flavor to nutrition as well as increase the current level of acceptance of dried foods in the market (An H., et al., 2010). Utilizing high temperature over 70^{o}C for water removal from foods will shorten the processing time and reduce moisture content for long storage. However, high temperature in drying could lead to some inherent disadvantages. The shrinkage on the surface of samples dried by conventional techniques was reported to be extremely high (Krokida et al., 1997). A greater shrinkage will result in a poor rehydration ratio and an unacceptable structure (Junlakan, 2014). Besides, the oxygen presence must be controlled in an extremely low range to have positive effects during drying and storage (Junlakan, 2014). If the oxygen level is high, the quality indicator of dried products as vitamin C will be degraded and rehydration capacity will be low. Therefore, low-temperature vacuum drying will offer a great potential for dehydration process. This method is recommended to create qualified dried products including fine structure (porosity and crispy), high nutritional properties (vitamin C retention) and good rehydration capacity. Furthermore, this new approach efficiently saves half of the energy cost as compared to sublimation drying (Bazyma and Kutovoy, 2005).

However, the application of low-temperature vacuum drying to create high-qualified dried banana chips have been untapped effectively. This matter occurs due to lack of equipment and experimental conditions to optimize the low-temperature vacuum drying process.

Therefore, the aim of this paper is modeling low-temperature vacuum drying process for banana by experimental designs. A multi-objective optimization problem describing for low-temperature vacuum drying is also established. The restricted area method with R*(Z) optimal combination criterion is utilized to solve the problem and ascertain the Pareto tests. In other words, solving this multi-objective optimization problem is a way to optimize the low-temperature vacuum drying and figure out the optimal drying conditions for

banana chips production. It is hypothesized that banana chips dried at these optimal parameters would meet all the quality standards such as minimal energy consumption, high nutritional values and low moisture content that could meet export requirements.

2. Materials and methods

2.1. Materials

Figure 1. Fresh banana slices.

Fresh banana used for experiments was Pisang Awak variety (*Musa acuminata x Musa balbisiana*), collected from Southwestern area of Vietnam. This was chosen because of its superb quality, popularity, and high yields in Vietnam. The amount of carbohydrate in bananas has changed constantly upon ripening due to the transformation from starch to sugar by enzymatic breakdown mechanism (Hettiaratchi et al., 2011; Mohapatra et al., 2010). Limiting the changes of chemical compositions and controlling the homogeneity of fresh bananas play a vital role associated with choosing the appropriate material (Monteiro et al., 2015; Tribuzi and Laurindo, 2014; Zotarelli et al., 2012). Thus, bananas were initially examined the ripeness before being washed, peeled, sliced to 5 mm thickness, and uniformly arranged on a tray (Figure 1).

The thickness of 05 mm was chosen because thinner slices could be shrinkable and brittle after drying, whereas thicker slices would obstruct the migration of water from inside of material. This hindrance might lead to longer drying time and higher energy consumption.

2.2. Methods

2.2.1. Drying equipment

Dehydration process was conducted by using the low-temperature vacuum dryer prototype DSV–03, fabricated and assembled by Professor Nguyen Tan Dzung and his colleagues in 2018 (Duong T., et al., 2018). This machine is automatically controlled by coding and IoT and is placed at laboratory of Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education, Vietnam (Figure 2).

Figure 2. The low-temperature vacuum drying system DSV – 03.

2.2.2. Determination of the chemical compositions in fresh banana

The following methods were used to determine some chemical compositions of Pisang Awak. Protein was measured by Kjeldahl method in FAO, Food & Nutrition, 14/7, 1986. Carbohydrate and lipid were estimated by the standard of Ref. EC 152-2009 and Ref. EC 996-06, in turn. Vitamin C was calculated by direct titration with iodine (Suntornsuk et al., 2002). For determination of the banana ripeness, refractometric method was applied corresponding to standard of TCVN 7771:2007.

2.2.3. Determination of the technological factors

Factors affecting the low-temperature vacuum drying of banana included:

- Temperature Z_1 (°C)
- Pressure Z_2 (mmHg)
- Time Z_3 (h)

Temperature and pressure were recorded by sensors located inside the drying chamber, while drying time was estimated by a timer integrated in the computer of DSV-03 system.

2.2.4. Determination of objective functions

Although dried banana chips must achieve all the quality criteria together with a long-term storage, products' energy consumption should also be abridged. Therefore, the four objective functions including energy consumption, the residual water content, loss of vitamin C and the rehydration capacity of products were considered.

- **The energy consumption (y_1, kWh/kg)**

The energy consumption (y_1, kWh/kg) for 1 kg of final product was determined by equation (1) (Dzung N.T., 2016), (Dzung N.T., 2012):

$$y_1 = \frac{P.\tau}{G} \text{ (kWh/kg)} \quad (1)$$

where: G (kg) – weight of final product; (s) – drying time; P (kW)–capacity shown on Watt meter.

- **The residual water content (y_2, %)**

The residual water content of final product was calculated by equation (2) (Dzung N.T., 2016), (Dzung N.T., 2012), (Dzung et al., 2016):

$$y_2 = 100 - \frac{G_o}{G_e}\left(100 - W_o\right) \quad (2)$$

where: G_o (g) – weight of raw material; G_e (g) - weight of final product; W_o (%) – the initial moisture content of sample.

- **The loss of vitamin C (y_3, %)**

The loss of vitamin C in final product was estimated by the formula (Dzung N.T., 2016):

$$y_3 = \frac{m_1 - m_2}{m_1}.100 \text{ (\%)} \quad (3)$$

where: m_1 and m_2 (mg) – the vitamin C content before and after drying, respectively.

- **The rehydration capacity (y_4, %)**

Determining the rehydration capacity of final product was determined by the below expression (Dzung N.T., 2016):

$$y_4 = \frac{G_1 - G_e}{G_o - G_e}100 \text{ (\%)} \quad (4)$$

where: G_o (g) – weight of fresh banana slices used for the experiment; G_1 (g) – weight of dried slices which were soaked into the water at 25 ^oC until the constant mass (*the saturation of water content*); G_e (g) – weight of banana slices before soaking (g).

2.2.5. Determination of microorganisms, mycotoxins, and heavy metals contents in dried products

The microbiological infection, the mycotoxins and heavy metal contents of dried products were measured by the following methods presented in Table 1, Table 2, and Table 3.

Table 1. Methods for the determination of microorganisms in dried bananas

No.	Parameter	Unit	Method
1	Total aerobic plate count	cfu/g	TCVN 4884-1:2015
2	*Coliforms*	cfu/g	TCVN 6848:2007
3	*Escherichia coli*	MPN/g	TCVN 6846:2007
4	*Staphylococcus aureus*	cfu/g	TCVN 4830-1:2005
5	*Clostridium perfringens*	cfu/g	TCVN 4991:2005
6	*Bacillus cereus*	cfu/g	TCVN 4992:2005
7	Total spores of yeast and mold	cfu/g	TCVN 8275-2:2010
8	*Salmonella*	per 25g	ISO 6579 – 1:2017

Table 2. Method for the determination of mycotoxins content in dried bananas

No.	Parameter	Unit	Method
1	Aflatoxin B_1	µg/kg	Ref. EN 15662-2018
2	Aflatoxin $B_1B_2G_1G_2$	µg/kg	Ref. EN 15662-2018

Table 3. Methods for the determination of heavy metals content in dried bananas

No.	Parameters	Unit	Method
1	Lead (Pb)	mg/kg	Ref AOAC 2015.01
2	Cadmium (Cd)	mg/kg	Ref AOAC 2015.01

2.2.6. Experimental planning method

It is proposed that there was a simultaneous impact on the objective functions y_1, y_2, y_3, y_4 from the technological factors Z_1, Z_2, Z_3 (Figure 3).

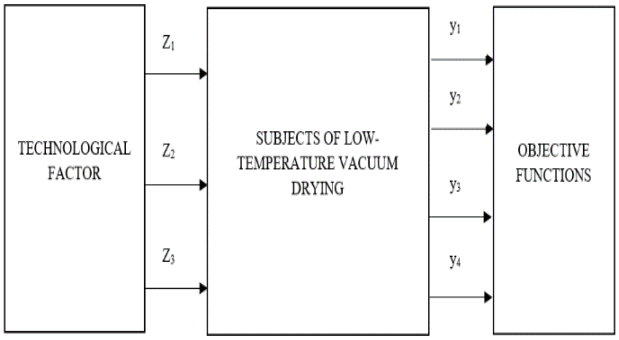

Figure 3. Diagram describing a relationship between the technological factors and the objective functions (Dzung et al., 2017)

An optimal experimental design was established to build a mathematical model assuring the accuracy, cost-saving, and a short experimental time. It was the quadratic orthogonal experimental plan, which k is the variables describing for the technological factors of the drying process (k = 3).

Assuming that x_1, x_2, x_3 were coded for variables Z_1, Z_2, Z_3. Thus, an experimental mathematical model was described as follow:

$$y_j = b_o + \sum_{j=1}^{k} b_j x_j + \sum_{\substack{j,i=1 \\ j \neq i}}^{k} b_{ij} x_i x_j + \sum_{j=1}^{k} b_{jj}\left(x_j^2 - \lambda\right) \quad (5)$$

- Code variable was calculated by equation below:

$$x_j = \frac{Z_j - Z_j^o}{\Delta Z_j}, \text{j} = 1 \div 3 \quad (6)$$

- Number of experiments:

$$N = 2^k + 2k + n_0 = 18 \quad (7)$$

- The star point (Dzung N.T., 2020):

$$\alpha = \sqrt{\sqrt{N.2^{k-2}} - 2^{k-1}} = 1.414 \quad (8)$$

- Orthogonal matrix's criterion was:

$$\lambda = \frac{1}{N}\left(2^k + 2.\alpha^2\right) = 0.667 \quad (9)$$

2.2.7. Optimization method

▪ *The one - objective optimization problems*

Every objective function $y_j = f_j(Z)$, j = 1÷4 depended on the technological elements including Z_1 (°C)- drying temperature; Z_2 (mmHg)- pressure of drying environment; Z_3 (h) - drying time. Obviously, each objective function had a relationship with technological elements to form the one-objective optimization problem.

These factors formed the vector of technological elements or the Z variable vector Z = (Z_1, Z_2, Z_3). These variables varied in the identified domain Ω_Z and the function values $f_j(Z)$ formed the domain of the objective function Ω_f (Dzung et al., 2017).

Therefore, to simplify without losing the generality, it was assumed that all objective functions could reach the minimum value. The one-objective optimization problem was hence stated as follow:

Determining the root of $Z^j = \{Z_i^{jopt}\} = (Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt}) \in \Omega_Z$ in order that:

$$\begin{cases} y_{j\min} = f_j(Z_i^{jopt}) \\ \quad = f_j(Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt}) \\ \quad = Min\left\{f_j(Z_1, Z_2, Z_3)\right\} \\ Z_i^{jopt} = (Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt}) \in \Omega_Z \\ i = 1 \div 3;\ j = 1 \div 4 \end{cases} \quad (10)$$

▪ *The multi - objective optimization problem*

In fact, not only did the technological factors such as Z_1 (°C), Z_2 (mmHg), Z_3 (h) influence each objective function discretely, but they also coincided with these functions $y_j = f_j(Z)$, j = 1÷4 to fulfil all the economic and technological

criteria. It was obvious that the multi-objective optimization problem had occurred in this research. Assuming the multi-objective optimization problem could be transformed into the problem to find the minimum value, the multi-objective optimization problem was stated:

Determining the root of $Z = Z^{opt} = (Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) \in \Omega z$ so that:

$$\begin{cases} y_{j\min} = f_j(Z_i^{opt}) \\ \quad = f_j(Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) \\ \quad = Min\left\{f_j(Z_1, Z_2, Z_3)\right\} \\ Z_i^{opt} = (Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) \in \Omega_Z \\ i = 1 \div 3; j = 1 \div 4 \end{cases} \quad (11)$$

Solving equation (10) to figure out the optimal root: $Z_i^{jopt} = (Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt})$ in order that: $f_{jmin} = f(Z_i^{jopt}) = Minf_j(Z)$, $\forall i = 1 \div 3$, $\forall j = 1 \div 4$ [9].

- If $Z_i^{jopt} = Z_i^{kopt} (\forall j, k = 1 \div 4; k \neq j)$, both the utopian root and the utopian optimal plan exist. In addition, the test $Z_i^{jopt} = (Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt})$ was also the root of the multi-objective optimization problem (11).

- If $Z_i^{j\,opt} \neq Z_i^{k\,opt} (\forall j, k = 1 \div 4; k \neq j)$, the utopian root does not exist whereas the utopian point $f^{UT} = (f_{1min}, f_{2min}, f_{3min}, f_{4min})$ can be normally determined. It has been reported that the multi-objective optimization problem (11) could be successfully solved by the restricted area method (Dzung et al., 2017).

Reality showed that most of the multi-objective optimization problems has its own objective functions $f_j(Z)$ subjected to the economic and technical conditions:

$$f_j(Z) < C_j; \forall j = 1 \div 4; \forall Z \in \Omega_Z \quad (12)$$

Expression (12) formed the restricted area:

$$C = \left\{f_j(Z) \geq C_j\right\} \quad (13)$$

The restricted area method suggested the $R^*(Z)$ optimal combination criterion to solve the multi-objective objective optimization problem (11), defined as (Dzung et al., 2017):

$$R^*(Z) = \left[\prod_{j=1}^{4} r_j(Z)\right]^{1/4} = \sqrt[4]{r_1(Z).r_2(Z).r_3(Z)r_4(Z)} \quad (14)$$

In which:

$$r_j(Z) = \frac{C_j - f_j(Z)}{C_j - f_{j\min}} \quad khi \quad f_j(Z) < C_J \quad (15)$$

$$r_j(Z) = 0 \quad khi \; f_j(Z) \geq C_j \quad (16)$$

According to (15), if $f_j(Z) \to f_{j\min}$ then $r_j(Z) \to r_{j\max} = 1$. By choosing R*(Z) as the objective function, the multi-objective optimization problem was restated as:

Finding the root $Z^R = \left(Z_1^R, Z_2^R, Z_3^R\right) \in \Omega_Z$ in order that:

$$\begin{cases} R^*_{\max} = R*\left(Z^R\right) = Max\{R*(Z)\} \\ \quad = Max\left\{\left[r_1(Z).r_2(Z).r_3(Z).r_4(Z)\right]^{\frac{1}{4}}\right\} \\ Z = \left(Z_1, Z_2, Z_3\right) \in \Omega_Z \end{cases} \quad (17)$$

The Pareto optimal root would be determined by solving (17). Hence, the Pareto effect was the optimal plan of the multi-objective optimization problem.

2.2.8. Statistical analysis

Microsoft Excel was utilized to calculate, solve, and build up the mathematical models describing for the low-temperature vacuum drying of bananas. In addition, MATLAB (2020) was also used to form the 3-D response surface plot simulating the objective functions from technological factors.

3. Results and discussions

3.1. Chemical constituents of raw material

Chemical constituents of fresh banana were summarized in Table 4.

Table 4. Chemical constituents per 100g of fresh banana pulp.

No.	Nutrient	Unit	Value
1	Moisture	%	68.1
2	Protein	%	0.87
3	Carbohydrate	%	24.90
4	Lipid	g	ND
5	Vitamin C	mg	10.50
6	Brix	%	22.9

**ND: not detected*

The amount of water in fresh bananas was approximately 68.1%, which resembled to that of Hettiaratchi et al. (2011) who also found the moisture content of Pisang Awak banana was about 68.2% (Hettiaratchi et al., 2011). Moreover, Dennis (1999) reported that under 20% of the residual water content of bananas is an ideal criterion to prevent foods from being spoiled by yeast, bacteria, molds, and enzymes (Dennis, 1999).

Regarding other chemical components of fresh bananas, they made up an insignificant ratio as compared to previous works (Qamar et al., 2018; Hettiaratchi et al., 2011; Mohapatra et al., 2010; Chandler, 2015). At limit of detection (LOD) value of 0.2g/100g, results also show that the lipid content was incredibly low in pulp. Nevertheless, the Brix value in this research was much higher than the other study (12-14%) (Rex Harrill, 1998). Differences in the chemical compositions of banana pulp in this research could be due to the differences in the degree of ripeness, the climatic conditions of cultivated areas, and the quality of fertilizers along with types of cultivar (Hettiaratchi et al., 2011).

3.2. Mathematical models describing the low-temperature vacuum drying of banana

Table 5. Parameter level design

Parameters		Z_1 (^{0}C)	Z_2 (mmHg)	Z_3 (h)
- α	-1.414	48.34	0.006	12.17
Low	-1	50	0.01	13
Central	0	54	0.02	15
High	+1	58	0.03	17
+ α	1.414	59.65	0.034	17.82
Deviation ΔZ_i		4	0.01	2

After conducting individual experiments for each technological factors Z_1, Z_2, Z_3 on the objective functions y_1, y_2, y_3, y_4, it could be presented that extrema domain of y_j ($j = 1 \div 4$) varied in identified domain Z_i ($i = 1 \div 3$). The results were summarized in Table 5.

According to Table 6, the low-temperature vacuum drying experiments of bananas were proceeded, followed by the quadratic orthogonal matrix with $k = 3$, $n_0 = 4$ to find out the objective functions y_j ($j = 1 \div 4$). The results were expressed in Table 6.

Table 6. Experimental matrix determining the objective functions.

No. of experiments		Coded variables			The objective functions			
N		x_1	x_2	x_3	y_1 (kWh/kg)	y_2 (%)	y_3 (%)	y_4 (%)
2^k	1	1	1	1	4.24	3.01	10.58	87.55
	2	-1	1	1	4.11	3.62	8.04	89.99
	3	1	-1	1	4.42	2.79	9.44	88.29
	4	-1	-1	1	4.33	3.51	7.80	92.27
	5	1	1	-1	3.46	4.11	6.88	89.06
	6	-1	1	-1	3.43	4.45	5.01	92.22
	7	1	-1	-1	3.44	4.23	6.36	89.91
	8	-1	-1	-1	3.40	4.62	4.01	93.69
2k	9	1.414	0	0	3.88	3.44	10.88	86.68
	10	-1.414	0	0	3.66	3.93	5.04	93.98
	11	0	1.414	0	3.62	3.68	4.81	91.39

	12	0	-1.414	0	4.14	2.76	4.01	92.25
	13	0	0	1.414	4.97	2.57	10.23	87.39
	14	0	0	-1.414	3.23	4.73	5.44	92.43
n_o	15	0	0	0	3.78	3.34	5.35	92.92
	16	0	0	0	3.81	3.18	5.41	92.88
	17	0	0	0	3.90	3.25	5.37	92.84
	18	0	0	0	3.81	3.17	5.79	92.26

From Table 6, the experimental data was calculated using statistical theory and Microsoft Excel 2020 software before identifying the coefficients of regression equations b_j, b_{jk} and b_{jj}. Furthermore, the fitness of the regression equations with the experimental results was tested by Fisher test after the significance of coefficients was checked by Student test. The mathematical models of low-temperature vacuum drying for banana were presented below:

- **Energy consumption:**

$$y_1 = 3.847 + 0.05x_1 - 0.09x_2 + 0.486x_3 - 0.067x_1^2 + 0.099x_3^2 \quad (18)$$

- **Residual water content:**

$$y_2 = 3.232 - 0.229x_1 + 0.112x_2 - 0.628x_3 + 0.268x_1^2 + 0.250x_3^2 \quad (19)$$

- **Vitamin C loss:**

$$y_3 = 5.505 + 1.388x_1 + 0.336x_2 + 1.698x_3 + 1.209x_1^2 - 0.575x_2^2 + 1.146x_3^2 \quad (20)$$

- **Rehydration capacity:**

$$y_4 = 92.522 - 1.974x_1 - 0.546x_2 - 1.159x_3 - 1.036x_1^2 - 1.247x_3^2 \quad (21)$$

Currently, there have been very few studies for drying banana in low-temperature vacuum environment so that none of similar mathematical models could be used to make comparison. In the other hand, mathematical models (18), (19), (20) and (21) were compatible with experimental data so ensuring that these regression equations were precise to describe for the drying process. Thus, such equations could be utilized to set up technological parameters of dehydrated banana for commerce and export.

3.3. Optimization the banana low-temperature vacuum drying process to establish the technological mode

3.3.1. Solving the one - objective optimization problems

The one-objective optimization problems were created after each object had been studied independently. These problems were found to achieve the minimum and maximum value including the energy consumption $y_{1min} = f_1(x_1, x_2, x_3)$; moisture content $y_{2min} = f_2(x_1, x_2, x_3)$; vitamin C loss $y_{3min} = f_3(x_1, x_2, x_3)$ and the rehydration capacity $y_{4max} = f_4(x_1, x_2, x_3)$ with the identified domain $\Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$. Consequently, the one-objective optimization problems were stated as follow:

Determining $(x_1^{opt}, x_2^{opt}, x_3^{opt}) \in \Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$, $j = 1 \div 4$ in order that:

$$\begin{cases} y_{1\min} = f_1\left(x_1^{1opt}, x_2^{1opt}, x_3^{1opt}\right) = Min\left\{f_1(x_1, x_2, x_3)\right\} \\ y_{2\min} = f_2\left(x_1^{2opt}, x_2^{2opt}, x_3^{2opt}\right) = Min\left\{f_2(x_1, x_2, x_3)\right\} \\ y_{3\min} = f_3\left(x_1^{3opt}, x_2^{3opt}, x_3^{3opt}\right) = Min\left\{f_3(x_1, x_2, x_3)\right\} \\ y_{4\max} = f_4\left(x_1^{4opt}, x_2^{4opt}, x_3^{4opt}\right) = Max\left\{f_4(x_1, x_2, x_3)\right\} \end{cases} \quad (22)$$

Expression (22) was solved by using the Excel – Solver software. This resulted in the optimal roots of the one-objective optimization problems, summarized in Table 7.

Table 7. Optimal roots of the one-objective optimization problems

j	x_1^{jopt}	x_2^{jopt}	x_3^{jopt}	y_j^{jopt}
1	-1.414	1.414	-1.414	**3.03**
2	1.414	-0.492	1.414	**3.00**
3	-0.452	0.000	-1.309	**4.87**
4	-0.533	-0.441	-0.590	**93.77**

As can be seen from Table 7, tests of the one-objective optimization problems from (18) to (21) satisfying all function values (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) ≠ (x_1^{kopt}, x_2^{kopt}, x_3^{kopt}) ∀j, k = 1 ÷ 4, j ≠ k could not be found. It was clear that cross tests of the one-objective optimization problems were inexistent. As a result, utopian roots and utopian optimal plans did not exist. Regardless of the inexistence of utopian roots, the utopian points were identified $y^{UT} = (y_{1min}, y_{2min}, y_{3min}, y_{4max})$ = (3.03; 3.00; 4.87; 93.77).

3.3.2. Solving the multi-objective optimization problem

Because all the one-objective optimization problems had none of cross tests fulfilling y_{1min}, y_{2min}, y_{3min}, y_{4max}, the multi-objective optimization problem had to be taken into account to find the optimal Paréto test for optimal Paréto effect $y_p^R = \left(y_{1p}^R, y_{2p}^R, y_{3p}^R, y_{4p}^R\right)$ closest to the utopian point and the furthest from the restricted area. The main purpose of this paper was finding both maximum and minimum value. However, it is necessary to transform all the objective functions y_1, y_2, y_3, y_4 into one type of finding minimum value to simplify in the solution. The expressions were then rewritten as following:

$$\begin{cases} I_1(x) = y_1(x) = f_1(x_1, x_2, x_3) \\ I_2(x) = y_2(x) = f_2(x_1, x_2, x_3) \\ I_3(x) = y_3(x) = f_3(x_1, x_2, x_3) \\ I_4(x) = 1/y_4(x) = 1/f_4(x_1, x_2, x_3) \end{cases} \quad (23)$$

From (23), the multi-objective optimization problem was restated below:

Determining the optimal roots $x^{opt} = (x_1^{opt}, x_2^{opt}, x_3^{opt}) \in \Omega_x$ in order that:

$$\begin{cases} I_{j\min}(x) = fj(x_1^{opt}, x_2^{opt}, x_3^{opt}) \\ \qquad\qquad = Min\left\{f_j(x_1, x_2, x_3)\right\} \\ I_j(x) < C_j; \forall j = 1 \div 4 \\ -1.414 \le x_1, x_2, x_3 \le 1.414 \end{cases} \quad (24)$$

The objective functions y_j (j = 1 ÷ 4) must subject to the technological conditions such as: $I_1 = y_1 \le C_1$, with C_1 = 5 kWh/kg; $I_2 = y_2 \le C_2$, with C_2 = 6 %; $I_3 = y_3 \le C_3$, with C_3 = 10 %; $y_4 \ge C'_4$ = 90% so $I_4 = 1/y_4 \le C_4$, with $C_4 = 1/C'_4$ = 1/90 = 0.011 (1/%), albeit y_j (j = 1 ÷ 4) were affected by the technological factors x_1, x_2, x_3. Therefore, the restricted area was investigated:

$$C = \{I_1 > C_1 = 5; I_2 > C_2 = 6; I_3 > C_3 = 10; I_4 > C_4 = 0.011\} \quad (25)$$

The R*(x) optimal combination criterion was created by the restricted area method:

$$\begin{cases} R^*(x) = \left[\prod_{j=1}^{4} r_j(x_1, x_2, x_3)\right]^{1/4} \\ \forall x = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\} \in \Omega_x \end{cases} \quad (26)$$

In which:

$$\begin{cases} r_1(x_1, x_2, x_3) = \dfrac{C_1 - I_1(x)}{C_1 - I_{1\min}} \quad khi \quad I_1 \le C_1 = 5 \\ r_1(x_1, x_2, x_3) = 0 \quad khi \quad I_1 > C_1 = 5 \end{cases}$$

$$\begin{cases} r_2(x_1, x_2, x_3) = \dfrac{C_2 - I_2(x)}{C_2 - I_{2\min}} \quad khi \quad I_2 \le C_2 = 6 \\ r_2(x_1, x_2, x_3) = 0 \quad khi \quad I_2 > C_2 = 6 \end{cases}$$

$$\begin{cases} r_3(x_1, x_2, x_3) = \dfrac{C_3 - I_3(x)}{C_3 - I_{3\min}} \quad khi \quad I_3 \le C_3 = 10 \\ r_3(x_1, x_2, x_3) = 0 \quad khi \quad I_3 > C_3 = 10 \end{cases}$$

$$\begin{cases} r_4(x_1, x_2, x_3) = \dfrac{C_4 - I_4(x)}{C_4 - I_{4\min}} \quad khi\, I_4 \le C_4 = 0.011 \\ r_4(x_1, x_2, x_3) = 0 \quad khi \quad I_4 > C_4 = 0.011 \end{cases}$$

According to the principle of building up the R*(x) optimal combination criterion (26), the multi-objective optimization problem was rewritten as follow:

Determining the optimal Paréto roots $x^R = (x_1^R, x_2^R, x_3^R) \in \Omega_x$ in order that:

$$\begin{cases} R^*_{max} = R^*\left(x^R\right) = R^*\left(x_1^R, x_2^R, x_3^R\right) \\ \quad = Max\left\{\left[\prod_{j=1}^{4} r_j\left(x_1, x_2, x_3\right)\right]^{1/4}\right\} \\ \forall x = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\} \in \Omega_x \end{cases} \quad (27)$$

The maximum value $R^*(x)$ in the optimal Paréto roots (28) was calculated by using the Excel-Solver software:

$$R^*_{max} = Max\ \{R^*(x_1, x_2, x_3)\} = R^*\left(x_1^R, x_2^R, x_3^R\right) = 0.822 \quad (28)$$

$$\text{Với } \begin{cases} x_1^R = -0.310 \\ x_2^R = -1.414 \\ x_3^R = -0.529 \end{cases} \quad (29)$$

Substituting these Paréto roots x_1^R, x_2^R, x_3^R into the regression equations (18), (19), (20) and (21), optimal Paréto effects were identified as the followings:

$$\begin{cases} y_1^R = 3.72 \\ y_2^R = 3.57 \\ y_3^R = 2.99 \\ y_4^R = 94.07 \end{cases} \quad (30)$$

Converting the optimal Paréto roots x_1^R, x_2^R, x_3^R (29) into the uncoded variables, the optimal technological parameters were then obtained:

$$\begin{cases} Z_1^{opt} = 52.76^o C \\ Z_2^{opt} = 0.006 mmHg \\ Z_3^{opt} = 13.94 h \end{cases} \quad (31)$$

After solving the four-objective optimization problem, the optimal roots (31) could be found as $Z_1^{opt} = 52.76\ ^oC$; $Z_2^{opt} = 0.006$ mmHg; $Z_3^{opt} = 13.94$ hours. Applying this technological mode, we acquired the value of $y_1 = y_1^R = 3.72$ kWh/kg; $y_2 = y_2^R = 3.57$ %; $y_3 = y_3^R = 2.99$ %; $y_4 = y_4^R = 94.07$ %.

3.4. Experiment to test the results of multi-objective optimization problem

Carrying out the low-temperature vacuum drying process of banana at the optimal technological parameters, results were determined as the energy consumption per product weight of $y_{1E} = 3.96$ kWh/kg, residual water content of $y_{2E} = 3.64$ %, vitamin C loss of $y_{3E} = 3.27$ % and rehydration capacity of $y_{4E} = 95.17$ %. It was obvious that the consequences (y_{1E}, y_{2E}, y_{3E}, y_{4E}) were a little higher than the optimal Paréto tests but the increase was not dramatic. These findings conformed with the economic and technical standards to preserve the quality of banana, prolong shelf-life of products for commerce.

3.5. Simulation of mathematical models on a 3-D response surface plot

MATLAB software 2020 was used to simulate the objective functions y_j ($j = 1 \div 4$) from technological factors. Below are the results expressed in Figure 4, 5, 6, 7.

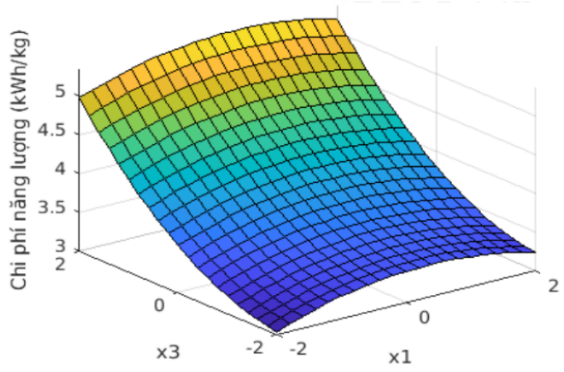

Figure 4. The energy consumption per weight product as $x_2 = -1.414$

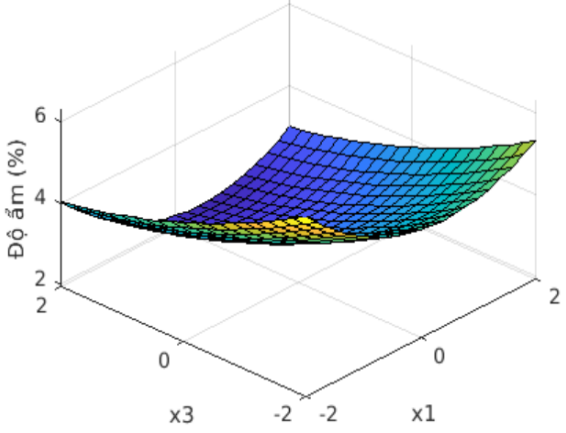

Figure 5. The residual moisture content as $x_2 = -1.414$

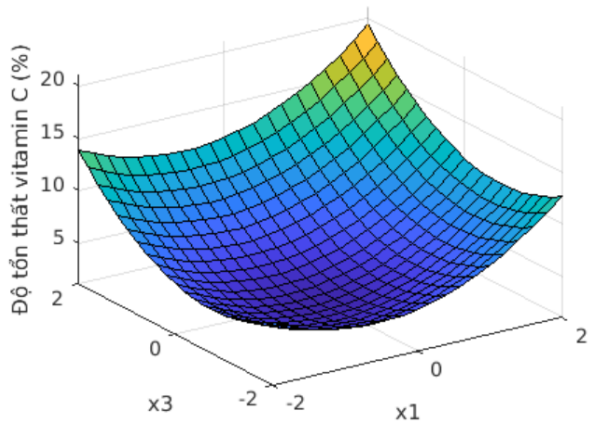

Figure 6. Vitamin C loss as x_2 = -1.414

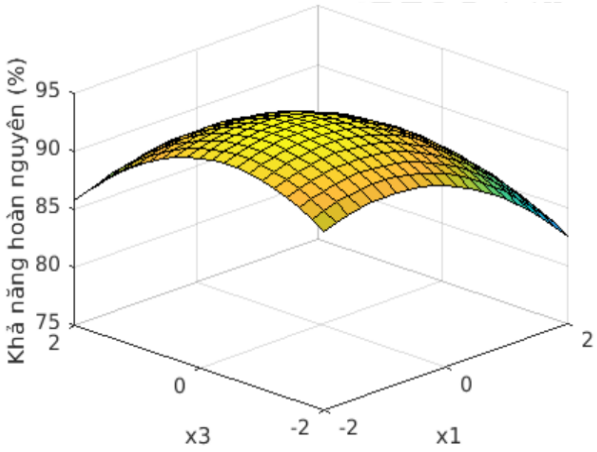

Figure 7. The rehydration capacity of products as x_2 = -1.414

By applying the optimal conditions for vacuum drying process (31) including Z_1 = 52.76 °C; Z_2 = 0.006 mmHg and Z_3 = 13.94 hours, final products reached minimum energy consumption per weight of 3.96 kWh/kg, made it become more economical as well as enhanced the probability for trade and export.

As regards to the energy consumption, remaining moisture content of dehydrated banana was 3.64%. It is clear that less than 6% of the amount of water in foods offers a great opportunity for reducing water activity, which presents a key factor of dried product sustainability. Furthermore, food spoilage can be caused by microorganisms. Their growth depends mostly on the amount of available water. Water removal from foods positively influences on microbiological instability of dried products.

Vitamin C is easily degraded in foods, and the vitamin C degradation depends on many factors such as pH, temperature, light, enzymatic process, and oxygen. Thus, vitamin C is considered as a quality indicator for foods (Moser and Bendich, 1991). In this paper, result of vitamin C loss proved that dried banana had achieved excellent quality, and this obtained data was better than previous works where vitamin C loss was ten times higher (Drouzas and Schubert 1996), (Jaya & Das, 2003). Regarding the vacuum drying process, high deficiency of vitamin C in foods also caused by the relatively high level of pressure (over 10mmHg) and temperature ranging from 60°C to 70°C, so that most of quality attributes decreased substantially after drying (Drouzas and Schubert 1996), (Jaya & Das, 2003). Besides, Junlakan (2014) and Sagar (2010) also reported when vacuum drying pressure had exceeded 10 mmHg, residual oxygen in chamber accelerated the oxidative reactions and lead to the intensification (Junlakan, 2014; Sagar and Kumar, 2010).

The rehydration capacity of 95.17 % also met the technological requirements collating from Junlakan's research (Junlakan, 2014). The author suggested that rehydration ratio of dehydrated banana should be controlled over 90% to maintain good structure (Junlakan, 2014). Measurement of rehydration capacity could help researchers get insights about the denaturation of chemical components and nutritional characteristics of product (Junlakan, 2014). The higher the rehydration ratio is, the more porous structure obtains. Its shrinkage phenomenon is minimized, chemical compositions are less denatured as well as dried banana will maintain the qualified properties. However, if rehydration capacity is low, it means that texture of products is soft, the surface is shrinkable, the chemical constituents are denatured, and the quality of dried banana will not satisfy the requirements for trade.

Experiments also presented that drying banana at 70°C or above would cause shrinkage (Figure 8) and the rehydration capacity only reached 30%. Drying banana under 55°C at optimal drying mode (31), products had less

shrinkage, rehydration capacity was over 90% and its quality was better than conventional dried products (Figure 9).

Figure 8. Infrared-dried banana in 70°C

Figure 9. The low-temperature vacuum dried banana at optimal drying mode

3.6. Evaluating nutritional quality of products

Bananas dried at optimal technological conditions in the low-temperature vacuum environment were tested for the nutritional quality. The results were shown in Table 8.

Table 8. Chemical compositions of vacuum dried banana per 100g dry weight

No.	Components	Unit	Value
1	Moisture	%	3.64
2	Protein	%	2.48
3	Carbohydrate	%	72.12
4	Lipid	%	0.39
5	Vitamin C	mg/100g	31.84

Once comparing with data in Table 4, the low-temperature vacuum dried banana accounted for a minority of nutritional loss. This statement could be demonstrated through the protein loss of 4.5%, carbohydrate loss of 7% and vitamin C loss of 3.27%. Thus, products had superb quality satisfying for market and export.

3.7. Evaluating the microorganisms and heavy metal criteria

The low-temperature vacuum dried banana was examined the microbiological infection, the limitation of mycotoxins and heavy metals. In case all the above criteria are fulfilled, the procedure of low-temperature vacuum drying of banana will be best suited for trade and export purposes. Results declaring for limitation of microbiological infection, mycotoxins and heavy metals were presented in Table 9, 10, and 11, respectively.

Table 9. Limit of microbiological infection

Criteria	Unit	Result	Allowable limit
Total aerobic plate count	cfu/g	$4,0.10^1$	$< 10^4$
Coliforms	cfu/g	ND	< 10
Escherichia coli	MPN/g	ND	0
Staphylococcus aureus	cfu/g	ND	< 20
Clostridium perfringens	cfu/g	ND	< 10
Bacillus cereus	cfu/g	ND	$< 10^3$
Total spores of yeast and mold	cfu/g	ND	10^2
Salmonella	per 25g	ND	ND in 25g

**ND: not detected*

Table 10. Limit of mycotoxins

No.	Criteria	Unit	Result	Allowable limit
1	Aflatoxin B_1	µg/kg	ND	2
2	Aflatoxin $B_1B_2G_1G_2$	µg/kg	ND	4

**ND: not detected*

Table 11. Limit of heavy metals

No.	Criteria	Unit	Result	Allowable limit
1	Lead (Pb)	mg/kg	ND	0,1
2	Cadmium (Cd)	mg/kg	ND	0,05

**ND: not detected*

Results summarized in Table 9, 10, 11 presented that all criteria complied with the allowance limits. These limits were specified in "Guidelines for Assessing the Microbiological

Safety of Ready-to-Eat Foods Placed on the Market" edited by Health Protection Agency, London (Health Protection Agency, 2009); Codex standards (Codex, 1997; Codex, 2003); Commission Regulation (EC) No. 2073/2005 and 1881/2006 (European Commission, 2005; European Commission, 2006). Therefore, low-temperature vacuum dehydrated banana chips in this study fulfilled all the prerequisites in terms of hygiene, food safety and export.

3.8. Production of low-temperature vacuum dried banana

After the technological mode had been determined, the entire procedure for banana chip production by low – temperature vacuum drying was standardized and presented in Figure 10.

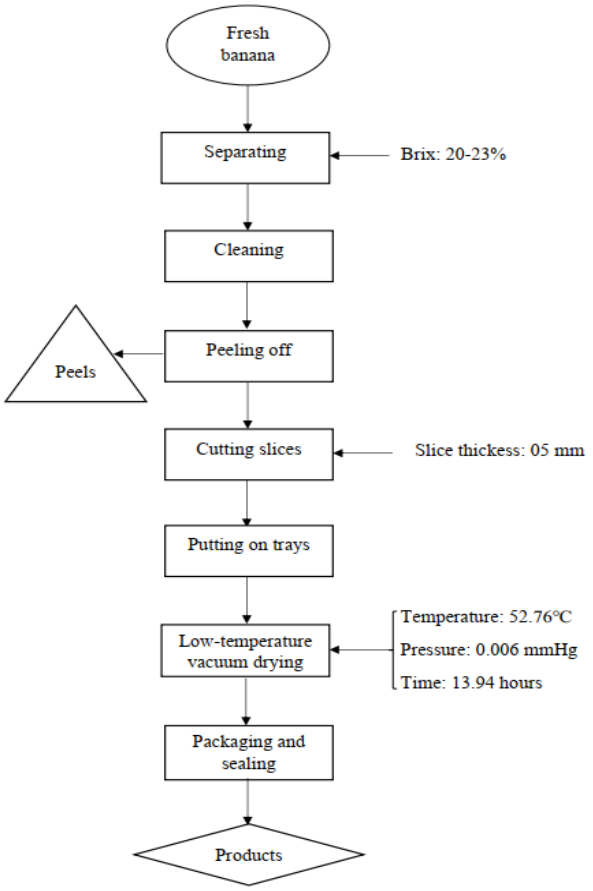

Figure 10. Flowchart of producing low-temperature vacuum dried banana

This procedure commenced when fresh banana was separated and removed the fingers spoiled by transportation. Following that, the ripeness was tested by refractometer (Brix≈20 ÷ 23%) before fresh bananas were cleaned to remove impurities on fruit skin and peeled off for chip production. Next, banana was cut into slices with 05 mm thickness and put on tray. After placing all trays in the drying chamber, closed the door tightly and started the drying process with parameters: ***52.76 °C, 0.006 mmHg and 13.94 hours.*** Finished products were put into PE bags and sealed (Figure 11).

Figure 11. The low-temperature vacuum dried banana at optimal technological mode

4. Conclusions

To form the mathematical models and solve it to find the technological mode for vacuum drying of bananas, this study had resolved some crucial initial targets:

- Determining chemical compositions of fresh banana to ensure that the starting material is the same and the banana quality is homogeneous before drying.
- Modeling the low-temperature vacuum drying process of banana by experimental planning method, shown by expressions (18), (19), (20) and (21). Therefore, these mathematical models can be easily used to establish the technological conditions for vacuum drying of banana slices.
- Optimizing the low-temperature vacuum drying process by building and solving the one-objective optimization problems and the multi-objective optimization problem via the restricted

area method. This resulted in the determination of optimal parameters including the temperature of drying 52.76 ^{o}C, vacuum pressure 0.006 mmHg and drying time of 13.94 hours. Final products positively obtained the energy consumption per 1 kg of final product was 3.96 kWh/kg, the residual moisture content was 3.64%, vitamin C loss was 3.27% and rehydration capacity was 95.17%. These consequences completely satisfied all the conditions regarding economy and technique of vacuum drying.

- Establishing the whole process of drying banana in low-temperature vacuum environment for trade and export (Figure 10).

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DEPTH EFFECT ON QUALITY CHARACTERISTICS OF TRADITIONAL SALTED-RIPENED ANCHOVY (ENGRAULIS ENCRASICOLUS) TAKEN FROM DIFFERENT PARTS OF THE SAME BARREL

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ABSTRACT

Samples from different depths of the same barrel of traditionally-produced salted anchovies (*Engraulis encrasicolus*) were assessed in terms of approximate composition, physicochemical, microbial and sensory attributes, underlining a new concept of "depth effect".

All samples showed low protein content, high levels of ash and salt, and a wide variation in moisture, total lipids, and dry matter contents. Saturated fatty acids were the most prevalent in all samples. Polyunsaturated fatty acids increased as depth increased. Zinc (Zn) was the main mineral noticed in all samples, followed by iron (Fe).

Microbiological analysis revealed a gradual increase in halophilic bacterial count from the surface to the bottom of the barrel. In terms of organoleptic properties, all samples were rated as regular, corresponding to a semi-ripened product. There was a depth effect on the overall quality characteristics of the fish, mainly related to the effect of pressure on the anchovy salting and ripening process.

1.Introduction

The anchovy (*Engraulis encrasicolus*) is a small pelagic species that belongs to the *Engraulidae* family. It is characterized by a very slim body and the standard color of pelagic fish: a dark back to hide from birds, and a silver belly, that looks like the water's surface once seen from below (Pons-Sánchez-Cascado *et al*., 2006).

Because of their abundance, anchovies have been caught and eaten by humans for a long time, and of the seventeen current genera, *Engraulis*, *Anchoa*, and *Stolephorus*are the most commercially important (FAO, 2014). The European anchovy features a high commercial worth and represents the foremost valuable pelagic fish resource in the Mediterranean Sea (Lleonart and Maynou, 2003).

Fresh anchovies are still in high demand in markets, even though there is an increasing orientation toward manufacturing prepared and preserved products. This fish is often consumed fresh during spring and summer time, or marinated in vinegar and ripened in saturated brines. Fish salting and ripening is a widespread and traditional practice utilized in European countries to lengthen different fish

species' shelf-life, like Atlantic herring (*Clupea harengus*), sprats (*Sprattus sprattus*), sardine (*Sardina pilchardus*), and anchovy (*Engraulis encrasicolus*). Among these varieties of products, salted and ripened anchovy has major importance in the worldwide market (Czerner and Yeannes, 2013). This salting and ripening process is mostly carried out, taking into consideration product safety and quality parameters. However, it is critical to understand the proximate composition of fish species in the application of various technological processes and as an aspect of raw material quality, sensory attributes, and storage stability (Huss, 1988; Sikorski *et al*., 1990).

The main objective of the present study was to assess and compare the quality of samples taken from three different depths of the same barrel of salted anchovies (*Engraulis encrasicolus*) produced using a traditional method, by evaluating their proximate composition, physicochemical, microbial, and sensory characteristics. The concept of the depth effect on the whole quality characteristics of the traditional salted-ripened European anchovy is being studied for the first time in this work.

2. Materials and methods

2.1. Preparation of salted-ripened anchovy and sample collection

Samples of salted anchovies prepared in an artisanal way were collected from the Jijelian (Algerian North-East) fish market and placed in thick-walled polystyrene boxes, packed in an icebox and delivered to the processing laboratory on the same day.

This artisanal method is a preliminary step in the wet salting process, which involves placing whole anchovies in bins and immersing them in saturated brine for 10 days. When compared to raw fish, water activity (aw) is reduced by 15 to 19% during this stage (Filsinger, 1987). Anchovies are beheaded, gutted, and placed in a 25 kg barrel alternating layers of fish and salt with a final salt-to-fish ratio of 1:3. Finally, the barrel is closed, and weights are set on top of the lid to press down on the salt and anchovies. After pressing, they are left in the barrel for four months at room temperature, until they have developed a reddish color and the wanted aroma.

To get reliable representation, three samples of 1 kg of salted anchovies were selected at random in triplicate and taken from three different parts (from top to bottom) of the same barrel:

Sample S: from the top of the barrel (at 5 ± 2 cm)

Sample I: from the middle of the barrel (at 15 ± 2 cm)

Sample D: from the bottom of the barrel (at 30 ± 2 cm).

Physical characteristics of the raw material (fresh anchovy) were determined by measuring the total length, weight, and number of fish pieces in 1 kg of each sample.

2.2. Proximate composition and physicochemical analysis

Salted anchovy was analyzed to determine chemical proximate composition. For this, all samples were analyzed for their protein, fat, moisture, dry matter and ash contents by standard analytical procedures of the Association of Official Agricultural Chemists, AOAC. The analysis was performed in triplicate and the average values were calculated and expressed as mean ± SD of triplicate observations.

Salted fish samples (10 g) were homogenized for 5 min in sterile blenders with 10 mL of distilled water to form a thick slurry. The slurry's pH was determined using a Hanna Hi 2210 pH meter (Hanna Glass Works, Medfield, MA) (AOAC, 2000).

To determine salt content in each sample (AOAC, 1995), 2 g of sample were homogenized with 18 mL of distilled water before being titrated with 0.1 M $AgNO_3$ using 10% (w/v) K_2CrO_4 solution as the indicator.

The moisture content was calculated by subtracting the fresh and dry weights of samples after drying them at 105 ± 1°C until they reached constant weight (AOAC, 1990). Crude protein, as the total nitrogen content, was

determined by the Kjeldahl method (AOAC, 1995). Crude fat was measured from the dried sample by Soxhlet extraction with petroleum ether (AOAC, 1995). And the crude ash fraction was obtained by incinerating the organic matter at 550 °C for about five hours until the sample was free from carbon particles (AOAC, 1990). Total titratable acidity (TTA) was determined by titrating against 0.1 M NaOH, and acidity was calculated as the percentage of lactic acid (AOAC, 1995). The peroxide index (PV) and the content of free fatty acids (FFA) or acid value were determined on the chloroform extracts of tissues (AOAC, 2000). The saponification index was measured according to the American Oil Chemists' Society (AOCS, 1993), where 1 g of ground sample was dissolved in 1.0 M alcoholic KOH and boiled gently but steadily in a water bath for 30 min for complete saponification. The solution was then titrated with 0.5 M HCl using phenolphthalein as an indicator. A blank determination was carried out concurrently with the sample.

For mineral composition, the ash solution of each sample in 10 mL of distilled water was hydrolyzed with 1 mL of HCl and boiled for 5 min in a water bath until the complete dissolution of ashes. The obtained solution was transferred into a 100 mL graduated flask where the volume is completed in 100 by adding distilled water (NF V04-404, 2001). From this final solution, lead (Pb), iron (Fe), chromium (Cr), manganese (Mn), copper (Cu), zinc (Zn), and cadmium (Cd) content were analyzed by atomic absorption spectrometry (Shimadzu AA-6200).

The fatty acid composition was determined in accordance with NF EN ISO 12966-4: 2015, which requires two preliminary stages, fatty acid extraction and esterification: 20 g of each lipid extract sample obtained previously using the Soxhlet method, were dissolved in 0.5 mL heptane and 0.2 mL of methanolic 2 M KOH was added. The mixture was boiled in a water bath for 2 min and 0.2 mL of 2 M HCl was added. After vigorous shaking and decantation, 100 µL of the upper phase was evaporated and the residue was reconstituted in 50 µL of heptane until the upper phase became clear and then injected into a gas chromatograph. The fatty acid methyl esters were analyzed by GC/MS on a Shimadzu QP2010 GC/MS equipped with a capillary column SE30 (30 m x 0.25 mm i.d., 0.25 µm film thickness) and helium as the carrier gas at a flow rate of 0.76 mL/min. Samples were injected into the split mode. The column was kept at 140 °C for 10 min and then programmed to increase by 1 °C/min up to 160 °C, then by 2 °C/min up to 220 °C then maintained for 15 min. By examining the elution order on the column and comparing the retention times to those of pure standards, the gas chromatogram peaks were identified as corresponding fatty acid methyl esters.

2.3.Microbiological analysis

Salted-ripened anchovies flesh was chopped and ground for 1 min using a Warning Commercial Blender (USA). Each sample (10 g) was collected aseptically and homogenized mechanically in 90 mL of sterile salt broth (meat extract, 3 g/L; meat peptone, 5 g/L; NaCl, 150 g/L) (ICMSF, 1983). As an enrichment step, the homogenate was incubated at 35-37 ºC for 30 min to recover stressed cells. Following that, decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of the same solution were prepared and spread in the growth media. Plate Count Agar (PCA) incubated at 30 °C for 48 h for aerobic mesophilic bacteria, Violet, Red Bile Lactose agar (VRBL) incubated at 30 °C and 44 °C for 24-48 h for total and thermotolerant coliforms, respectively, Man Rogosa-Sharpe agar (MRS) incubated at 37 °C for 48-72 h for lactic acid bacteria, Chapman agar incubated at 37 °C for 48 h for staphylococci, Meat-Liver agar incubated at 46 °C for 24-48 h for sulfito-reducing *Clostridium*, Oxytetracycline Glucose Agar (OGA) at 25 °C for 5 days for yeasts and moulds (AOAC, 1995; NF ISO 4832: 2006).

The halophilic bacteria count was determined using the Halophilic Medium (HM) of Torreblanca *et al*. (1986) containing (per

liter of distilled water): 5 g peptone (Difco), 5 g yeast extract (Difco), 22 g agar at two final total salt concentrations of 5% and 10% (w/v).The stock solution of total salts of 30% (w/v) was prepared as described by Subov (1931): (NaCl, 234 g; $MgCl_2.6H_2O$, 42 g; $MgSO_4.7H_2O$, 60 g; KCl, 6g; $CaCl_2.2H_2O$, 1 g; NaBr, 0.7 g; $NaHCO_3$, 0.2 g; $FeCl_3$, 0.005 g and 1000 mL of distilled water). The enumeration of these microorganisms was performed in duplicate and the mean value of each count was expressed as the number of colony-forming units/g (CFU/g).

2.4. Sensory assessments

A sensory evaluation of the ripening progress was performed by a six-member trained panel using the Table proposed by Filsinger *et al*. (1982) in which the parameters flavor, odor, flesh color, flesh consistency, and flesh adhering to the backbone are assessed using an 8-point quality scale. Each factor was assigned a score to the fish based on the descriptions in the table. The fish's score was determined by taking the average of the five factors. The maturation scale's minimum value is zero, representing the sensory characteristics of raw fish just before the ripening process begins. Point 6 denoted the optimal level of ripening, while point 8 denoted spoilt or overripe anchovies.

2.5. Statistical analysis

The data were subjected to one-way analysis of variance and expressed as the mean ± the standard deviation (SD). The Statistical Package for Social Sciences, version 23.0, was used for all statistical analyses (SPSS for Windows; SPSS Inc., Chicago, IL). The significance level for differences was defined at $p < 0.01$.

3.Results and discussions

3.1. Physical characteristics of raw fish

The characteristics of fish used in the present investigation in terms of length, weight, and the number of pieces in 1 kg are shown in Table 1. The average length of the fish in the current study was almost the same for all the three samples (13 ± 0.00 to 13 ± 0.82 cm), though the average weight varied slightly, indicating their sexual and seasonal maturity. These values are lower than those found by Šimat and Bogdanović (2012) who recorded an average length of 15.23 ± 0.68 cm and an average weight of 24.72 ± 3.64 g, and higher than those found by Sofoulaki *et al*. (2018) who registered an average length between 8.71 ± 0.46 cm and 11.33 ± 0.52 cm and an average weight between 3.9 ± 0.6 g and 8.2 ± 1.3 g.

Table 1. Quality characteristics of different anchovy samples.

Characteristics	Samples		
	S	I	D
Physical characteristics of fresh anchovies			
Length (cm)	13 ± 0.82^a	13 ± 0.00^b	13 ± 0.00^c
Weight (g)	13.4 ± 2.97^a	13 ± 0.71^a	13.5 ± 1.73^a
Number of fish pieces in 1 kg	70 ± 1.00^a	66 ± 0.57^a	66 ± 0.57^a
Physicochemical characteristics			
pH	5.81 ± 0.04^a	5.75 ± 0.03^b	5.66 ± 0.02^c
TTA (°D)	0.06 ± 0.1^a	0.06 ± 0.1^a	0.08 ± 0.1^a
Moisture (%)	57.5 ± 0.1^a	55.0 ± 0.2^b	52.5 ± 0.1^c
Dry matter (%)	42.5 ± 0.1^b	45.0 ± 0.2^a	47.5 ± 0.1^c
Crude protein (%)	1.37 ± 0.63^a	1.32 ± 0.19^a	1.28 ± 0.35^a
Total lipid (%)	4.25 ± 0.08^b	4.69 ± 0.12^a	5.38 ± 0.15^c
Ash (%)	35.0 ± 0.1^a	36.0 ± 0.3^b	40.5 ± 0.1^c

Salt (%)	34.7 ± 0.9^{a}	35.1 ± 0.2^{b}	40.3 ± 0.3^{c}
PV (meq O_2/kg of fat)	0.5 ± 0.00^{a}	0.5 ± 0.00^{b}	0.75 ± 0.05^{c}
FFA (% of Oleic acid)	5.74 ± 0.5^{a}	7.17 ± 0.5^{a}	7.89 ± 1.0^{a}
Saponification index (mg KOH/g)	102.38 ± 0.7^{a}	75.73 ± 0.4^{b}	91.27 ± 0.5^{c}
Microbiological characteristics (CFU/g)			
Total plate count	$4.0\times10^{4} \pm 1.05^{a}$	$3.3\times10^{4} \pm 0.07^{b}$	$2.0\times10^{4} \pm 1.00^{c}$
Total coliforms	00	00	00
Thermotolerant coliforms	00	00	00
Lactic acid bacteria	$1\times10^{6} \pm 0.7^{a}$	$2.7\times10^{6} \pm 1{,}27^{a}$	$1.32\times10^{7} \pm 0.13^{a}$
Staphylococci	Abs	Abs	Abs
Sulfito-reducing *Clostridium*	00	00	00
Yeasts and moulds	10	00	00
Halophilic count **5%** **10%**	$1.23\times10^{3} \pm 0.83^{a}$ $1.12\times10^{3} \pm 1.00^{a}$	$2.40\times10^{3} \pm 0.12^{b}$ $1.21\times10^{3} \pm 0.05^{b}$	$2.64\times10^{3} \pm 0.55^{c}$ $1.43\times10^{3} \pm 0.59^{c}$
Sensory characteristics			
Mean scores	3.76 ± 0.5^{a}	3.12 ± 1.05^{b}	4.08 ± 0.09^{c}

S: Sample taken from the Superficial part, I: Sample taken from the Intermediate part, D: Sample taken from the Deep part. TTA: Total Titratable Acidity, PV: Peroxide Value, FFA: Free Fatty Acid, CFU: Colony Forming Unit, Abs: Absence.
$^{a\text{-}c}$ Values in the same line and labelled with different letters differ significantly ($p < 0.01$).

Besides, the number of fish pieces in 1 kg of each sample, was 70 ± 1.00, 66 ± 0.57, and 66 ± 0.57 pieces for the samples S, I, D, respectively. According to Arrignon (1966), 1 kg of anchovy has to contain 50 elements of fish. These elements measure between 8 and 19 cm and have an average length and an average weight of 14 cm and 20 g, respectively. Our results correlate with this data regarding length. However, the average weight was lower with a difference of about 6 g, which explains the low number of fish pieces in 1 kg. The number of elements may be decreased, thereby decreasing the total mass of the elements.

3.2. Proximate composition and physicochemical analysis

The pH values were significantly different in the three samples ($p < 0.01$). There was no significant difference between TTA values ($p > 0.01$). These results are in agreement with most of the reported literature data (Hernández-Herrero *et al*., 1999; 2002; Llorente Holgado *et al*., 2007; Ababouch and El Marrakchi, 2009). According to Ababouch and El Marrakchi (2009), the pH of anchovy muscle decreased from 6 to 5.4 after 3-4 months of the ripening process. This decrease, which is essentially attributed to the accumulation of free fatty acids, is made in spite of a significant increase in nitrogenous bases, in particular NH_3. On the other hand, the reason for the lower pH values of the product may also be attributed to the fact that the samples were fermenting rather than spoiling. In the same analogy, the high value of TTA seemed to be due to the production of various organic acids, including free fatty acids. This implies that the fish underwent sufficient fermentation with endogenous and/or exogenous (microbial) enzyme systems (Majumdar and Basu, 2010).

The significant differences in pH values between the three samples could be also explained by the difference in salt concentration and depth from where each sample was collected. It has been suggested that salt concentration influences not only water activity, but also the pH (Rodríguez-Jerez *et al*., 1993; Hernández-Herrero *et al*., 1999). The decrease in pH value is explained by the

increase of the ionic strength of the solution within the cells (Goulas and Kontominas, 2005; Ormanci and Colakoglu, 2015).

The peroxide and saponification indexes showed a significant variation in the three samples ($p < 0.01$). The FFA content was in the range of 5.74 ± 0.50-7.89 ± 1.00 of oleic acid. The low-value PV index indicates that oxidation is well developed and that hydroperoxides have already been decomposed and transformed (Adrian *et al*., 1998), indicating that salting conditions accelerate lipid oxidation, which is consistent with previous reports (Smith *et al*., 1988). Furthermore, it was also reported that a significant role in the oxidative deterioration of salted fish was played by enzymatic oxidation (Cho *et al*., 1989). The highest value of the saponification index was registered for sample S, reflecting its high content of short and medium-chain fatty acids.

Furthermore, the highest free fatty acid content was obtained in Sample D. Previous research found that salt had no inhibitory effect on lipases, which are responsible for the release of free fatty acids (Perez-Villareal and Pozo, 1992). This could mean that more unsaturated fatty acids were produced and were subjected to oxidative hydrolysis at the double bonds. The resulting substances, primarily ketones, and aldehydes, appear to be largely responsible for the products' flavor, odor, and taste (El-Sebaiy and Metwalli, 1989).

Moreover, the proximate composition showed that the protein content varied between 1.28 ± 0.35% and 1.37 ± 0.63%. But the total lipid, moisture, and ash contents showed a wide variation.

The fat content of anchovy flesh can differ not only between species but also within species from a single catch. This is due to the various stages of maturity (Pigott and Tucker, 1987). The difference in the observations could also be attributed to the higher salt content in sample D than in others. In most fish, particularly pelagic species, the total sum of the two main constituents (moisture and lipid) accounts for approximately 78-80% of the total weight. This sum is lower for the three samples in the present study. Earlier studies have also found that lipid content varies slightly (Shiriskar *et al*., 2010a; 2010b).

The data also revealed an inverse relationship between moisture content and fat content, which is consistent with previous studies that investigated the relationship between fat and water content (Gökoglu *et al*., 1999; Šimat and Bogdanović, 2012). The authors reported a very strong negative correlation between these two parameters and concluded that the existence of such a relationship between fat and water content would allow the fat content to be estimated using the moisture content. This would not replace the standard procedures for precise measurement of fat content; however, it would allow processors to make an accurate estimate of fat content.

The dry matter varied significantly between 42.5 ± 0.1% and 47.5 ± 0.1%. The value of sample S was among the aforementioned samples. As shown in the table, there is a clear relation between salting, moisture, and dry matter contents, which is an inversely proportional relation.

Lower protein content and higher levels of ash and salt compared with previously reported analyses were found (Hernández-Herrero *et al*., 1999). On one hand, higher values of ash content, which estimate the mineral composition as well as the amount of residual salt in fish products could be attributed to high salt content, fish size, and possibly bone fragments that were not removed from anchovy samples during processing (Selmi *et al*., 2010). On the other hand, the protein content was found to be lower. Previous research has shown that solubilized proteins (as a result of the salting process) have an additional osmotic effect and contribute to the equilibrium state (Czerner and Yeannes, 2010). According to Nketsia-Tabiri and Sefa-Dedeh (1995), the tilapia salting process results in protein loss. High salt concentration inside the fish during salting causes a loss of soluble proteins and a

loss of water holding capacity, resulting in protein denaturation (Kong *et al*., 2008).

The salt content of the three samples varies significantly as well. In sample S, the salt content was the lowest and the highest was in sample D. These high levels of salt content can be attributed to traditional preparing conditions as well as moisture uptake due to salt's hydrostatic moisture during storage (Dewi *et al*., 2011). The process of anchovy salting includes the diffusion of salt into the fish and the elimination of water via osmosis. The loss of moisture from the fish due to osmosis resulted in a decrease in moisture content as well as an increase in ash and salt content in the final product (Hernández-Herrero *et al*., 2002; Majumdar and Basu, 2010). Furthermore, the rate of salt penetration in the muscle is highly influenced by fat content, the freshness of the fish, flesh thickness, surface/volume ratio, and temperature (Clucas, 1982). We suggest that the freshness of the fish in sample S slightly reduced the rates of absorption of salt. Fish chemical composition varies greatly between species and individuals, depending on starvation and intensive food intake periods, as well as external factors such as temperature and salinity (Huss, 1995; Zlatanos and Laskaridis, 2007). The salt content in our study is considerably higher than that reported in numerous other studies (Ababouch and El Marrakchi, 2009; Czerner and Yeannes, 2014; Zang *et al*., 2019). This could be due to the uncontrolled and unregulated salting process applied according to the artisanal method.

Table 2. Values of trace mineral elements and heavy metals in salted-ripened anchovies.

	Samples		
	S	**I**	**D**
Trace mineral elements (ppm)			
Zinc (Zn)	0.2219 ± 0.002^{a}	0.4056 ± 0.032^{b}	0.3344 ± 0.015^{c}
Manganese (Mn)	0.0562 ± 0.001^{a}	0.0351 ± 0.005^{b}	0.0066 ± 0.003^{c}
Iron (Fe)	0.1351 ± 0.010^{a}	0.2091 ± 0.022^{b}	0.0777 ± 0.034^{c}
Copper (Cu)	0.0247 ± 0.017^{a}	0.0332 ± 0.022^{a}	0.0570 ± 0.013^{a}
Heavy metals (ppm)			
Lead (Pb)	0.0264 ± 0.002^{a}	0.0966 ± 0.004^{b}	0.1142 ± 0.009^{c}
Chromium (Cr)	0.0256 ± 0.092^{a}	0.0584 ± 0.111^{a}	0.0531 ± 0.102^{a}
Cadmium (Cd)	0.0212 ± 0.001^{a}	0.0119 ± 0.003^{a}	0.0132 ± 0.001^{a}

[a-c] Values in the same line and labelled with different letters differ significantly ($p < 0.01$).

Mineral composition

Fish is a good source of minerals that are highly needed for the normal functioning of the body. Trace minerals Zn, Mn, Fe, and Cu, were present in the range of 0.0066 ± 0.003 to 0.4056 ± 0.032 ppm in the fish (Table 2). Mineral and metal content can vary depending on the surrounding environment (Sen *et al*., 2011). Zn was the main mineral noticed in all samples with the highest level found in sample I, followed by iron (Fe) with an important amount recorded for sample I. Our results are lower than those reported by several authors (Mol, 2011; Galaţchi *et al*., 2017; Afandi *et al*., 2018; Sofoulaki *et al*., 2018).

As shown in the table above, cadmium and lead were lower than the allowed levels of the EU legislation (0.30 µg/g or 0.30 ppm) and chromium was lower than the content registered by Chandrashekar and Deosthale, (1993) and Afandi *et al*. (2018) who reported a value of 69.3 µg/100g and 0.27 ± 0.442 µg/g, respectively.

Variations in metal bioaccumulation between species have been attributed primarily to different diets and trophic levels (Metian *et al*., 2013; Renieri *et al*., 2014). During its

reproductive cycle, anchovy consumes only foods with high-energetic value, which increases its feeding intensity (Karachle and Stergiou, 2013). Finally, various intrinsic parameters such as body size, age, sex (Sarkar *et al*., 2008) as well as proximate composition (lipid and protein contents) (Kalantzi *et al*., 2016; Sofoulaki *et al*., 2018) have also been proposed to explain differences in bioaccumulation or metal load among different species.

Fatty acid composition

There were changes in the fatty acid profiles (Table 3). Saturated Fatty Acids (SFA) are the most common fatty acids found in all samples. The major saturated fatty acids (SFA) were C16:0 and C18:0 with the highest percentage of C16:0. This finding is consistent with previous research, which reported C16:0 to be the most abundant fatty acid in anchovies (Bayir *et al*., 2006) and almost all fish species (Özogul *et al*., 2007). Similarly, the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contents showed a higher proportion in the anchovies collected from the surface and deep layers, respectively. The PUFA increased as the depth increased. Among the individual fatty acids, C16:0 and C18:1 were predominant in all samples. C2:0, C16:0, and C18:1 decreased with depth, whereas C18:0 and C18:2 increased. In addition, only sample D contained Docosahexaenoic acid (DHA; C22:6) with a percent of 2.42% while the Eicosapentaenoic acid (EPA; C20:5) content, which was present only in the two samples I and D, showed a higher content in sample D, with a percentage of 1.18 ± 0.24%. According to Bayir *et al*. (2006), anchovy contains 11.68% EPA and 25.85% DHA. In addition, Zlatanos and Laskaridis (2007) found that EPA and DHA levels in anchovy ranged from 2.46 to 12.4%, and 12.23 to 32.46%, respectively, depending on the season. Saglik and Imre (2001) determined EPA (0.86 g/100g) and DHA (1.56 g/100g) in anchovy. Our results are closer to those obtained by Zlatanos and Laskaridis (2007) in DHA value and Saglik and Imre (2001) in EPA content.

Table 3. Fatty acid composition of salted-ripened anchovies.

Fatty acids (%)	**Samples**		
	S	**I**	**D**
Saturated fatty acids (SFA)			
Oxalic acid (C2:0)	1.47 ± 0.00^{a}	0.61 ± 0.01^{b}	0.41 ± 0.01^{c}
Caplrylic acid (C8:0)	nd	nd	0.17 ± 0.00
Capric acid (C10:0)	2.02 ± 0.08	nd	0.18 ± 0.13
Lauric acid (C12:0)	nd	nd	0.26 ± 0.08
Pentadecylic acid (C15:0)	nd	nd	1.63 ± 0.11
Palmitic acid (C16:0)	42.03 ± 0.10^{a}	38.24 ± 0.06^{a}	36.21 ± 0.15^{a}
Margaric acid (C17:0)	nd	nd	1.73 ± 0.13
Stearic acid (C18:0)	8.78 ± 0.18^{a}	11.11 ± 0.27^{b}	13.19 ± 0.07^{c}
Arachidic acid (C20:0)	nd	nd	0.35 ± 0.15
Monounsaturated fatty acids (MUFA)			
Hexadecanoic acid (C16 Δ^{7})	nd	nd	0.65 ± 0.00
Palmitoleic acid (C16Δ^{9})	1.99 ± 0.01	nd	0.23 ± 0.01
Oleic acid (C18 Δ^{9})	29.46 ± 0.00^{a}	22.42 ± 0.03^{b}	22.24 ± 0.07^{c}
Vaccenic acid (C18Δ^{11})	nd	nd	3.60 ± 0.05
Gondoic acid (C20Δ^{11})	nd	nd	0.27 ± 0.00
Polyunsaturated fatty acids (PUFA)			
Octadecadienoic acid (C 18 $\Delta^{8, 11}$)	2.05 ± 0.33^{a}	4.01 ± 0.08^{b}	4.70 ± 0.03^{c}

Eicosapentaenoic acid (C 20 Δ $^{5, 8, 11, 14, 17}$)	nd	1.10 ± 0.10	1.18 ± 0.24
Docosahexaenoic acid (C 22 Δ $^{4, 7, 10, 13, 16, 19}$)	nd	nd	2.42 ± 0.17
∑ SFA	54.30 ± 0.36	49.96 ± 0.34	54.13 ± 0.83
∑ MUFA	31.45 ± 0.01	22.42 ± 0.03	26.99 ± 0.13
∑ PUFA	2.05 ± 0.33	5.11 ± 0.18	8.30 ± 0.44

nd: not detected, $^{a-c}$ Values in the same line and labelled with different letters differ significantly ($p < 0.01$).

Anchovy, Atlantic herring, and both farmed and wild salmon contain, in decreasing order, 2055 to 1840 mg/100g EPA+DHA, while Atlantic mackerel, bluefish, Atlantic sardines, and trout contain from 1203 to 936 mg/100g (Mozaffarian and Wu, 2011).

Therefore, it is very important to underline that there was a depth effect on the whole quality characteristics of the fish related to the effect of pressure on the salting and ripening process of anchovies. According to previous reports, the main issue with the vat or barrel salting method is product irregularity because the height of the barrel and the pressure exerted by weight can create different salt concentrations at different levels in the same barrel. To avoid this, the upper layers should receive nearly twice the amount of salt as the bottom layers. Furthermore, brining, at a level of about 70%, is a faster method of preserving fish. The lower the salt concentration and the fatter the fish is, the longer the period of brining required to ensure extended shelf life will be. The principal problem encountered here is that, as the fish absorbs salt from the brine and releases water from the tissues, the brine becomes diluted. Salt must therefore be added regularly (periodically) and the solution stirred frequently to ensure that the added salt does not settle down (Curtis, 1993). In our case, and based on an informal discussion with the vendor, this depth effect was mainly ascribed to the processing technique as to how the salting was applied. His information indicates that the upper layers received the same amount of salt as the bottom layers.

Previous studies, as previously mentioned, had found a negative correlation between fat and moisture; in our study, we found the same kind of relationship, but not only between the aforementioned two parameters. There was also an inverse relationship between ash and moisture, salt and moisture, and free fatty acid and moisture contents. The other thing to observe is the direct relation between crude protein and moisture content. It is necessary to note that all of these results are deeply connected with the difference in depth levels, from the superficial to the deeper one. In the same line, we noticed a clear increase with increasing depth levels in the poly-unsaturated fatty acid profile. There is no recent data that explains clearly this influence on the proximate composition of our traditional salted-ripened anchovy, but it is clear that the difference in level from which we collected our samples was the significant factor.

3.3. Microbiological flora analysis

As shown in Table 1, counts of aerobic mesophilic bacteria ranged from $2.0\times10^4 \pm 1.0$ to $4.0\times10^4 \pm 1.1$ CFU/g, and lactic acid bacteria counts were between $1\times10^6 \pm 0.7$ and $1.3\times10^7 \pm 0.1$ CFU/g. Yeasts and mould colonies were only found in sample S. None of the total and thermotolerant coliforms, staphylococci, and sulfito-reducing *Clostridium* were detected. The obtained results were in agreement with the microbiological criteria limits fixed by the Official Journal of the Algerian Republic (2017). It is clear from the results that the almost complete absence of spoilage and pathogenic flora is mainly due to the preservative effect of high levels of salt, which results in a decrease in water activity, thereby promoting less availability to microbial attack and an improvement of functional properties (Santiago and Maurizio, 2002).

The count of halophilic bacteria in final salt concentrations of 5% and 10% (w/v) was between $1.23\times10^3 \pm 0.83$ and $2.64\times10^3 \pm 0.55$ CFU/g and $1.12\times10^3 \pm 1.00$ and $1.43\times10^3 \pm 0.59$ CFU/g, respectively (Table 1). Previous data reported that the halophilic flora varies

between 1.0×10^4 CFU/g and 6.4×10^4 CFU/g at salt concentrations between 5 and 10% after 73 days of anchovy's maturation (Czerner and Yeannes, 2014). This halophilic population of anchovy represents the bacterial load present in the marine environment; halophilic bacteria naturally occur in the outer layer of the skin, on the gills, and intestines of marine fish (Prescott *et al*., 1996). Processing environments such as handling, storage, and practical methods for preserving quality, including the salting process, could also affect halophilic count in fish.

A gradual increase was observed in halophilic bacterial count from the superficial to the deeper part (Table 1). This variation in counts could be firstly related to the initial concentration of microorganisms in the pre-salting stage where halophilic bacteria from salt could develop to a certain extent before being in contact with anchovies (Perez *et al*., 2018), and secondly due to the gradual increase in salinity created by the depth and the salting processing technique, which therefore gives higher to a lower count in halophilic microflora. During the salted anchovy ripening process, the halophilic microflora was found to be dominated by moderate and extremely halophilic bacteria, showing an important role in the ripening process (Czerner and Yeannes, 2014; Felix *et al*., 2016; Perez *et al*., 2018; 2020).

3.4. Sensory analysis

In terms of organoleptic quality, the overall acceptability of fish, as measured on an 8-point quality scale (Filsinger *et al*., 1982), was 4.20, 4.76, and 5.80 for samples I, D, and S, respectively. The fish was rated regular organoleptically, though the average sensory scores of the samples varied slightly between 3.12 ± 1.05 and 4.08 ± 0.09, corresponding to a semi-ripened product. This could be due to variations in the fish's time-temperature history. Our scores are lower than those reported by Czerner and Yeannes (2013), who recorded a mean sensory score of 5.6, 5.1, and 4.5.

4. Conclusions

In conclusion, salted-ripened anchovy samples showed low protein content, high levels of ash and salt, and a wide variation in lipid content. These findings could be attributed to the traditional preparing conditions. As a result, traditional methods of preparation should be inspected, especially regarding the amount and the quality of added salt, raw material, as well as fish to salt ratio. On the other hand, there was an evident correlation between the depth levels from where the samples were collected, the halophilic bacterial count, and the poly-unsaturated fatty acid profile, which could constitute very useful information to initiate a comprehensive study on this topic.

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SUPERIORITY OF GERMINATED OVER RAW SAMPLE IN PROXIMATE COMPOSITION AND OVER BOTH RAW AND FERMENTED IN MINERALS OF *ZEA MAYS* L. DK 818

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ABSTRACT

This research report dealt with proximate and mineral analyses of raw (B11), germinated (B22) and fermented (B33) maize grains from the same source. Values of dry matter, organic matter and carbohydrate were high at (g/100g): 94.2-95.3, 92.9-94.6 and 74.4-75.8 respectively. Crude protein had these values (g/100g): B11 (10.9) < B22 (13.0) < B33 (13.2). Crude fat was moderate at 5.05-5.59 g/100g. On concentration levels, the following were observed in the proximate composition: B22 > B11; B22 ≡ B33; B11 > B33. The energy from Z. mays was majorly from the carbohydrate (kcal/100g): B11 (303, 77.3%); B22 (297, 74.4%) and B33 (302, 75.5%). These minerals were generally high in the samples (mg/100g): K (550-661), Mg (220-235), P (369-401) whereas values were low for Fe, Cu, Co, Mn, Zn, Se and < 0.001 in Pb. The mineral density per sample ran thus (mg/100g): B11 (1227)< B22 (1403) > B33 (1313). On the whole, B22 > B33 >B11 as follows: B22> B11, 12/12 = 100%; B22>B33, 10/12 = 83.3%; B33 > B11, 8/12 = 66.7%. In the mineral ratios determined, only Zn/Cu values of 7.80-10.7 were close to reference balance ideal of 8.00. All the calculated mineral safety index (MSI) were lower than the standard Table values. At both proximate and mineral levels, the pairs: B11/B22, B11/B33 and B22/B33 were significantly different at r=0.01. All the index of forecasting efficiency (IFE) were high making it possible for one of the pairs to carry out the other pair metabolic functions and vice versa.

1. Introduction

The word “maize” derives from the Spanish form of the indigenous Taino word for the plant *mahiz* (Maize, 2012). It is known by other names around the world. Seed of maize contains endosperm which is a food storage organ and consists primarily of starch which is digested into sugar when germination occurs and growth begins. Maize may be divided into vaious groups differing in endosperm character of the seeds. These groups or types are (Obi,1991):

-Flour corn: *Zea mays* var. *amylacea* Sturt.
-Popcorn: *Zea mays* var. *everta* Sturt.
-Dent corn: *Zea mays* var. *indentata* Sturt.
-Flint corn: *Zea mays* var. *indurate* Sturt.
-Sweet corn: *Zea mays* var. *saccharata* Sturt and *Zea mays* var. *rugosa*.
-Waxy corn: *Zea mays* var. *ceratina* Kulesh.
-Amylomaize: *Zea mays*
-Pod corn: *Zea mays* var. *tunicate* Sturt Larranaga ex A. St. Hil.
-Striped maize: *Zea mays* var. *japonica*

This is an artificial classification that is not indicative of natural relationships; however, these subspecies are sometimes classified as various subspecies related to the amount of starch each has. Dent and Flint account for the bulk of world production; pop, sweet and flour corn are used almost entirely for human consumption; pod and waxy are not important as food stuffs, however, waxy maize is used industrially in the United State of America (Obi, 1991).

1.1. Maize scientific classification

Kingdom (Plantae), *Clade* (Tracheophytes), *Clade* (Angiosperms), *Clade* (Monocots), *Clade* (Commelinids), Order (Poales), Family (Poaceae), Genus (*Zea*), Species (*Z. mays*), Binomial name (*Zea mays* L.).

Maize is widely cultivated throughout the world and a greater weight is produced each year than any other grain (International Grains Council, 2013). In 2018, total world production was 1.15 billion tonnes, led by the USA with 34.2% (392.5) of the total. China produced 22.4% (257.3) of the global total (FAOSTAT, 2020). Actual world total was 1147.6 (millions of tonnes). Nigeria was number 14 world producer (10.2 million tonnes) which is about 0.889%. Maize has a very high yield of energy more than wheat and rice. Maize of about 93% extraction has an average yield of 5.4 million calories per hectare, thus ranking wheat (85% extraction) and rice (70% extraction) which gave average yield of 3.2 and 4.3 million calories per hectare, respectively (FAO, 1968). In Africa, maize consumption accounts for about 64% of the total daily calorie intake of the rural dwellers especially during the hunger "period". In Southern Nigeria, maize has been used primarily as human food (Obi, 1991). It is eaten as whole grain when boiled or roasted and used in its prepared form as pap (*ogi*) or eko (*Agidi*) which is an extracted starch meal obtained after a prolonged soaking of maize. Maize consumption in the Western States of Nigeria varies from 2.6-2.8kg per person per week (Agboola, 1979). In Nigeria, Anasonwu-Bello (Anazonwu – Bello, 1986) published over forty recipes from maize to encourage maximum use of maize as food-crop and to give it a new outlook. Of these recipes "Ogi" (Yoruba) or "Agidi" (Igbo) and "Akamu" (Igbo) are corn diets eaten by almost all the ethnic groups of Nigeria. Obi (1991) had further elaborated the maize recipes of other countries. In Ghana, "Kenkey" is the principal corn diet of the people. In Benin Republic (corn fritters); in Cameroon (Koga); in Malawi (roasted and parched maize); in Kenya ("Posho" or Gruel", "Ugali" and "Gbenga"); in the Republic of South Africa (Mahewa or Magou, a non-alcoholic drink, is made from sorghum which is supplemented with corn); in Central and South America, "Tortilla" as "Echilada" or "Taco" or "Tamele" are corn diets of the people. From Central and South America, other food preparations of corn are "Atole", "Penolillo", "Chicheme", "Colada" and "Chicha dulee". In Nigeria, *akamu* or *ogi* has a consistency similar to that of American pudding (Kulp, 2000). *Ogi/Akamu* in Nigeria is generally accompanied with *moinmoin*, a bean pudding of *akara* which is a bean cake.

Maize grains have long been shown to be of poor quality protein and consequently of poor nutritive value due to high levels of zein. The protein fraction of maize (zein) has insignificant amounts of Lys and Trp (Jose, 1966). The deficiency of these amino acids has resulted in negative nitrogen balance and poor growth of animals and humans fed on unbalanced maize meals. The economic importance of developing a good quality protein maize is immense. It will go a long way in helping to solve human nutritional problems especially kwashiorkor among babies weaned *akamu* or *ogi* diet.

The major aim of this research was to see if processing the maize grains would improve their nutritional quality. To this end, similar grains of maize (*Zea mays* L. Dk 818) were subjected to fermentation and sprouting, dried, pulverized and analysed for proximate and mineral contents. Data results were discussed comparatively and conclusions drawn.

2. Materials and methods

2.1. Collection of samples

Samples of maize grains were collected from the Department of Crop, Soil and Wildlife, Ekiti

State University, Ado-Ekiti, Nigeria. About 1.5kg of the grains were used for the experiments. After removing stones, damaged grains, manually, the maize grains were divided into three equal parts for use as raw, steeped (fermented) and germinated (sprouted) maize samples. Raw samples were labeled as B11 and no further treatment after cleaning. It was however dried to constant weight.

2.2. Sample treatment

For steeping, 0.50kg grains were placed in a plastic container, covered with distilled water and left in the laboratory at ambient temperature (30.9^{o}C) at 0.41 Im^2/ft light intensity. After four days, grains were washed with distilled water, dried in sun to constant weight and stored in covered plastic container; this was labeled B33 (Fig-1). For germination of samples, 0.5kg were soaked in water at room temperature for 24 h; then spread on a damp fabric, protected from direct sunlight, for approximately 48 h until 5.04cm long sprouts developed. Germinated grains were dried in sun for three days until constant weight; sprouts were manually removed and desprouted grains were stored in plastic container (FAO, 1976); this was labeled sample B22 (Fig-1). Each sample was homogenized, sieved using 200 mesh size and kept in the refrigerator (2.8^{o}C). Triplicates of raw, steeped and germinated grains were used for the proximate and mineral compositions.

2.3. Proximate composition determination

Moisture content was estimated gravimetrically by drying the flours at 100^{o}C in ventilated oven to express moisture in g/100g. Crude protein (N × 6.25) of the flours was evaluated following the method of micro-Kjeldahl (Pearson, 1976). Crude fat was extracted with chloroform/methanol (2:1 v/v) mixture using Soxhlet extraction apparatus (AOAC, 2006). Carbohydrate was calculated by difference.

Carbohydrates (g/100g) = [Protein (g/100g) + Lipids (g/100g) + Fibre (g/100g) +Ash (g/100g) + Moisture (g/100g)] (1)

Gross energy (kcal/kJ/100g) was calculated using Atwater factors (Muller and Tobin, 1980).

Gross energy (kcal/100g) = (Protein × 4) + (Lipid × 9) + (Carbohydrate × 4) (2)

Gross energy (kJ/100g) = (Protein × 17) + (Lipid × 37) + (Carbohydrate × 17 (3)

Utilizable energy due to protein (%): UEDP % = %protein energy in gross energy × 60% (4)

Energy requirement for infants per day: calculation = 100/Total energy value × 740 kcal (5)

Water required for complete protein metabolism for each sample: X × 3 = y; 3.5 × X = Z; Z-Y = water required (6)

where 3 = 1 calorie of protein requires 3.0ml of water for excretion of the urea and sulphate formed from it; X = protein energy in kcal/100g; 3.5 = water deficit (350/100 = 3.5).

Conversion of lipid to total fatty acid (TFA): Crude fat × 0.72 = TFA (Greenfield H. & Southgate, 2003) (7)

2.4. Mineral analysis

Minerals were determined using the solutions obtained by ashing the samples at 550^{o}C and dissolving it in 10% HCl and (25ml) and 5% lanthanum chloride (2ml), boiling, filtering and making up to standard volume with deionized water. Phosphorus was evaluated colorimetrically using a Spectronic 20 (GallenKamp, London, UK) instrument with KH_2PO_4 as the standard (AOAC, 2006). Na and K were determined by flame photometry, Model 405 (Corning, Halstead Essex, UK) using NaCl and KCl to prepare standards. All other elements (Ca, Mg, Fe, Zn, Ni, Co, CU, Pb and Se) were determined by atomic absorption spectrophotometry, Model 403 (perkin-Elmer, Norwalk, Connecticut, USA). All chemicals used were of analytical grade, and were products

obtained from British Drug House (BDH, London, UK). Detection limits for the metals in aqueous solution had been determined just before the mineral analyses using the methods of Varian Techtron (Varian, 1975) giving the following values in µg/ml: Fe (0.01), Cu (0.002), Na (0.002), K (0.005), Ca (0.04), Pb (0.08), Mg (0.002), Zn (0.005), Mn (0.01), Co (0.05), Mn (0.01) and Se (0.15). The optimal analytical range was 0.1-0.5 absorbance units with coefficients of variation from 0.9-2.2%. From the mineral elements determined, further calculations were made.

2.5. Mineral ratios

Ratios of Ca/Mg, Ca/K, Zn/Cu, Ca/P, Fe/Cu, Ca/Pb, Fe/Pb, Fe/Co, Na/Mg, K/Co, K/[(Ca + Mg)], Na/K and Zn/Pb (Hatcock, 1985; Watts, 2010; ARL, 2012) were calculated.

Mineral safety index (MSI)

The mineral safety index (MSI) (Hatcock, 1985) of Fe, Ca, P, Mg, Zn, Na and Se were calculated using the formula:

MSI = MSIs/RAI × Research data result (8)

where MSI = mineral safety index of Table (standard); RAI = recommended adult intake.

2.6. Statistical analyses

Both descriptive and inferential statistics were used to discuss the analytical results. The descriptive statistics used were mean, standard deviation (SD) and coefficient of variation percent (CV%). For the inferential statiatics, Pearson's moment correlation coefficient (r_{xy}) mode was used (Oloto, 2001). Further to the r_{xy} calculation were the determination of variance (r_{xy}^2), regression coefficient (R_{xy}). Also determined were the coefficient of alienation (C_A) and index of forecasting efficiency (IFE) (Chase, 1976). The level of significance of r_{xy} was determined at a critical level of $r_{= 0.01}$.

$$C_A = \sqrt{1-(r_{xy})^2} \quad (9)$$

$$IFE = (1 - C_A)100 \quad (10)$$

2.7. PubChem CID for mineral elements

Mineral elements studied in this report were: Copper/Cu (PubChem CID: 23978); Iron/Fe (PubChem CID: 23925); Zinc/Zn (PubChem CID: 23994); Magnesium/Mg (PubChem CID: 5462224); Calcium/Ca (PubChem CID: 5460341); Cobalt/Co (PubChem CID: 104730); Manganese/Mn (PubChem CID: 23930); Sodium/Na (PubChem CID: 5360545); Potassium/K (PubChem CID: 5462222); Phosphorus/P (PubChem CID: 5462309); Selenium/Se (PubChem CID: 6326970); Lead/Pb (PubChem CID: 5352425). PbChem is a database of chemical molecules and their activities against biological assay. The system is maintained by the National Centre for Biotechnology Information (NCBI). A component of the National Library of Medicine, which is part of the United States National Institute of Health (NIH). Hence we can talk of PubChem Compound ID (CID) (PubChem and ACS, 2018).

3. Results and discussions

The proximate profiles of the maize samples were shown in Table 1. Total ash of 0.73-1.34 had mean value of 1.10±0.327 g/100g with highest CV% of 29.7 showing the highest disparity of values in the three samples. Ash value reduced from raw maize (B11) down to 0.73 g/100g (B33) shown as follows in g/100g: 1.34 (B11) > 1.24 (B22) > 0.73 (B33). The next highest CV% came from crude fibre (CV% = 18.5) with low levels of fibre having values of (g/100g): 1.07 (B11) ≡ 1.07 (B22) > 0.76 (B33). Dry matter (94.2-95.3 g/100g) with mean of 94.9 ± 0.635, CV% (0.669); organic matter (92.9-94.6g/100g), mean of 93.9 ± 0.874 and CV% (0.931) had the highest concentration values but least variations of 0.669-0.931%. The carbohydrate was also high at 74.4-75.8g/100g, mean of 75.3 ± 0.757g/100g and CV% of 1.01. The protein value was relatively low in each sample with values of 10.9-13.2g/100g, mean of 12.4 ± 1.27 and CV% of 10.3. The crude fat was low at values range of 5.05-5.06g/100g, mean of 5.23 ± 0.309g/100g with CV% of 5.90. Moisture was low and had CV% of 12.2. We recall that the samples underwent these treatments: raw (B11) had no

special treatment, steeped (B33) had the seeds soaked in water for some days with probably high microbial activities likely due to the high enabling environment due to interplay of water, air and enzyme activities. These scenario might have happened in total ash where we observed (g/100g): B11 (1.34) > B22 (1.24) > B33 (0.73) respectively; crude fire: B11 (1.07) ≡ B22 (1.07) > B33 (0.76). However, treatment enhanced dry matter where B11 < B22 ≡ B33, organic matter: B11 < B22 < B33 and protein: B11 < B22 < B33. No definite trend existed in moisture, carbohydrate and crude fat. The reduced moisture content in B22 and B33 was important as it would lead to lower microbial activities and therefore longer shelf life for the grains, it would reduce the bulk of the samples. Protein was enhanced along the line of treatment as B33 > B22 > B11; this might be because microbial activities were more prolonged in B33 (steeped) processing, some chelated protein materials being released in the treatment process which was more in B33 than in B22 and none in B11. The fat content had the trend: B11 (5.06) < B22 (5.59) > B33 (5.05) that is B22 > B11 by a value of 9.48% and B22 > B33 by a value of 9.66%. The high increase in crude fat in B22 showed that some proximate components could have been used up by microorganisms to elevate the fat content level; also, it could have been possible for lipolytic microorganisms to have decomposed some of the fat in the steeped (B33) sample.

Table 1. Proximate composition of the maize samples: raw maize (B11), germinated maize (B22) and steeped maize (B33) at g/100g value on dry weight basis

Parameter	B11	B22	B33	Mean	SD	CV%
Total ash	1.34	1.24	0.73	1.10	0.327	29.7
Moisture	5.79	4.71	4.72	5.07	0.621	12.2
Crude fibre	1.07	1.07	0.76	0.967	0.179	18.5
Carbohydrate	75.8	74.4	75.6	75.3	0.757	1.01
Crude protein	10.9	13.0	13.2	12.4	1.27	10.3
Crude fat	5.06	5.59	5.05	5.23	0.309	5.90
Dry matter	94.2	95.3	95.3	94.9	0.635	0.669
Organic mater	92.9	94.1	94.6	93.9	0.874	0.931

For example, a diversity of moulds such as *Aspergillus* spp., *Penicillium* spp. (Ogundiwin *et al*., 1991; Lefyedi, 2006) and bacteria such as *Pseudomonas aeruginosa* (Ilori *et al*., 1991; Ahmed, 2013) have been identified as microorganisms associated with sorghum grains and malt. This could be similar with maize grains. The lowest level of fibre (0.76g/100g) in B33 could be due to highest level of protein in B33. The carbohydrate trend could not had been out of place as it is the first source of energy for organisms and since the process was raw → steeped → germinated; the diminishing level of carbohydrate would have followed that path.

Maize protein had been classified into five groups based on its solubility in various solvents (Obi, 1991). They are: (i) prolamines (soluble in 70-80% ethanol). Prolamine is mainly Zein that accounts for about 50% of the total protein in the normal maize seed. Zein is deficient in Trp and Lys which are essential amino acids. Zein is located in the endosperm. It has an economic value in that it can be converted into a protein fibre called "vicara". Vicara is used in blends with wool for manufacturing socks, sweaters and swimming suits (Milner, 1954). Number (ii) the globulins (soluble in neutral salt solution, e.g. 5% NaCl); (iii) glutelins (soluble in sodium hydroxide, e.g. 0.2% NaOH); (iv) albumins (soluble in water, e.g. 50ml distilled water/g of defatted endosperm); (v) scleroproteins (insoluble in aqueous solvents), it amounts to about 4.3% of the endosperm protein of normal maize.

Profiled in Table 2 were the differences and the percentage differences in B11 – B22; B11 – B33

and B22 – B33. The highest percentage difference was B11 – B33 (+49.2%) in total ash followed by B11 – B33 ≡ B22–B33≡ 29.0% in crude fibre. The third major difference was in crude protein where B11-B33 = -20.5%; for the signs, positive (+) meant the left hand value was higher than the right hand value and vice versa of the compared pair. On the whole we have this distribution: B22>B11 = 57.1% / 42.9%; B22≡ B33≡ 50.0%; B11> B33 = 62.5% / 37.5% in terms of concentration ratios.

Table 2. Proximate composition differences in the maize samples as B11-B22, B11-B33 and B22-B33

Parameter	B11 – B22(%)	B11 – B33 (%)	B22 – B33 (%)
Total ash	+0.100 (+7.46)	+0.610 (+49.2)	+ 0.510 (+41.1)
Moisture	+ 1.08 (+18.7)	- 0.010(- 0.212)	- 0.010 (-0.212)
Crude fibre	0.00 (-)	+0.310 (+29.0)	+0.310 (+29.0)
Carbohydrate	+1.46 (1.93)	+0.24 (+0.317)	- 1.22 (-1.64)
Crude protein	-2.11 (-19.3)	-2.24(-20.5)	- 0.130 (-0.997)
Crude fat	- 0.530 (-10.5)	+0.010 (+0.198)	+0.540 (+9.66)
Dry matter	-1.08 (-1.15)	+0.010(+0.010)	+0.010 (+0.010)
Organic matter	- 1.18 (-1.27)	-0.500(-0.532)	-0.500(-0.532)
Summary: B22 > B11 = 57.1% / 42.9%; B22 ≡ B33 ≡ 50.0%; B11 > B33 = 62.5% / 37.5% in terms of concentration ratios			

+ = in the two compared values, when sample in the left hand is higher than the right hand, the sign is positive and vice versa

The lipid distribution of the samples were depicted in Table 3. The crude fat was converted to total fatty acid (TFA) by multiplying the crude fat by 0.72. The TFA or g/100g Ep (edible portion) ranged between the 3.64 – 4.02g/100gEp, mean of 3.77± 0.219 and CV% of 5.82%; with TFA distributed as (g/100g EP): B11 ≡ B33 ≡ 3.64 < B22 (4.02). Other lipids without TFA in the samples ranged from 1.41-1.57g/100g, mean of 1.47±0.09g/100g and CV% of 6.11. The other lipids would be made up of sterols, phospholipids, etc. The percentage value for each TFA was 72.0% whereas other lipids percent was 28.0% in each sample. The values of constant percentage of 72 (TFA) and 28 (other lipids) was due to the constant conversion factor of 0.72. The crude energy range was 45.5-50.3 kcal/100g and 187-207 kJ/100g; for g/100gEp (TFA), we have 32.7-32.8 kcal/100g and 135 - 149kJ/100g; whereas other lipids had 12.7-14.1kcal/100g and 52.3-57.9 kJ/100g. The total energy from TFA and other lipids (kcal/100g) was 45.6 (B11) = 45.5 (crude fat); 50.3 (B22) = 50.3 (crude fat); 45.4 (B33) =45.5 (crude fat); each case giving virtually equivalent values. For both kcal and kJ energy percentages, each group recorded similar values: %TFA = 36.0 for all and % other lipids =14.0 for all.

Table 3. Fat (g/100g) distribution of the maize samples.

Parameter	B11	B22	B33	Mean	SD	CV%
Crude fat	5.06	5.59	5.05	5.23	0.309	5.90
Crude fat x 0.72* (Total fatty acid)	3.64	4.02	3.64	3.77	0.219	5.82
Other fats	1.42	1.57	1.41	1.47	0.090	6.11
%TFA	72.0	72.0	72.0	72.0	0.00	0.00
% other fats	28.0	28.0	28.0	28.0	0.00	0.00
Energy (kcal) crude fat (E)	45.5	50.3	45.5	47.1	2.77	5.88
TFA (E)	32.8	36.2	32.7	33.9	1.99	5.88

Other lipids (E)	12.8	14.1	12.7	13.2	0.781	5.92
Total E (kcal)	91.1	101	90.9	94.3	5.77	6.12
% Crude fat (E)	49.9	50.0	50.1	50.0	0.100	0.200
%TFA (E)	36.0	36.0	36.0	36.0	0.00	0.00
% other fats (E)	14.1	14.0	14.0	14.0	0.058	0.411
Energy (kJ) Crude fat (E)	187	207	187	194	11.5	5.96
TFA (E)	135	149	135	140	8.08	5.79
Other lipids (E)	52.4	57.9	52.3	54.2	3.20	5.91
Total E (kJ)	374	414	374	387	23.1	5.96
% crude fat (E)	49.9	50.0	50.0	50.0	0.058	0.116
% TFA (E)	36.1	36.0	36.1	36.1	0.058	0.160
% other lipids	14.0	14.0	14.0	14.0	0.00	0.00

B11= raw maize; B22 = germinated maize; B33= steeped maize; 0.72 = conversion of crude fat to fatty acid

Table 4. Energy density in raw maize (B11), germinated maize (B22) and steeped maize (B33) from carbohydrate, crude Fat and crude protein

Parameter	B11 (%)	B22 (%)	B33 (%)	Mean	SD	CV%
Energy (kcal/100g)in: Protein	43.7 (11.1)	52.2 (13.0)	52.7 (13.2)	49.5	5.06	10.2
Carbohydrate	303 (77.3)	297 (74.4)	302 (75.5)	301	3.21	1.07
Crude fat	45.5 (11.6)	50.3 (12.6)	45.5 (11.4)	47.1	2.77	5.88
Total	392	400	400	397	4.62	1.16
Energy (kJ/100g)in: Protein	186 (11.2)	222 (13.1)	135 (8.40)	181	43.7	24.2
Carbohydrate	1289 (77.6)	1264 (74.7)	1285(80.0)	1279	13.4	1.05
Crude fat	187 (11.3)	207 (12.2)	187 (11.6)	194	11.5	5.96
Total	1662	1693	1607	1654	43.6	2.63
UEDP (%)in: Kcal	6.66	7.80	7.92	7.46	0.695	9.32
kJ	6.72	7.86	5.04	6.54	1.42	21.7

UEDP = utilization of 60% of proportion of total energy due to protein percent

The total energy density for each sample was shown in Table 4. Both crude fat and protein contributed low values into the energy density whereas carbohydrate contributed very high percentage. The total energy density (kcal/100g) in the samples ran thus: B11 (392) with percentage contribution of 11.1% (protein), 11.6% (crude fat) and 77.3% (carbohydrate); in B22, total energy was 400 having distribution of 13.0% (protein), 12.6% (fat) and 74.4% (carbohydrate); and in B33 we have total energy of 400 kcal/100g with protein being 13.2%, fat (11.4%) and carbohydrate (75.5%). For energy in kJ/100g, total energy was B11 (1662), B22 (1693) and B33 (1607) with percentage distribution virtually similar to the observation in kcal/100g. On the whole CV% showed that the energy values were close. CV% range was 1.05-24.2. The total energy values of 1.61-1.69mJ/100g were close to the literature energy ranges of cereals put at 1.61-1.71 MJ/100g (Paul and Southgate, 1978). About 50-60% of somebody's total daily calories should come from carbohydrate.

Carbohydrate contains mostly glucose and gives the quickest form of energy. The body has the capacity to change 100% carbohydrate to glucose.

This is even lower than the recommended safe level of 8% for adult man that requires 55 protein per day with 60% utilization (Femi *et al*., 2015). This is not high enough to prevent energy malnutrition in children and adults that depend solely on maize as the main protein source. It is important to note that *ogi* produced from cereals is inherently deficient in nutrients, especially protein and cannot guarantee an adequate supply of nutrients (Femi *et al*., 2015). Such deficies may result in protein malnutrition among *ogi* consumers particularly the young children who are fed with the product as weaning food. *Ogi* has been reported as contributing to the prevalence of kwashiorkor among infants owing to its high energy density (due to carbohydrate) and reduced proteins (Sengev and Nwobi, 2016). The recommended PEF% from food sources is 30% of the total energy requirement (NACNE, 1983) or the value of 35% (COMA, 1984) for total energy intake. The present PEF% value of 11.4 -12.6 were much lower than the two extreme energy levels. This might be an advantage The utilizable energy due to protein (UEDP%) was low at 5.04 -7.88 (kJ model) and 6.66 – 7.92 (kcal model), assumption of 60% of protein utilization. and useful to people wishing to adopt the guidelines for a healthy diet.

For energy need, the daily energy requirement for infants is 740 kcal (Bingham, 1978). From Table 5, this translated that an infant would have to consume about 189g (raw), 185g (germinated) and 185g (steeped) to satisfy its needs per day. Sample total in kcal/100g was also indicated in the Table 5. As changes occur in dietary, nutritional status and age of an animal, appreciable shifts occur in the tissue compartments water and protein levels (Cowgwill, 1958). For effective utilization and conversation of food within the human body, water is indispensable (Snively Jr., and Wessener, 1954), this is because the water content of the body changes with the types of diet (White House Conferences, 1932). This important connection of water with other food substances is the fact that the biochemical basis for this relationship arises from the fact that the water deficit created by protein metabolism is about seven times that for equivalent calories of carbohydrates or fat.

Table 5. Calculated energy requirements for infants and water required for complete metabolism by the samples

Parameters	B11	B22	B33	Mean±SD	CV%
Daily infant energy requirement (kcal)	740	740	740	740±0.00	0.00
Sample total kcal/100g	392	400	400	397±4.62	1.16
Sample equivalent/gramme	189	185	185	186±2.31	1.24
Protein energy (kcal/100g; X)	43.7	52.2	52.2	49.5±5.06	10.2
Kcal equivalent for water excretion; X× 3^a(= y)	131	157	158	149±15.3	10.3
Water deficit 3.5b × X (= Z)	153	183	184	173±17.6	10.2
Water required for complete metabolism = Z-Y (cm^3)	21.9	26.1	26.4	24.8±2.52	10.1

B11 = raw maize seed; B22 = germinated maize; B33 = steeped maize; [a] = 1calorie of protein requires 3.0ml water for excretion of the urea and sulphate formed from it; [b] = water deficit = 350/100 (3.5); SD = standard deviation; CV = coefficient of variation percent

Therefore, in young children an increase in calories from carbohydrate causes hydration; whereas an increase in calories from proteins causes dehydration (Pratt and Snyderman, 1953). The increased output of ketones and acids that accompanies a shift to high-fat diets is associated

with increased water loss that can be offset by increase in carbohydrate intake. Protein quality as well influences the degree of tissue hydration. Albanese (1959) had estimated grammes of water needed for complete metabolism of 100 calories of some food substances. Food materials (protein, starch and fat) all have pre-formed water of 0.00ml in each case; water gained by oxidation: 10.3 (protein), 13.9 (starch) and 11.9 (fat); lost in dissipating heat: 60.0 for each of the food materials; water lost in excreting end products (1 calorie of protein requires 3.0ml of water for the excretion of the urea and sulphate formed from it, 1g of ash requires 65ml of water for its excretion): 300 (protein), both 0.00 in starch and fat; deficit : 350 (protein), 46 (starch) and 48 (fat). Shown in Table 6, the following protein energy values were shown: B11 (43.7 kcal), B22 (52.2 kcal) and B33 (52.7 kcal) g/100g sample. Column 3 in Table 6 showed the kcal equivalent of water needed for urea and sulphate excretion and column 4 showed the water deficit. To balance for the water deficit, column 5 showed the values to range from 21.9 -26.4 ml. Distribution of values ran thus (ml): B11 (21.9) <B22 (26.1) < B33 (26.4). These water deficit values were low because the protein content of each sample was low.

In Table 6 we have the statistical evaluation of the data from Table 1. The comparisons were B11/B22, B11/B33 and B22/B33. In all the comparisons, r_{xy} was values positively high and significant with values ranging between 0.9996 – 0.9999 with the trend being B11/B22 > B11/B33 >B22/B33. These high r_{xy} levels were followed by similar r_{xy}^2 (variance) levels of 0.9992 – 0.9999. In the R_{xy}, we B11/B22 (0.9922) < B11 / B33 (1.01) ≡ B22 / B33 (1.01).

Table 6. Statistical analysis of the data from Table 1 concerning the proximate composition of raw (B11), germinated (B22) and steeped grains of *Zea mays*

Statistics	Raw/Germinated			Raw / Steeped			Germinated / Steeped		
	B11		B22	B11		B33	B22		B33
r_{xy}		0.9996			0.9997			0.9999	
R_{xy}^2		0.9992			0.9994			0.9999	
Rxy		0.9922			1.01			1.01	
Mean	35.9		36.2	35.9		35.9	36.2		36.2
SD	43.3		43.4	43.3		43.3	43.4		43.8
CV%	121		120	121		121	120		121
C_A		0.0284			0.0241			0.0100	
IFE		0.9716			0.9759			0.9900	
Remark		*			*			*	

r_{xy} = correlation coefficient; r_{xy}^2 = variance; R_{xy} = regression coefficient; C_A = coefficient of alienation; IFE = index of forecasting efficiency; * = Results were significantly different a t n-2 and $r_{=0.01}$ (critical value = 0.834) [NOTE: n-2 = 8-2 = 6df)

The mean values were close at 35.9 $\pm$ 43.3 – 36.2$\pm$ 43.4 g/100g whereas the CV% values were also very close at 120-121. The C_A (coefficient of alienation) was generally low at 0.0100-0.0284 with corresponding high but inverse relationship of 0.9716 – 0.9900. The values of 0.9716 -0.9900 were index of forecasting efficiency (IFE) values. IFE is a reverse of C_A; also, IFE + C_A = 1.0 or 100%. Whereas C_A designates error value in forecasting the relationship between two compared entities, IFE represents the reduction in the error of predicting the relationship between two compared entities. When C_A <IFE, prediction of relationship is easy but vice versa when the C_A >IFE. From Table – 7, reduction in error of prediction of relationship ranged between 97.3 – 99.0. Since IFE > C_A in each case, each member of a pair would be able to carry out the metabolic functions of the other pair member and vice versa.

In Table 7, we have the display of the mineral profiles of the samples. The least concentrated mineral was Pb which had similar value of <0.001 mg/100g in each of the samples. Minerals of high concentration were K (550 – 661mg/100g). P (369-401mg/100g) and Mg (220-235mg/100g) with B22 predominating in each of the samples. Moderate mineral values were observed in Na (62.2 – 81.9mg/100g) and Ca (13.3 – 20.9mg/100g); low levels were in Fe (4.27 5.59mg/100g and Zn (4.24 – 6.60mg/100g). Very low mineral levels were in Cu, Co, Mn and Se. Enhanced minerals in B22 greater than B11 and B33 were Fe, Cu, Co, Mn, Zn, Mg, K, P and Se whereas similar minerals in B33 were Ca and Na. The CV% values were generally low with highest CV% being 25.8 in Mn and CV% of 0.00 in Pb (being the lowest CV%). The total sample loads of the samples were (mg/100g): B11 (1227) < B22 (1403) > B33 (1313). The percentage values of each mineral in each sample were indicated in the Table 8.

Table 7. Mineral profiles (mg/100g) of raw (B11) germinated (B22) and steeped (B33) grains of *Zea mays*

Mineral	**B11 (%)**	**B22 (%)**	**B33 (%)**	**Mean**	**SD**	**CV%**
Fe	4.42 (0.361)	5.59 (0.398)	4.27(0.325)	4.76	0.723	15.2
Cu	0.543 (0.044)	0.811 (0.0580	0.513(0.040)	0.628	0.158	25.2
Co	0.008 (0.001)	0.012 (0.001)	0.009 (0.001)	0.010	0.002	21.5
Mn	0.864 (0.070)	1.28 (0.092)	0.817 (0.062)	0.987	0.255	25.8
Zn	4.24 (0.345)	6.60(0.471)	5.68(0.432)	5.51	1.19	21.6
Pb	<0.001 (0.0001)	<0.001 (0.0001)	<0.001 (0.0001)	0.001	0.00	0.00
Ca	13.3 (1.09)	16.5(1.17)	20.9 (1.59)	16.9	3.82	22.6
Mg	220 (17.9)	235 (16.8)	230 (17.5)	228	7.64	3.34
K	550 (44.8)	661 (47.1)	600 (45.7)	604	55.6	9.21
Na	62.2 (5.07)	74.8 (5.34)	81.9 (6.23)	73.0	9.98	13.7
P	371 (30.2)	401 (28.6)	369 (28.1)	380	17.9	4.71
Se	0.028 (0.002)	0.036 (0.003)	0.032 (0.002)	0.032	0.004	12.5
Total	1227	1403	1313	1314	88.0	6.70
Ratio	B11/B22 = 0.875:1.00; B11/B33 = 0.935:1.00; B22/B33 = 1.07:1.00					
Superiority of B22 over B11 and B33 B22 > B11; 12/12 = 100%; B22 > B33; 10/12 = 83.3%; B33> B11; 8/12 = 66.7%. i.e. B22 > B33 > B11						

Minerals of significant percentage levels were: Mg (16.8 – 17.9%), K (44.8-47.1%) and P (28.1-30.2%). Values of percentage levels greater than 1.0 were observed in Na (5.07-6.23%) and Ca (1.09 – 1.59%) whilst all others were less than 1.00% each. The superiority (in concentration) of B22 over B11 and B33 ran thus: B22 > B11, 12/12 = 100%; B22>B33, 10/12 = 83.3%; B33>B11, 8/12 = 66.7%; that is B22 > B33 > B11.

The improvement status of the minerals was qualitatively displayed in Table 8. These minerals were under "definite improvement (++)" in B22: Fe, Cu, Co, Mn and K whereas it was only Na in B33 since they were considerably improved in their corresponding samples. Minerals classified under

"usually some improvement (+)" cut across the samples (B11, B22, B33) and no improvement (-) was observed only in Pb for the samples.

Table 8. Improvement status of the minerals of maize grains during sprouting and fermentation

Mineral	Raw grains	Germinated grains	Steeped grains
Fe	+	++	+
Cu	+	++	+
Co	+	++	+
Mn	+	++	+
Zn	+	+	+
Pb	-	-	-
Ca	+	+	++
Mg	+	+	+
K	+	++	+
Na	+	+	++
P	+	+	+
Se	+	+	+
Total	+	+	+

++ = definite improvement; + = usually some improvement; - = no improvement

Minerals are necessary for life. Mn has always been low in the Nigerian food sources. Examples: in eight organs of African giant pouch rat (*Cricetomys gambianus*) (Adeyeye and Adesina, 2018), Mn was not detected in them but recorded 1.86mg/100g (muscle) and 0.01mg/100g (skin) in the same animal; it was 1.9 $\pm$ 0.04mg/kg (meat pie), 1.0 $\pm$ 0.00mg/kg (doughnut), 2.9 $\pm$ 0.01 mg/kg *(moin moin*) and 2.80 $\pm$ 0.01mg/kg (cake) (Adeyeye *et al*., 2012). Both Co and Cu are minor but essential minerals which were also low in the samples under discussion. Fe was at moderate level in the samples ranging from 4.27 – 5.59mg/100g; these values were low to the needed Fe in human metabolism. Usually about 1-10% of Fe from plant sources is usually absorbed by the body although this value can be improved upon when plants are consumed with meat or other animal Fe source (Adeyeye *et al*., 2012). Minimum Zn allowance (about 15-20 mg/day) could not be met by any of the samples. Zinc is a major constituent of the body tissues and it is a component of more than 50 enzymes (Adeyeye *et al*., 2012). Calcium is an important constituent of body fluids being a coordinator of inorganic elements particularly K, Mg or Na where Ca is capable of assuming a corrective role when such metals are in excessive amount in the body (Fleck, 1976). Ca, P and vitamin D combine together to avoid rickets in children and osteoporosis (bone thinning) among older people (Adeyeye *et al*., 2012). A dietary regime of adequate dietary Ca over the years would be a deterrent to this condition. Phosphorus has always been found with Ca in the body, both contributing to the supportive structures of the body. Phosphorus exists in cells and in blood as soluble phosphate ion, as well as in lipids, proteins, carbohydrate and energy transfer enzymes (Adeyeye *et al*., 2012). Mg was the third highest concentrated mineral in the samples; it is an activator of many enzyme systems and also maintains the electrical potential in nerves. Potassium is primarily an intercellular cation in large part being bound to protein and together with Na influences osmotic pressure and contributes to normal pH equilibrium (Adeyeye *et al*., 2012).

In Table 9, differences in the mineral profiles of maize samples between B11/B22, B11/B33 and B22/B33 were shown, and accompanied by the percentage differences. Percentage differences of 50 and >50 were observed in Co (-50.0, B11 –B22)

and Zn (-55.9, B11 – B22), all being positive towards B22; in B11-B33, Ca and value of -56.7% (being positive towards B33) and none in that range for B22-B33. The low level of differences showed the low differences between the compared samples.

Table 9. Differences in the mineral profiles of *Zea mays* between raw/germinated (B11-B22), raw/fermented (B11-B33) and germinated / fermented (B22-B33) grains

Mineral	B11 – B22 (%)	B11 – B33 (%)	B22 – B33 (%)
Fe	-1.16 (-26.2)	+0.150 (+3.39)	+1.31 (+23.5)
Cu	-0.268 (-49.4)	+0.012 (+2.21)	+0.280 (+34.5)
Co	-0.004 (-50.0)	-0.001 (-12.5)	+0.003 (+25.0)
Mn	-0.419 (-48.5)	+0.047 (+5.42)	+0.466 (+36.3)
Zn	-2.37 (-55.9)	-1.44 (-34.1)	+0.924 (+14.0)
Pb	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ca	-3.13 (-23.5)	-7.55 (-56.7)	-4.42 (-26.8)
Mg	-15.2 (-6.89)	-10.0 (-4.59)	+5.07 (+2.15)
K	-110 (-20.0)	-50.0(-9.09)	+60.2 (+9.11)
Na	-12.7 (-20.4)	-19.7 (-31.7)	-7.05 (-9.42)
P	-30.5 (-8.33)	+1.92 (+0.519)	+32.4 (+8.08)
Se	-0.008 (-28.6)	-0.004 (-14.3)	+0.004 (+11.1)
Total	-176 (-14.3)	-86.7 (-7.07)	+89.2 (+6.36)

+ = in the two compared values, when sample in the left is higher than the right hand, the sign is positive and vice versa

A study by Zhang et al. (Zhang *et al*., 2015) on buckwheat showed that phytic acid in buckwheat decreased with increase in the germination time due to activation of phytase which hydrolyses phytic acid into phosphoric acid and myinositol thereby making minerals more bioavailable (Liang *et al*., 2008; Mbithi *et al*., 2000). Mineral availability had been said to be grain specific with highest availability for Fe in wheat, Zn in rice and wheat, Mn in rice and soybean and Ca in soybean, rice and faba beans (Luo *et al*., 2015). The difference in mineral availability from different cereals and also legumes after germination for similar period may be related to differences in phytate content, phytase activation, extent of binding of minerals within the matrix, or interaction of these factors. Malting of sorghum, foxtail and chickpea significantly increased the content of Na, K, P, Ca and Mg (Desal *et al*., 2010; Idris *et al*., 2007; Laxmi *et al*., 2015) but decreased Ca and Fe (Desal *et al*., 2010; Laxmi *et al*., 2015; Ogbonna *et al*., 2012). This difference could be accounted for by different processing methods such as steeping times and freeing of bound minerals during malting (Onyango *et al*., 2013).

Table 10 contained the various computed mineral ratios which were: Ca/Mg, Ca/K, Zn/Cu, Ca/P, Fe/Cu, Ca/Pb, Fe/Pb, Fe/Co, Na/Mg, K/Co, Na/K, Zn/Pb and K [(Ca+Mg)]. Mineral ratios normally reveal the important balance between the elements, provision of information regarding the many factors they may be represented by a disruption of their relationships, such as disease states, physiological and developmental factors, the effects of diet and drugs (Watts, 2010).

Table 10. Mineral ratios of the minerals analyzed for in the variously treated maize samples

Appropriate minerals	Mineral ratio	(Reference balance ideal)	Acceptable ideal range	B11	B22	B33
Fe	Ca/Mg	7.00	3 to 11	0.061	0.070	0.091
Cu	Ca/K	4.20	2.2 to 6.2	0.024	0.025	0.035
Co	Zn/Cu	8.00	4 to12	7.80	8.14	10.7
Mn	Ca/P	2.60	1.5 to 3.6	0.036	0.041	0.057
Zn	Fe/Cu	0.90	0.2 to 1.6	8.15	6.89	8.05
Pb	Ca/Pb	84.0	126 to 168	13318	16451	20866
Ca	Fe/Pb	4.40	6.6 to 8.8	4424	5585	4274
Mg	Fe/Co	440	-[a]	553	465	475
K	Na/Mg	4.00	2 to 6	0.282	0.318	0.356
Na	K/Co	2000	-[a]	68807	55054	66718
P	K/[(Ca + Mg)]	2.2	- [a]	4.72	5.25	4.78
	Na/K	2.40	1.4 to 3.4	0.113	0.113	0.136
	Zn/Pb	-[a]	-[a]	4235	6603	5679

-[a] = not available

Of all the ratio values, only Zn/Cu (7.80-10.7) fell within the acceptable ideal range of 4-12 whereas all other ratios fell much below the acceptable ideal range. The advantages / disadvantages of such lower than ideal acceptable range had been variously discussed (Watts, 2010; Adeyeye *et al*., 2017; Nieman *et al*., 1992; Adeyeye *et al*., 2012; National Research Council, 1989).

Table 11. Statistical analysis of the data from Table 8 concerning the mineral profiles of raw (B11), germinated (B22) and fermented grains of *Zea mays*

Statistics	Raw Versus Germinated			Raw Versus Fermented			Germinated Versus Fermented		
	B11		B22	B11		B33	B22		B33
r_{xy}		0.9983			0.9986			0.9995	
r_{xy}^2		0.9966			0.9972			0.9991	
R_{xy}		1.16			1.06			0.9132	
Mean	102		117	102		109	117		109
SD	183		212	183		194	212		194
CV%	179		181	179		177	181		177
C_A		0.0586			0.0532			0.0303	
IFE		0.9414			0.9468			0.9697	
Remark		Significant			Significant			Significant	

r_{xy} = correlation coefficient; r_{xy}^2 = variance; R_{xy} = regression coefficient; SD = standard deviation; CV% = coefficient of variation percent; C_A = coefficient of alienation; IFE = index of forecasting efficiency; r_{xy} = significant at n-2 and $r_{0.01}$ (critical value = 0.708) [NOTE: n-2 = 12-2 =10 df]

The statistical analysis results of the data from Table 7 had been profiled in Table 11. All the r_{xy} values were positively high and significant at $r_{=0.01}$; this was followed by high values of r_{xy}^2. R_{xy} were high at 0.9132 – 1.16. Both mean, SD and CV% were higher than observed in the proximate statistical results. The C_A values were higher than reported for the proximate values but still lower than their corresponding IFE levels in the minerals; this made comparison between B11/B22, B11/B33 and B22/B33 easy since all C_A < all IFE.

Figure 1 had all the mineral safety index (MSI) values of Fe, Ca, P, Mg, Zn, Cu, Se and Na in the maize samples. Levels of MSI within the 10th unit range were in Mg (8.26-8.82), Cu (5.84 – 8.92), Se (5.60 – 7-20), P (3.07 – 3.34) and Fe (1.91 – 2.49), whereas only Zn was in the 20th unit range (9.32 – 14.5); other MSI for Ca and Na had values less than 1.00. Highest MSI values were observed in B22 for Fe, P, Mg, Zn, Cu and Se.

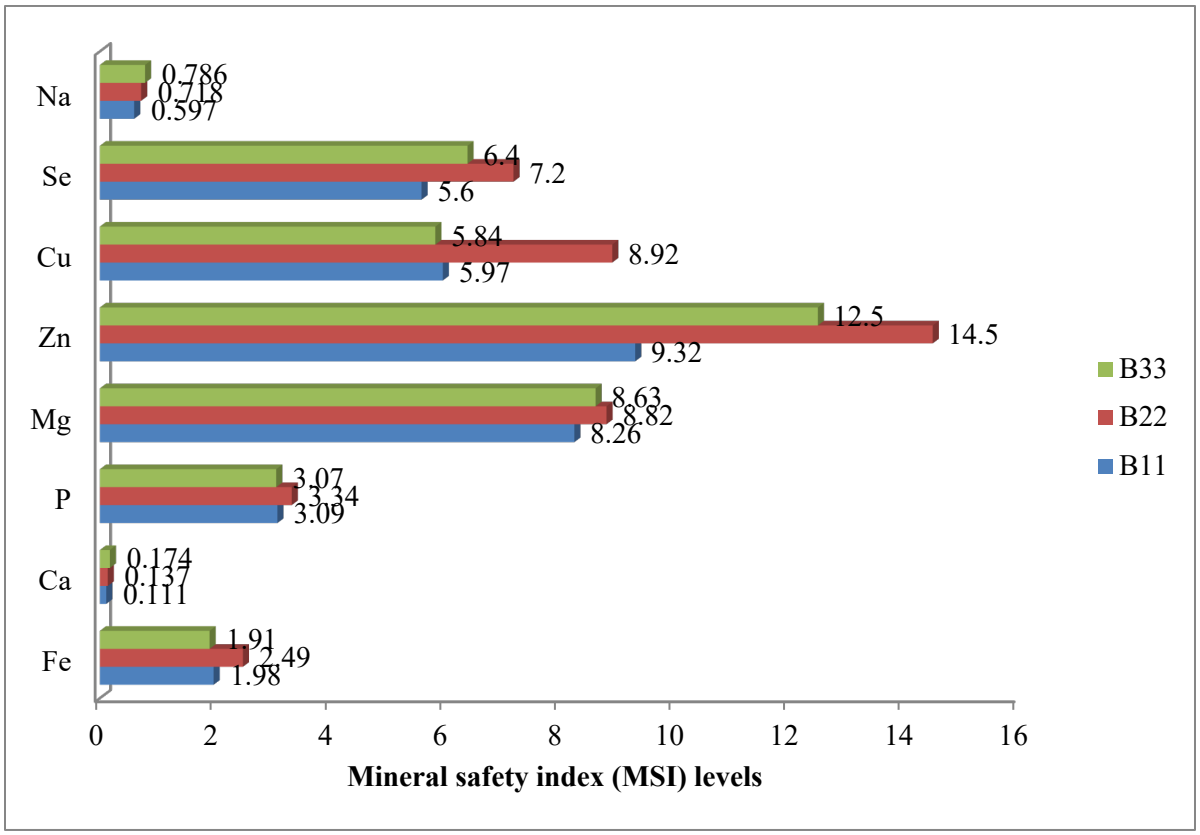

Figure 1. Mineral safety index (MSI) of Fe, Ca, P, Mg, Zn, Cu, Se and Na of *Zea mays* grains

In Figure 2, the MSI differences between the standard MSI values and the sample calculated MSI values from *Zea mays* samples were profiled. All the calculated MSI values were less than the standard MSI values. The implication of this would be that none of the minerals would have any deleterious effect on any of the sample consumers. The percentage differences showed that the following trends could be observed: Ca (98.3 – 98.9%), Na (83.6 – 87.6%), Cu (73.0 – 82.3%), Fe (62.8 – 71.5%), Zn (56.1 – 71.8%) whereas others were less than 70.0%.

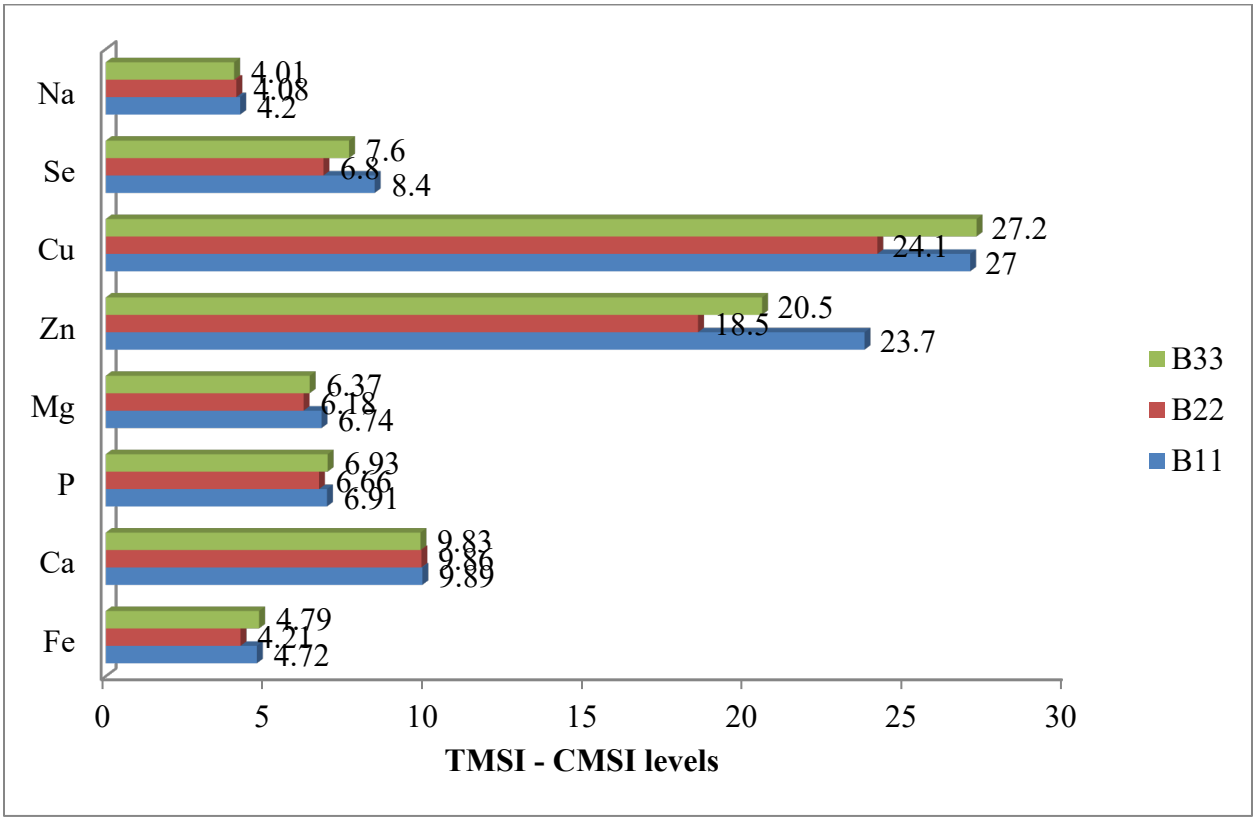

Figure 2. Mineral safety index (MSI) differences between the Table MSI (TMSI) values and sample calculated MSI (CMSI) values from *Zea mays* samples

4. Conclusions

The three differently processed samples of *Zea mays* L.Dk 818 grains exhibited high and positive significant differences among their compared groups both in the proximate and mineral results. The variations between parameters (both in proximate and minerals) in each sample were low as seen in the CV%. There was evidence of likely microbial influence in both the steeped and germinated samples both in proximate and minerals. All sample pairs: raw (B11)/ germinated (B22), raw (B11) / steeped (B33) and germinated (B22) / steeped (B33) had low C_A but corresponding high IFE showing that biochemical vice versa functions can occur in B11/B22, B11/B33 and B22/B33 both in proximate and mineral levels. To show the better improvement of germination over raw maize, we have B22> B11 = 57.1% / 42.9%; B22 over B33, we had B2≡ B33 ≡ 50.0%/50.0%; but B11 > B33 = 62.5% / 37.5% in terms of concentration ratios in the proximate composition. For mineral composition, we had a reverse observation; B22 > B11, 12/12 = 100%; B22 > B33, 10/12 = 83.3%; B33 > B11, 8/12 = 66.7%; i.e. B22 > B33 > B11.

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PRODUCTION OF PAPAYA FLAVORED ICE-CREAM WITH PUREE, AND SPRAY-DRIED PAPAYA POWDER

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ABSTRACT

Ice-cream is one of the most consumed dairy products. However, the commercial ice-cream product is low in natural antioxidants, dietary fibers, and minerals. Therefore, highly nutritious papaya can be incorporated into ice-cream. This study aims to produce ice-cream using papaya puree (20-80% w/v) and spray-dried papaya powder (20-80% w/v). The sensory properties (QDA and nine points hedonic scale), and physicochemical and proximate analysis of the ice-cream were performed. From sensory evaluation, ice-cream formulated with 20% (v/v) papaya puree gained the highest rate on overall acceptability and was most preferred among the formulated papaya ice-creams. Meanwhile, ice-cream formulated with 20% (v/v) papaya puree contains 23.53 ± 2.33% moisture, 2.30 ± 0.10% protein, 0.88 ± 0.06% ash, and 3.04 ± 0.81% fat. Therefore, ice-cream formulated with 20% (v/v) papaya puree might be suitable and more accepted by the consumers and may have a potential marketable value.

1.Introduction

Incorporation of fruits, dietary fibers, natural antioxidants into ice-cream to improve nutritional attributes has been one of the interests in recent years (Erkaya *et al.*, 2012). Based on a study conducted by Santana *et al.* (2003), the addition of papaya pulp into ice-cream showed higher averaging corresponding to the terms of the hedonic scale "liked moderately" for all attributes. The study showed that papaya ice-cream was considered as a nutritious dessert and also an excellent alternative to the use of fruit, thus opening new opportunities for a manufactured product of papaya.

However, in Malaysia, papaya ice-cream is not available in the market, although the production quantity of papaya was 50519 tonnes in the year 2018 (FAOSTAT, 2020). Papaya is commonly known for its nutritional values and is rich in antioxidants, vitamins, minerals, and fibers (Krishna *et al.*, 2008). Application of enzyme in juice is common, where it serves to increase extraction yield before processing. The juice yielded will have better color and aroma (Bhat, 2000). Conversion of fruit into powder form can reduce the increased product stability, reduce wastage, and cost from transportation and storage (Chew *et al.*, 2019). Spray drying is usually applied to produce fruit juice powder (Phisut, 2012). The spray-drying method was applied in the production of 'Terung Asam,' papaya, kuini mango, 'cempedak', 'Bintangor' orange, kedondong and pineapple powder (Chang *et al.*, 2020a; Chang *et al.*, 2020b; Loo and Pui, 2020; Pui *et al.*, 2020; Yu and Pui, 2021; Chang *et al.,* 2021; Wong *et al.*, 2015). The powder produced can serve as a functional ingredient or incorporated into food as a colorant or natural flavorings.

Hence, this study aims to incorporate papaya puree and papaya powder into ice-cream. The work includes the production of papaya puree using enzyme treatment and the production of papaya powder using a spray-dryer. Papaya ice-cream that is made of spray-dried papaya powder and papaya puree will be tested using sensory evaluation techniques, including Quantitative Descriptive Analysis (QDA) and Hedonic Test.

2. Materials and methods

2.1. Materials

Fresh "Sekaki" papaya was purchased from local fruits stall in Serdang, Selangor, Malaysia. The ingredients used to make ice-cream include whole milk powder (Brand: Nestlé), whipping cream (Brand: Emborg), egg yolk (Brand: Nutriplus), caster sugar, and salt.

2.2. Preparation of papaya puree and powder

Ripe papaya fruit was rinsed to remove dirt on its surface. The skin of papaya was peeled using a peeler, and the seed was removed using a spoon. The pulp was sliced and cut into smaller pieces with dimensions approximately 5 cm x 4 cm x 3 cm. The cut fruit pulps were subjected to a blender (Warring, USA) and blended at speed 3 for 1 minute until no observable solid form. Then, 200 g of blended papaya was poured into a 250 mL beaker and weighed using an electronic weighing balance (Mettler Toledo, Switzerland).

For the production of papaya puree, 2% (v/w) of enzyme pectinase, Pectinex® Ultra SP-L (Novozymes, Denmark) was added into 200 g of the blended papaya pulp. The mixture was placed into a small water bath at 50 °C and incubated for two hours with shaking at speed 4.5. It was then placed in a hot water bath (Memmert, Germany) at 90 °C for five minutes, followed by ice water for five minutes (Wong et al. 2015).

On the other hand, to produce spray-dried papaya powder, the papaya puree was added with 100 g of maltodextrin (Bronson and Jacobs, Australia) diluted in 500 g of warm filtered water. The concentration of maltodextrin added was 20% (w/v) of water weight, and the ratio of water to puree was 1:1 (Pui et al., 2020). The inlet temperature of the spray-dryer (Büchi, Switzerland) was preset at 160 °C. Compressed air was set to a level of 4 cm with gas spray flow at 600 L/h and a feed flow rate of 6 mL/min. The papaya powder was then collected in the product collection vessel and vacuum packed.

2.3. Preparation of papaya ice-cream

Table 1 shows the respective formulations with the number of ingredients used expressed in the form of a percentage. The mixture was stirred until fully solubilized and then pasteurized in a water bath at 74 °C for 10 minutes with shaking at speed 3. After pasteurization, the mixture was allowed to cool for 10 minutes.

Subsequently, the pasteurized mix was homogenized (Ika, Germany) at speed 13.8min×1000 for 5 minutes, followed by 3.4/min×1000 for 5 minutes. The mix was then cooled and undergone aging in a refrigerator at 4°C overnight. After that, the mix was removed from the refrigerator and poured into an ice-cream maker (Kenwood, Malaysia). Within 15 minutes, the ice-cream was set, and the ice-cream was removed from the ice-cream maker using a plastic spatula into a plastic container and stored in the freezer (Ardo, Belgium) at -18°C.

2.4. Properties of papaya puree and reconstituted powder

2.4.1 Total Soluble Solid (TSS) and pH

The TSS of papaya puree and reconstituted powder were evaluated using a portable Hand Refractometer (B+S, UK) with a scale of 0-32 °Brix (Wong *et al.*, 2015). The pH value of papaya puree and reconstituted powder were evaluated with pH meter (Mettler Toledo, Switzerland).

2.4.2 Color Determination

The color of papaya puree and reconstituted powders were evaluated by a colorimeter (ColorFlez EZ, Hunter Associates Laboratory Inc, USA). Results were expressed in L* (lightness), a* (redness), and b* (yellowness) values (Chang *et al.*, 2020b).

Table 1. Formulations of papaya ice-cream

Formulations	Ingredients in percentage (%) (w/w)							
	Whole milk powder	Sugar	Salt	Egg yolk	Whipping cream	Water	Papaya powder	Papaya puree
F0	7.8	5.9	0.2	7.1	20.2	58.8	-	-
F1	7.8	5.9	0.2	7.1	20.2	58.8	20	-
F2	7.8	5.9	0.2	7.1	20.2	58.8	50	-
F3	7.8	5.9	0.2	7.1	20.2	58.8	80	-
F4	7.8	5.9	0.2	7.1	20.2	58.8	-	20
F5	7.8	5.9	0.2	7.1	20.2	58.8	-	50
F6	7.8	5.9	0.2	7.1	20.2	58.8	-	80

F0 (Control) = ice-cream without addition neither papaya puree nor papaya powder; F1 = ice-cream added with 20% (w/v) of papaya powder; F2 = ice-cream added with 50% (w/v) of papaya powder; F3 = ice-cream added with 80% (w/v) of papaya powder; F4 = ice-cream added with 20% (v/v) of papaya puree; F5 = ice-cream added with 50% (v/v) of papaya puree; F6 = ice-cream added with 80% (v/v) of papaya puree

2.5. Sensory evaluation of papaya ice-cream

8 trained panelists evaluated the ice-cream with Quantitative Descriptive Analysis (QDA). A total of seven samples, including control (ice-cream without papaya puree or powder), were given to panelists one at a time. Three formulations with the best result were used to conduct a hedonic test. The hedonic test was carried out with 100 untrained panelists from UCSI University. A total of four samples, including one commercial ice-cream and three best-formulated ice-creams based on QDA, were used in the testing. The ice cream's appearance, aroma, flavor, texture, and overall acceptability evaluation were evaluated. Participants are encouraged asked to rank their preferences on a nine-point hedonic scale and willingness to purchase the samples to examine the market potential.

2.6. Properties of selected papaya ice-cream

2.6.1. Total Solid Contents

The total solid contents of ice-cream were determined using the AOAC official method 941.08. The ice-cream test portion (2 g) was weighed into a round, flat-bottom porcelain dish with a diameter bigger than 5 cm. It was heated on a steam bath for 3.5 hours at 100 °C, cooled in a desiccator, and weighed. Repeat drying, cooling, and weighing samples until a constant weight was obtained. The percentage of total solid content in papaya ice-cream was expressed as in Equation 1.

$$\text{Total solid content, \%} = (A - B)/C \times 100 \quad (1)$$

Where:

A = Total weight of porcelain dish, lid, and sample after drying in gram

B = Weight of porcelain dish, and lid in gram

C = Initial weight of sample in gram

2.6.2. Titratable Acidity

The titratable acidity of papaya ice-cream was evaluated using the method described by Pui *et al.* (2018). Ice-cream (9 g) was melted to room temperature and mixed thoroughly in a 100 mL beaker. Then, 18 g of distilled water was weighed and mixed with the melted ice-cream sample. A volume of 0.5 mL of phenolphthalein indicator was added into the beaker followed by titration with 0.1 N sodium hydroxide until a pink color was shown. The titratable acidity of ice-cream was calculated.

2.6.3. Meltdown Test

The meltdown test was performed, according to Goh *et al.* (2006). Ice-cream samples were stored at -18°C overnight before the meltdown test. A hardened ice-cream sample in block shape weight approximately 158 ± 5 g (11 cm x 8 cm x 2 cm) was

placed on a metric text sieve suspended over a weighing balance. They were allowed to stand at ambient temperature (20°C ± 2°C). The dripped ice-cream was collected and weighed at every 5 minutes up to 60 minutes. Pictures of the ice-cream were taken every 20 minutes to see the effect on the rate of melting on the deformation of shape (Muse and Hartel, 2004). The time (min) was plotted against the weight of dripped ice-cream (g), and the slope is representing the melting rate.

2.7. Proximate analysis

The moisture content, protein, ash, and fat) of ice-cream added with 20% (v/v) of papaya puree, and control (ice-cream without addition neither papaya puree nor papaya powder) were analyzed according to the method stated in AOAC (2000).

2.8. Statistical Analysis

The analyses were conducted in triplicate. Results obtained from QDA, Hedonic test, and physicochemical analysis were analyzed by One-way ANOVA from statistical analyzing software, SPSS (Statistical Package for the Social Sciences 20). Post-Hoc tests were performed, significantly different at $p \leq 0.05$. Meanwhile, results obtained from the proximate analysis were analyzed with a T-test.

3.Results and discussions

3.1. Properties of papaya puree and reconstituted papaya powder

The reconstitution of spray-dried powder was evaluated to determine the potential of the juice powder in resembling the original juice as close as possible upon reconstitution (Simha *et al.*, 2012). Table 2 shows the properties of papaya puree and reconstituted papaya powder. In terms of pH value, there was no significant difference ($p > 0.05$) between papaya puree and reconstituted papaya powder. This may be explained by no further release or reduction of the carboxylic acids and galacturonic acid content upon spray-drying of papaya puree (Akesowan and Choonhahirun, 2013).

Based on Table 2, reconstituted papaya powder was (14.94 ± 1.09) lighter while showing a decrease in redness and yellowness in as compared to papaya puree (Table 4). This may result from pigment degradation, especially carotenoids, during spray-drying (Fratianni *et al.*, 2010).

3.2. Sensory evaluation of papaya ice-cream

The mean intensity ratings of papaya ice-cream's appearance, flavor, texture, and aroma (from QDA) were exhibited in Figure 1. According to the trained panelists, color is defined as the intensity of it from white to light yellow, followed by orange under white light. The intensity for the color attribute of ice-cream formulated with papaya puree and papaya powder ranged from 8.60 cm to 12.19 cm, with F2 (ice-cream formulated with 50% (w/v) papaya powder) having the highest intensity.

Trained panelists define glassiness as the number of sharp abrasive pieces in ice-cream. Ice-cream formulated with 80% (w/v) papaya powder (F3) showed to have the least intensity in glassy attributes, among the ice-cream, which has a glassy attribute from 1.48 cm to 8.20 cm. This may be due to its high mix viscosity. The addition of papaya powder increases the viscosity of the ice-cream mix and reduces the amount of free water to be frozen (Rincón *et al.*, 2006).

Separation is defined by trained panelists, as the cream appears on the top layer of ice-cream. With the separation attribute ranging from 2.74 cm to 4.98 cm, with both ice-cream formulated with 50% (v/v) papaya puree (F5) and 80% papaya puree (F6) showed to have the lowest degree of separation. This may be explained by the homogenization processing steps that cause a reduction in particle size and aggregation of fat globules (Sun-Waterhouse *et al.*, 2013).

Sweetness is defined as the fundamental taste sensation contributed by either sugar or sweeteners (Dooley *et al.*, 2010). Ice-cream formulated with papaya powder was found to have a higher intensity of sweetness than ice-cream formulated with papaya puree. This may be explained by the addition of papaya powder that increases the solid content in ice-cream, attributing to maltodextrin added (Abdallah *et al.*, 2017).

The sweetness of ice-cream formulated with 20% (w/v) of papaya powder (F2) showed to have

the highest intensity. Based on the work done by Santana *et al.* (2003), papaya ice-cream found to have high sugar content indicated by total soluble solid (TSS) contents at above 31°Brix. There was no increasing trend of ice-cream samples for sweetness attributes. The reason could be due to the trained panelists' difficulty to distinguish between varying sugars concentrations at high overall sweetness level (Bolenz *et al.*, 2006).

Both ice-creams formulated with 50% (v/v) papaya puree (F5) and 80% papaya puree (F6) showed to have the highest intensity in the scoopability attribute. The force required to cut the sample with a spoon was known as scoopability (Dooley *et al.*, 2010). It was influenced by the microstructure of ice crystal (Wildmoser *et al.*, 2004). A smaller size of ice crystal can improve the scoopability of ice-cream.

Ice-cream mix formulated with papaya powder freeze faster than the ice-cream mix formulated with papaya puree because it has lower water content and lower freezing point (Li and Sun, 2001). Therefore, ice-cream formulated with papaya powder had a smaller size of ice crystal.

Table 2. Properties of papaya puree and reconstituted powder

Analysis	Papaya Puree	Reconstituted Papaya Powder
TSS (°Brix)	10.23 ± 0.80^{a}	10.17 ± 0.07^{a}
pH	4.42 ± 0.19^{a}	4.32 ± 0.32^{a}
Color (L*)	31.68 ± 1.72^{a}	14.94 ± 1.09^{b}
Color (a*)	27.95 ± 2.71^{a}	0.77 ± 0.76^{b}
Color (b*)	41.27 ± 1.71^{a}	14.19 ± 2.28^{b}

Data on TSS, pH, and color of reconstituted papaya powder are means ± standard deviations where n = 3. Different letters in the same row indicate a significant difference at $p \leq 0.05$. Abbreviations: TSS = total soluble solids, L* = degree of lightness, and darkness, a* = degree of redness or greenness, and b* = degree of yellowness or blueness.

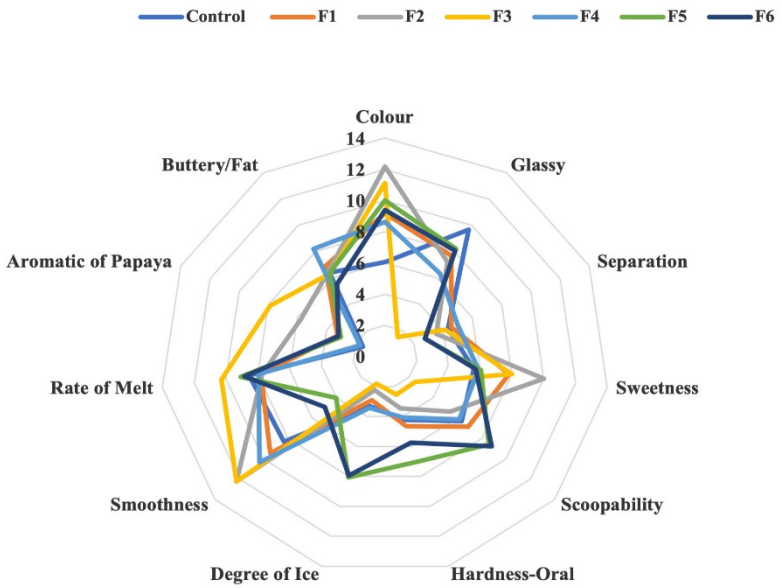

Figure 1. Spider diagram of the mean intensity ratings for the sensory attributes of the formulated papaya ice-cream samples

Other than that, ice-cream formulated with 50% (v/v) papaya puree (F4) has the highest intensity in the hardness-oral attribute. This means that more force is needed to compress the ice-cream between the tongue and mouth roof (Dooley *et al.*, 2010). Ice-cream formulated with papaya puree had higher intensity for hardness-oral attribute than ice-cream formulated with papaya powder. The water content of puree is more. Hence when frozen, the ice crystals network is more compact, hence harder (Wildmoser *et al.*, 2004).

The intensity of the degree of ice was lowest in ice-cream formulated with 80% (w/v) papaya powder (F6). The degree of ice is defined as the number of ice crystals felt in the mouth when the ice-cream is chewed (Dooley *et al.*, 2010). Ice-cream formulated with papaya puree freeze slower will lead to the formation of a larger size of ice crystal. Hence, the degree of ice was higher in the ice-creams formulated with papaya puree than ice-creams formulated with papaya powder.

Both ice-creams formulated with 50% (w/v) papaya powder (F3) and 80% (w/v) papaya powder (F4) showed to have high intensity in smoothness attributes. According to Li and Sun (2001), the fine ice crystal present in ice-cream is crucial in maintaining its smooth and creamy texture. Ice-creams formulated with papaya powder were smoother than ice-cream formulated with papaya puree.

On the other hand, ice-cream formulated 50% (w/v) papaya puree (F2) has the least intensity in the rate of melt attribute. The rate of the melt is defined as the rate in which the ice-cream change forms from solid to liquid state (Dooley *et al.*, 2010). Ice-cream formulated with 80% (w/v) papaya powder (F6) melts fastest among the ice-cream samples. The melting rate is influenced by the difference in air cell and size of ice crystal (Wildmoser *et al.*, 2004; Temiz and Yeşilsu, 2010). The addition of papaya powder potentially increases the sugars and organic acids contents, leading to an impact on the depression of freezing point, which causes the increase in unfrozen liquid in ice-cream, causing it to melt faster.

For aromatic of papaya, ice-cream formulated with 80% (w/v) papaya powder (F3) has the highest intensity, where it was found to have pleasant, sweet, and mellow flavor (Fuggate *et al.*, 2010). The addition of a high concentration of papaya powder increases the soluble solids of papaya in the ice-cream (Abdallah *et al.*, 2017). Ice-creams formulated with papaya puree have lower intensity than ice-creams formulated with papaya powder. This may due to the ice-cream mix is diluted by a high amount of free water despite its high amount of solid contents in papaya puree.

Ice-cream formulated with 20% (v/v) papaya puree (F1) was found to have a higher intensity of buttery/fat than other ice cream samples. From the different attributes, the three best-formulated ice-creams (F2, F3, and F4) were chosen for further investigation in the Hedonic test for the determination of consumer affectiveness. They appeared to have a smoother texture as compared to other ice-cream samples. However, sensory attributes such as color, glassy, separation, sweetness, scoopability, hardness-oral, degree of ice, rate of melt, aromatic of papaya, and buttery/fat were taken into consideration as well. Other than the three formulations, a commercial ice-cream was also included in the nine-point hedonic scale test.

Table 3 shows the mean of hedonic ratings for consumer's acceptance of formulated papaya ice-cream samples. Based on the scores showed in Table 3, consumers liked the less intense color as F4 has a lighter color compared with F2, and F3. Less intense color in ice-cream formulated with papaya puree may change due to the dilution of solids content in papaya puree. According to Patel *et al.* (2010), the ideal color of the ice-cream should be neither too pale, not too intense.

Table 3. Mean of hedonic ratings for consumer's acceptance of formulated papaya ice-cream samples

Attributes	Sample[2]			
	F2	F3	F4	Commercial
Appearance	5.66 ± 1.55^{a}	4.70 ± 1.70^{b}	6.46 ± 1.34^{c}	6.49 ± 1.57^{c}
Aroma	4.85 ± 1.71^{a}	4.25 ± 1.73^{b}	5.39 ± 1.39^{a}	7.22 ± 1.28^{c}
Flavor	4.10 ± 1.98^{a}	3.38 ± 1.92^{b}	5.09 ± 1.75^{c}	7.16 ± 1.24^{d}
Texture	5.24 ± 1.73^{a}	4.35 ± 1.90^{b}	5.73 ± 1.53^{a}	6.95 ± 1.26^{c}
Overall Acceptability	4.52 ± 1.76^{a}	3.73 ± 1.63^{b}	5.29 ± 1.57^{c}	7.17 ± 1.12^{d}
Preference	2.20 ± 0.89^{a}	1.61 ± 0.89^{b}	2.64 ± 0.80^{c}	3.54 ± 0.92^{d}

Values represent means ± standard deviation; n = 100

Hedonic ratings are based on 9-point hedonic scales with descriptors: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely.

[2] Commercial – 'Corn flavor; F2 – ice-cream added with 50% (w/v) of papaya powder; F3 – ice-cream added with 80% (w/v) of papaya powder; F4 – ice-cream added with 20% (v/v) of papaya puree

[a-c] Different letters in same row indicate a significance difference at $p \leq 0.05$

In terms of aroma, the commercial sample got the highest scores for aroma attribute followed by ice-cream formulated with 20% (v/v) papaya puree F4. This is similar to flavor. Table 3 showed consumers liked the flavor of ice-cream formulated with 20% (v/v) papaya puree among the papaya ice-cream formulations. According to Kilara and Chandan (2007), the flavor of ice-cream will be more apparent when it undergoes melting. The serving temperature should not be too cold, which may lead to a deadened palate.

The texture is defined as how an ice-cream sample reacts in the mouth (Yilsay *et al.*, 2006). The commercial sample got the highest scores for texture attribute followed by ice-cream formulated with 20% (v/v) papaya puree (F4), while ice-cream formulated with 50% (w/v) papaya powder (F2) got the score more than average. Meanwhile, ice-cream formulated with 80% (w/v) papaya powder (F3) got the least scores. There was no significant difference ($p > 0.05$) for the appearance attribute existed between F2 and F4 (Table 3). The texture of ice-cream is affected by overrun and fat content (Muse and Hartel, 2004; Murtaza *et al.*, 2004). The commercial product got the highest scores because the fat content is high in the commercialized product.

Based on Table 3, the scores for overall acceptability varied from 3.73 to 7.17, with the commercial sample was rated superior to the other formulated samples. However, ice-cream formulated with 20% (v/v) papaya puree (F4) was found to have well-accepted compared with ice-cream formulated with 50% (w/v) papaya powder (F2) and 80% (w/v) papaya powder (F3). This demonstrates that papaya puree was more preferred for the production of ice-cream, although the rating was lower compared to the commercial sample.

The commercial sample got the highest scores for appearance attribute, while F4 (ice-cream formulated with 20% (v/v) papaya puree) was the only one rated similar to the commercial sample, thus indicating that 20% (v/v) papaya puree can be used in the ice-cream production without any appearance defect (Temiz and Yeşilsu, 2010).

3.3. Physicochemical properties of papaya ice-cream

Table 4 shows the total solid contents and titratable acidity for the chosen papaya ice-cream as compared to the control ice-cream. Based on Table 4, there was no significant difference in the percentage of total solid content between F4 (ice-cream added with 20% (v/v) of papaya puree) and F0 (without the addition of papaya puree). This

could be explained by the addition of 20% (v/v) of papaya puree that did not increase the total solids in ice-cream. In terms of percentage of titratable acidity, ice-cream added with 20% (v/v) papaya puree was higher (0.153 ± 0.006%) than control (0.113 ± 0.006%).

Figure 2 shows the meltdown characteristics of ice-cream, where the melting rate of ice-cream added with 20% (v/v) of papaya puree (F4) was faster than ice-cream without the addition of papaya puree (control, F0). Schmidt (2004) reported that the addition of purees of kiwifruit with green, gold, and red flesh into ice-cream altered the physical form of proteins in ice-cream and causes a change in melting properties. Moreover, the addition of papaya puree increases the sugars and organic acid contents, which eventually increase the amount of unfrozen liquid (Temiz and Yeşilsu, 2010). Therefore, ice-cream added with purees melts faster. Besides, the melting rate can be reduced by high overrun (higher air volume) (Muse and Hartel, 2004). Meanwhile, ice-cream added with 20% (v/v) of papaya puree retained their shape well during melting, which parallels to control ice-cream.

3.4. Proximate analysis of papaya ice-cream

Proximate composition of control ice-cream (F0) and ice-cream formulated with 20% (v/v) papaya puree (F4) were shown in Table 5. The moisture content of control, F0 ice-cream (27.93 ± 0.16%), was found to be higher than the ice-cream formulated with 20% (v/v) papaya puree, F4 (23.53 ± 2.33%). Meanwhile, the increase in total solids in ice-cream reduces the amount of free water that is present in the ice-cream, thus decreasing its moisture content (Rincón *et al.*, 2006). From Table 5, there was no significant difference ($p > 0.05$) found in protein, ash and fat content in both ice-cream.

Table 4. Mean of total solid contents and titratable acidity for the chosen papaya ice-cream samples

Analysis	Control	F4
Total Solid Content (%)	25.28 ± 0.53^{a}	25.31 ± 0.26^{a}
Titratable Acidity (%)	0.113 ± 0.006^{a}	0.153 ± 0.006^{b}

Data on total solid content and titratable acidity are means ± standard deviations where n = 3. Different letters in the same row indicate a significant difference at $p \leq 0.05$. Abbreviations: Control = ice-cream without addition neither papaya puree nor papaya powder F4 = ice-cream added with 20% (v/v) of papaya puree.

Table 5. Mean of moisture content, protein content, ash content, and fat content for the chosen papaya ice-cream samples

Analysis	Control	F4
Moisture Content (%)	27.93 ± 0.16^{a}	23.53 ± 2.33^{b}
Protein Content (%)	2.32 ± 0.25^{a}	2.30 ± 0.10^{a}
Ash Content (%)	0.51 ± 0.09^{a}	0.88 ± 0.06^{a}
Fat Content (%)	3.71 ± 0.76^{a}	3.04 ± 0.81^{a}

Data on total solid content and titratable acidity are means ± standard deviations where n = 3. Different letters in the same row indicate a significant difference at $p \leq 0.05$. Abbreviations: Control = ice-cream without addition neither papaya puree nor papaya powder F4 = ice-cream added with 20% (v/v) of papaya puree.

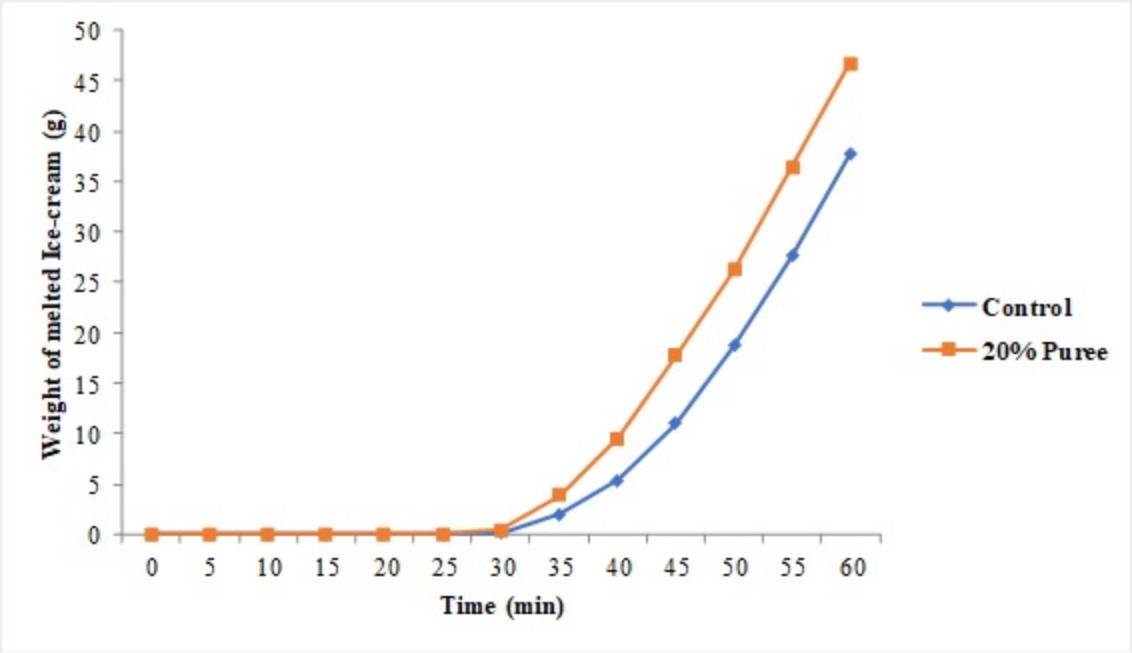

Figure 2. Meltdown characteristics of ice-cream samples, ( ) control, and ( ) ice-cream added with 20% (v/v) papaya puree. Each data point is an average value obtained from three samples.

4. Conclusions

Papaya ice-creams were produced with incorporation of papaya purees and papaya powder at 20%, 50%, and 80% concentrations. QDA panelists selected F2 (ice-cream with 20% papaya powder), F3 (ice-cream with 50% papaya powder) and F4 (ice-cream with 20% papaya powder). Among the 3 formulations, when conducted with 100 panelists for the Hedonic test, F4 was found to be 2nd most preferable, after commercial ice-cream. The ice-cream formulated with 20% (v/v) papaya puree (F4) can be produced commercially and has a high potential marketable value because papaya flavored ice-cream is not available in the market. When compared with F0 (without papaya puree), F4 (ice-cream with 20% papaya puree) is higher in titratable acidity and lower in moisture content.

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BUILDING AND SOLVING THE HEAT TRANSFER MODELS TO DETERMINE SUITABLE FRYING CONDITIONS FOR INSTANT NOODLES

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ABSTRACT

The purpose of this study is to build a mathematical model of unstable heat transfer describing the frying process of instant noodle products, and this unstable heat transfer mathematical model was solved to determine the relationships between frying temperature and radius of fried noodle strands, between frying temperature and frying time. The obtained results were then used to simulate the frying kinetics, establish the frying conditions for instant noodles, and could be used to design instant noodle frying equipment. The results of solving the mathematical model established the frying conditions for instant noodles as follows: instant noodle strands had a radius of 1 mm, frying temperature was 150 ºC in oil, and frying time was 90 s. At these conditions, experimental results confirmed that the temperature of frying noodles reached 150 ºC, and the moisture content of the fried products was satisfactorily 1.8 %; the fried noodles were crispy with a beautiful yellow color, the reconstitution ability in boiling water was significantly improved.

1. Introduction

In the hurry of modern life, instant noodles have become indispensable food products not only in Vietnamese but also in other countries (Saguy and Dana, 2003). Currently, a number of factories produce instant noodles according to the manufacturing process shown in **Fig.1**.Instant noodle making process.

Ingredients: The most popular types of noodles in the world are Chinese-, Japanese- and European-style instant noodles. Basic ingredients of instant noodles include wheat flour, starch, water, salt or *kansui* (an alkaline salt mixture of sodium carbonate, potassium carbonate and sodium phosphate) and other ingredients that help improve the texture and flavor of the noodles. *Kansui* is normally used in Chinese instant noodles but not in Japanese noodles. European-style noodles are usually made from semolina, a type of flour that is coarsely ground from durum wheat. In addition, it is possible to mix other flours with wheat flour to create different types of instant noodles such as buckwheat flour (10 - 40%) in the production of soba (Benu, Adhikari, Xiaotian, Zhang, Min, Zhang., 2020).

Preparation of the dough: The first step in noodle production is dissolving salt or *kansui*, starch, flavoring and other ingredients (except wheat flour) into water. Wheat flour is then added and the mixture is kneaded until a dough of desired toughness is obtained. After that, the dough is rested for 20 – 40 mins to make it smoother and less streaky after sheeting (Pankaj and Keene., 2017).

Sheeting – Slitting – Waving: After incubation, the dough is passed to a sheeter to gauge the dough mass into a sheet. The dough sheet then passes through a series of 4-6 pairs of rollers with decreasing gaps to facilitate the development of the gluten network and to reduce the thickness of the dough sheet to that

required for slitting. The wavy pattern of noodle strands is created by setting the conveyor speed to be slower than the cutting roll speed in the previous step. In addition, the interference by the metal blocks of the cutting machine also contributes to the noodle waves (Akinsola et al., 2018; Shashi K.Pankaj, Kevin MKeene., 2017).

Steaming: Sometimes, noodle strands are dipped into a mixture of liquid spices before being quantitatively cut and molded into blocks or shapes which are suitable for consumption purposes. The noodles are then steamed at 100ºC for 1-5 minutes to gelatinize the starch and improve the texture of the noodle strands (Akinsola et al., 2018).

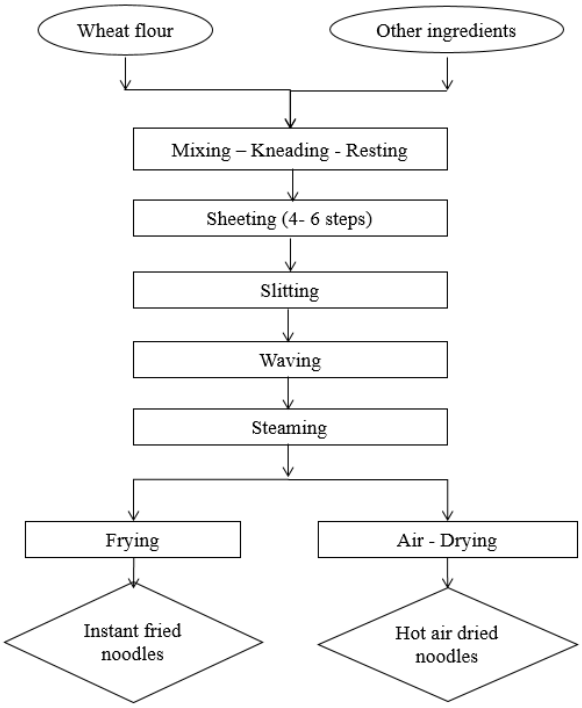

Figure 1. Instant noodle making process **(** Akinsola et al., 2018)

Drying of noodle (Frying or Drying)

*Frying/drying***:** After steaming stage, noodles are dried by frying in oil (Fried instant noodles) or drying with hot air (Non-fried instant noodles). Frying noodles in oil at 140-160 ºC for 1-2 minutes reduces the moisture content of noodles from 30-50 % (after steaming) to about 2-5 %. Palm oil is commonly used in Asia, while the mixture of canola oil, cottonseed oil and palm oil is often used in North America A. (Akinsola et al., 2018).

With the drying method, noodles are kept in hot air at 70-90 °C for 30-40 minutes to reach 8-12% of moisture. Both the frying or drying process improves the starch gelatinization and the spongy texture of the noodles. Frying method is more preferred than drying, and up to 80% of the noodle products on the market are fried noodles. The disadvantage of air-drying process is the uneven exposure noodle strands to the hot air and this adversely affects the texture of the finished noodles (Bouchon, P., et al., 2003; Akinsola, et al., 2018). Non-fried noodles also require a longer cooking time. However, one drawback of frying is that the fried noodles contain about 15 - 20% of lipid as compared to that of air-dried noodles (about 3%). Thus, fried noodles are more susceptible to lipid oxidation and deterioration. Nevertheless, antioxidants could be used to extend the shelf-life of fried noodles (Bouchon, P., et al., 2003).

Cooling and packaging: the dried noodles are quickly cooled, and then examined for the moisture content, color, shape and other quality properties. The finished blocks of noodles will be packed with different pouches of seasonings. There are two types of product packaging: the bag-type and the cup-type (Akinsola, et al., 2018).

Today, instant noodles are availabe in a wide range of flavors such as pork, beef, chicken, shrimp flavors as well as other vegetarian variations to serve diverse needs of consumers. Due to their low moisture content, instant noodles have high stability and a shelf life of 4 - 6 months in the tropics and about 6-12 months in the Northern hemisphere. They can be used immediately after boiling in water for 1- 2 minutes or soaking in hot water for 3- 4 minutes (Costa, R. M., et al., 1999).

Through the literature review it can be seen that the frying process in which noodles are fried in cooking oil at 140-160ºC for 1-2 minutes to reduce the moisture content from 35-55% (at the steaming stage) to about 2-5% has not been substantially studied in terms of

building methamatical models to accurately determine suitable frying conditions (Costa et al., 1999; Akinsola et al., 2018). This is an important stage to decide the quality of the product because if the frying temperature is low, the fried time is short, the fried noodles would not reach the required moisture content; if the frying temperature is high, the noodles would be burnt, leading to an increase in production cost (Bouchon, P., et al., 2003; Gertz, C., 2014). Hence, it is necessary to to establish and solve mathematical models to determine the frying conditions (e.g. frying temperature and fyring time) for instant noodles, so that fried the noodles could obtained good quality and production cost is reduced.

2.Matherials and methods

Building and solving descriptive mathematical models for the frying process

2.1. Assumptions

- *Problem analysis*

The frying process increases the noodle temperature from $t_0 = 25\ ^0C$ (room temperature) to $t_e = 150^0C$ (the boiling point of cooking oil, or fried temperature). Hence, the noodles strands are cooked and microorganisms are kills through this heating process. The research questions here are: (1) How does the average temperature of noodle strands vary over frying time?, and (2) How long should the frying time be? Because if the frying time is not enough, the noodles are undercooked; on the other hand, long frying time would adversely affect quality of the final product. These are hence very important issues in instant noodle production technology (Dzung, N.T., 2012).

- *Model the system as an object model, determine the actual geometrical dimensions of the object model*

Assume that the instant noodle strands have cylindrical shape with the following dimensions:

- Radius: R = 1 mm = 0.001 m.
- Length: infinitely long (L >> R).
- Assume that shape and size of noodle strands are unchanged during the frying process.
- Noodles have concentric isothermal surfaces, meaning that a set of points that are equidistant from the center, with the same inner radius will have the same temperature.

- *Set up assumptions of physicochemical systems associated with actual conditions before building the mathematical model, determine the physical and chemical properties affecting the technological process*

- The temperature field is distributed in the noodle in the radial direction $T(r, \tau)$, whose isothermal surfaces are concentric cylindrical surfaces.
- The initial temperature of the noodle strand is $T_0 = 25\ °C = 298\ K$, and it is suddenly placed into an environment with constant temperature $T_e = 150\ °C = 443\ K$.
- Frying conditions: use shortening oil whose temperature remains constant during heat transfer and is equal to the boiling point of shortening oil, $150\ ^0C$ ($T_e = 443\ K = const$).
- The internal heat source of the noodle strand at the beginning is $q_v = 700 – 0.1T$, $W.m^{-3}$ (Heldman and Lund, 1992).
- The process may be assumed to be an unstable heating in the symmetrical cylinder, under boundary condition type 1, with the same heat emission coefficient of the environment surrounding the object, and the boiling point of shortening is considered constant during the heat transfer.

- Heat emission coefficient of shortening is α = 100 $W.m^{-2}.K^{-1}$, (Heldman and Lund, 1992).
- Physical conditions: thickness $\delta = 2R$ = 2 mm = $2x10^{-3}$ m; density ρ = 1100 $kg.m^{-3}$; thermal conductivity λ = 0.45 $W.m^{-1}.K^{-1}$; specific heat c_p = 2930.79 $J.kg^{-1}.K^{-1}$, (Heldman and Lund, 1992).
- To facilitate the calculation process, it is assumed that the total frying time is: τ_o = 90s.

2.2. Building mathematical model

Based on the energy balance equation, the heat transfer model in the case of having an internal heat source is established as follows (Holman, 1992):

$$\begin{cases} \dfrac{\partial T(r,\tau)}{\partial \tau} = a\left(\dfrac{\partial^2 T(r,\tau)}{\partial r^2} + \dfrac{1}{r}\dfrac{\partial T(r,\tau)}{\partial r}\right) + \dfrac{q_v}{\rho C_p} \\ -R \le r \le R \end{cases} \quad (1)$$

Initial and boundary conditions to solve equation system (1):

- Initial condition:

$$T_0(r, 0) = 25°C = 298K = \text{const} \quad (2)$$

- Frying temperature:

$$T(r, \tau) = T_e = \text{const} \quad (3)$$

- Boundary conditions:

$$\left.\frac{\partial T(r,\tau)}{\partial r}\right|_{r=R} = -\frac{\alpha}{\lambda}\left[T_e - T(r,\tau)\right] \quad (4)$$

$$\left.\frac{\partial T(r,\tau)}{\partial r}\right|_{r=0} = 0 \quad (5)$$

2.3. Solving mathematical model

Using Fourier variable separation method to solve the system of equations (1) - (5), the dimensionless temperature θ was found as follows (Holman, 1992):

$$\bar{\theta} = \frac{t(\tau) - t_o}{t_e - t_o} = 1 + \frac{P_o}{4}\left(1 + \frac{2}{Bi} - \frac{r^2}{R^2}\right) - \sum_{n=0}^{\infty}\left(1 + \frac{P_o}{\mu_n^2}\right) A_n J_o\left(\mu_n \frac{x}{R}\right)\exp\left(-\mu_n^2 Fo\right) \quad (6)$$

where:

$$P_o = \frac{q_v R^2}{\lambda(T_e - T_o)} = \frac{(700 - 0.1T)R^2}{\lambda(T_e - T_o)} \quad (7)$$

$$Fo = \frac{a\tau}{R^2} \quad (8)$$

The dimensionless average temperature $\bar{\theta}$ of the cylindrical noodle strands is:

$$\bar{\theta} = \frac{\bar{t}(\tau) - t_o}{t_e - t_o} = 1 + \frac{P_o}{8}\left(1 + \frac{4}{Bi}\right) - \sum_{n=0}^{\infty}\left(1 + \frac{P_o}{\mu_n^2}\right) B_n \exp\left(-\mu_n^2 Fo\right) \quad (9)$$

with:

$$A_n = \frac{2.Bi}{J_o(\mu_n)[\mu_n^2 + Bi^2]}; \quad (10)$$

$$B_n = \frac{4.Bi^2}{\mu_n^2[\mu_n^2 + Bi^2]} \quad (11)$$

μ_n - roots of specific equation:

$$\frac{J_0(\mu_n)}{J_1(\mu_n)} = \frac{\mu_n}{Bi} \quad (12)$$

Bi: Biot number

$$Bi = \frac{\alpha R}{\lambda} \quad (13)$$

Fo: Fourier number

$$Fo = \frac{a\tau}{R^2} \quad (14)$$

$J_0(\mu_n)$, $J_1(\mu_n)$: Bessel functions type 1 of the zero and first order.

$$J_0(x) = 1 - \left(\frac{1}{2}x\right)^2 + \frac{\left(\frac{1}{2}x\right)^4}{1^2.2^2} - \frac{\left(\frac{1}{2}x\right)^6}{1^2.2^2.3^2} + \frac{\left(\frac{1}{2}x\right)^8}{1^2.2^2.3^2.4^2} - \ldots \quad (15)$$

$$J_1(x) = -J_0'(x) = \frac{1}{2}x - \frac{(\frac{1}{2}x)^3}{1^2.2} + \frac{(\frac{1}{2}x)^5}{1^2.2^2.3} - \frac{(\frac{1}{2}x)^7}{1^2.2^2.3^2.4} + ... \quad (16)$$

2.4. Materials

This research only studied the frying process of raw noodle strands after steaming step. Raw noodle strands were provided by Asiafood Company, which had been prepared by the following steps: mechanical dough mixing, sheeting, cutting into strands, waving, shaping and steaming. Raw noodle strands were uniform in size and shape, with the radius R = 1 mm = 0.1 cm (***Figure 2***). They were then fried to dehydrate and to increase the crispness.

Figure 2. Fresh, un-waved and un-fried noodle strands

2.5.Apparatus

- The deep fryer DVF-04 made by Ho Chi Minh City University of Technology and Education, Vietnam in 2019 was used to perform the study. This is a conveyor frying system (***Figure 3***).

- The DVF-04 can automatically measure the technological parameters as: temperatures of frying oil and products, frying time, conveyor speed. The equipment was controlled by programs on the computer.

Figure 3. The deep fryer DVF--04

2.5. Methods

- In this study, the problem analysis and systematic approach were used to build mathematical models (Figura and Teixeira, 2007).

- Modeling and optimization methods were used to build and solve descriptive mathematical models for instant noodle frying process (Figura and Teixeira, 2007).

- The moisture content was determined by drying to a constant weight, the result was calculated using the following equation (Figura and Teixeira, 2007):

$$W_e = 100 - \frac{G_o}{G_e}(100 - W_o) \quad (17)$$

where: G_o (g) - weight of the sample before frying; G_e (g) - weight of the sample after frying; W_o (%) - initial moisture content of the sample before frying; W_e (%) - moisture content of the sample after frying.

- Mathematical tools and softwares such as Microsoft Excel 2020, Matlab 7.0 and Visual Basic 8.0 were used to establish and solve the mathematical models (Dzung et al., 2012a; 2012b).

3.Results and discussions

3.1.Solving specific equation (12)

With the division algorithm programmed by Visual Basic 8.0 software, the solution of the characteristic equation were found as (12):

$\mu_1 = 2.4048$; $\mu_2 = 5.5201$; $\mu_3 = 8.6538$; $\mu_4 = 11.7915$; $\mu_5 = 19.256$;

with:

$$a = \frac{\lambda}{c_p \rho} = \frac{0.45}{2930.79 \times 1100} = 1.395 \times 10^{-7}\ \text{m}^2/\text{s}$$

Substitute a = 1.395×10^{-7} m^2/s into equation (14) to determine the Fourier's standard number.

Substitute (7), (13), (14) and roots of specific equation (12) in Table 1 into equation (9). The results showed that: equation (9) quickly converged to 0 when n > 4.

$$\sum_{n=0}^{\infty}\left(1+\frac{P_o}{\mu_n^2}\right)B_n \exp\left(-\mu_n^2 Fo\right) \to 0$$

Therefore, at n = 4, roots of specific equation (12) are presented in Table 1 as follows:

Table 1. Roots of specific equation (12)

μ_1	μ_2	μ_3	μ_4
2.4048	5.5201	8.6538	11.7915

3.2. Determination of frying temperature of instant noodle

- Substitute equations (11), (13) and (14), roots of specific equation (12) in Table 1 into equation (9). Using the Visual Basic language programmed in Macro in Microsoft Excel 2020 (Dzung et al., 2012a), $\bar{\theta}$ was calculated and it was found out that the average temperature of noodles strands $\bar{t}(r, \tau)$ varied according to the radius from the fixed center of the noodle strand and the frying time.
- Given the frying time at 10 different values as τ = 10s, 20s, 30s, 40s, 50s, 60s, 70s, 80s, 90s, 100s to calculate $\bar{t}(r, \tau) = \bar{t}(r)$ at each frying time; the obtained results are shown in ***Table 2a, Table 2b*** and ***Table 2c***:

Table 2a. Variation of temperature according to r at different frying times

r	Temperature after τ (s) of frying noodles			
	10s	20s	30s	40s
0.00000	61.04	109.49	131.92	141.93
0.00005	61.33	109.64	131.98	141.96
0.00010	62.21	110.08	132.18	142.05
0.00015	63.66	110.80	132.50	142.19
0.00020	65.69	111.80	132.95	142.39
0.00025	68.27	113.06	133.52	142.65
0.00030	71.39	114.58	134.20	142.95
0.00035	75.03	116.34	134.98	143.30
0.00040	79.18	118.32	135.87	143.69
0.00045	83.78	120.49	136.84	144.13
0.00050	88.81	122.84	137.89	144.60
0.00055	94.22	125.34	139.00	145.09
0.00060	99.96	127.96	140.17	145.62
0.00065	105.97	130.68	141.39	146.16
0.00070	112.20	133.47	142.63	146.71
0.00075	118.56	136.29	143.89	147.27
0.00080	125.00	139.13	145.15	147.84
0.00085	131.44	141.95	146.41	148.40
0.00090	137.80	144.71	147.64	148.95
0.00095	144.01	147.41	148.84	149.48
0.00100	150.00	150.00	150.00	150.00

Table 2b. Variation of temperature according to r at different frying times

r	Temperature after τ (s) of frying noodles		
	50s	60s	70s
0.00000	146.40	148.40	149.28
0.00005	146.42	148.40	149.29
0.00010	146.45	148.42	149.29
0.00015	146.52	148.45	149.31
0.00020	146.61	148.49	149.33
0.00025	146.72	148.54	149.35
0.00030	146.86	148.60	149.37

0.00035	147.01	148.67	149.41
0.00040	147.19	148.75	149.44
0.00045	147.38	148.83	149.48
0.00050	147.59	148.92	149.52
0.00055	147.81	149.02	149.56
0.00060	148.04	149.13	149.61
0.00065	148.29	149.24	149.66
0.00070	148.53	149.35	149.71
0.00075	148.78	149.46	149.76
0.00080	149.04	149.57	149.81
0.00085	149.29	149.68	149.86
0.00090	149.53	149.79	149.91
0.00095	149.77	149.90	149.95
0.00100	150.00	150.00	150.00

Table 2c. Variation of temperature according to r at different frying times

r	**Temperature after τ (s) of frying noodles**		
	80s	90s	100s
0.00000	149.68	149.86	149.94
0.00005	149.68	149.86	149.94
0.00010	149.69	149.86	149.94
0.00015	149.69	149.86	149.94
0.00020	149.70	149.87	149.94
0.00025	149.71	149.87	149.94
0.00030	149.72	149.88	149.94
0.00035	149.74	149.88	149.95
0.00040	149.75	149.89	149.95
0.00045	149.77	149.90	149.95
0.00050	149.79	149.90	149.96
0.00055	149.81	149.91	149.96
0.00060	149.83	149.92	149.97
0.00065	149.85	149.93	149.97
0.00070	149.87	149.94	149.97
0.00075	149.89	149.95	149.98
0.00080	149.91	149.96	149.98
0.00085	149.94	149.97	149.99
0.00090	149.96	149.98	149.99
0.00095	149.98	149.99	150.00
0.00100	150.00	150.00	150.00

▪ Given the distance from the center to the fixed isothermal surfaces as r = 0.0000, 0.0002, 0.0004, 0.0006, 0.0008, 0.0010 to calculate $\bar{t}(r, \tau) = \bar{t}(\tau)$, the obtained results are shown in ***Table 3a*** *and* ***Table 3b*** as followings:

Table 3a. Variation of temperature over time at different radius values

τ (s)	**Temperature of the noodle strand** $\bar{t}(r, \tau) = \bar{t}(\tau)$,		
	r = 0.0000	r = 0.0002	r = 0.0004
10s	61.04	65.69	79.18
20s	109.49	111.80	118.32
30s	131.92	132.95	135.87
40s	141.93	142.39	143.69
50s	146.40	146.61	147.19
60s	148.40	148.49	148.75
70s	149.28	149.33	149.44
80s	149.68	149.70	149.75
90s	149.86	149.87	149.89
100s	149.94	149.94	149.95

Table 3b. Variation of temperature over time at different radius values

τ (s)	**Temperature of the noodle strand** $\bar{t}(r, \tau) = \bar{t}(\tau)$,		
	r = 0.0006	r = 0.0008	r = 0.0010
10s	99.96	125	150
20s	127.96	139.13	150
30s	140.17	145.15	150
40s	145.62	147.84	150
50s	148.04	149.04	150
60s	149.13	149.57	150
70s	149.61	149.81	150
80s	149.83	149.91	150
90s	149.92	149.96	150
100s	149.97	149.98	150

- With the data in Table 2a, Table 2b and Table 2c, Matlab 8.0 software was used to simulate the frying process of instant noodles. The results obtained are shown in Figure 4.

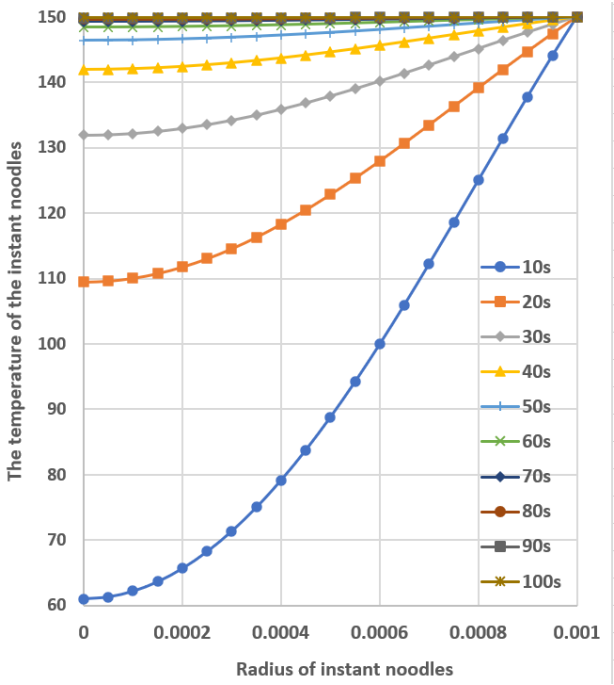

Figure 4. Relationship between frying temperature and radius of the noodle strands

- From the data in Table 3a and Table 3b, Matlab 8.0 software was used to simulate the frying process of instant noodles. The results obtained are shown in Figure 5 as following:

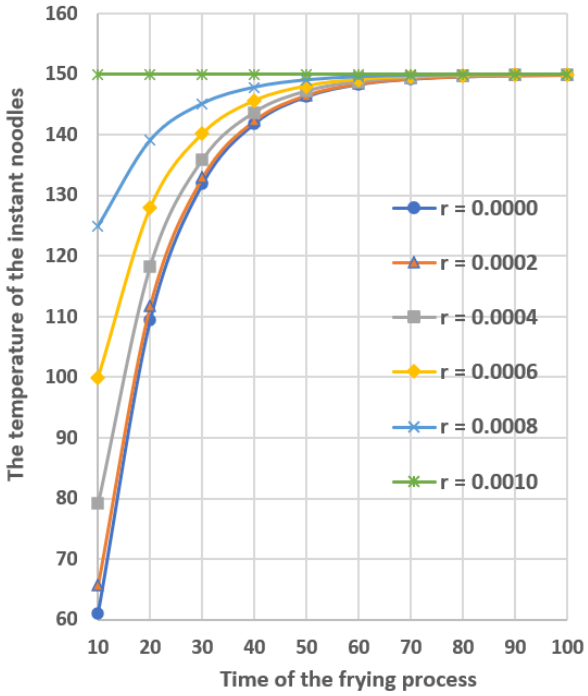

Figure 5. Relationship between frying temperature and frying time

- To validate the compatibility of the mathematical model, the error function Er (%), the error between calculated data and experimental data, is normally used. Er (%) of the model was calculated by the following equation:

$$Er = \frac{|T_M - T_E|}{T_E}.100\% \quad (18)$$

- If Er ≤ Er(p), where Er(p) is the permissible error, the mathematical model is considered compatible.

- If Er > Er(p), the mathematical model is not compatible.

Technically, the maximum error of mathematical model is Er(p) = 10% = 0.1.

On that basis, the surface temperature of the noodle strand with the radius R = 1 mm =

0.001 m was determined by using the calculated data T_M (°C) and by experimental data T_E (°C). Equation (18) was used to calculate the temperature (Table 5).

Table 5. Temperature determined by mathematical model T_M(°C) and by experiments T_E(°C) when r = 0.001m (at the surface of noodle strand)

τ (s)	Temp, T_M (°C)	Temp, T_E (°C)	Error, Er (%)
00s	25	25	0.00
20s	150	138	8.70
30s	150	142	5.63
40s	150	146	2.74
50s	150	147	2.04
60s	150	150	0.00
70s	150	150	0.00
80s	150	150	0.00
90s	150	150	0.00
100s	150	150	0.00

Table 5 shows that, the error of the mathematical model was Er = 8.7% < Er (p) = 10%. Thus, the mathematical models from (1) to (5) and their roots as equation (9) were compatible with the experimental data. Therefore, model (9) can be used to calculate and establish the frying conditions for the instant noodle.

Figure 4 and ***Figure 5*** show that when the frying time was more than 60s, the temperature variation according to the radius of the noodle calculated by the mathematical model (9) reached the oil frying temperature, 150 °C. These temperature curves were almost superimposable, which was completely consistent with the experimental results obtained in ***Table 5***.

- Mathematical model evaluation: The advantage of model (9) is that it is simple and easy to use to calculate the frying process of instant noodles, to calculate and establish the frying conditions. The disadvantage of this model is that such physical parameters as heat-generating coefficient, thermal conductivity coefficient, density, specific heat, heat source q_v…, are approximate values based on assumed compositions of noodles. Therefore, the result calculated by model (9) was different from experimental data at frying time of 20 seconds; the error value was 8.7%, which was higher than 5% (***Table 5***). However, when the frying time increased, the error between calculated data and experimental data was reduced. As the frying time was greater than 60s, the data calculated by model (9) were identical with the experimental data (***Table 5***). This shows that at the beginning of frying process when heat was provided for intensive heating and moisture removal, the boundary conditions of the model (9) would change as compared to the actual frying conditions, leading to a large error. As the frying time increased, the temperature of the noodle strands gradually reached the temperature of frying oil, the moisture content of the noodles hence reached the equilibrium moisture content, the boundary conditions of model (9) were more stable, hence the accuracy of model (9) increased and the calculated data were consistent with the experimental data.

3.3. Determination the frying moisture of the instant noodles

After being fried at different times, the moisture content of the products was then determined. The obtained results are presented in ***Table 6***.

Table 6. Moisture contents of the noodles before and after frying at oil temperature of 150 °C(the radius of noodle strands was r=0.001m)

Time of frying τ(s)	Moisture content of noodles before frying, Wo (%)	Moisture content of noodles after frying, We (%)
00s	54.8	54.8

10s	54.8	12.5
20s	54.8	5.4
30s	54.8	3.2
40s	54.8	2.9
50s	54.8	2.7
60s	54.8	2.4
70s	54.8	2.3
80s	54.8	2.1
90s	54.8	1.8
100s	54.8	1.8

It can be seen from ***Table 6*** at two different frying times of 90s and 100s, the two noodle samples had similar equilibrium moisture content (1.8%). This moisture content was satisfactory for the exported product (Saguy S. and Dana, D., 2003), and the temperature of noodle strands reached the temperature of frying oil which was 150°C. After frying, the noodles were well cooked and had a fine yellow color as shown in ***Figure 6***.

Figure 6. Instant noodles after frying for 90s, the moisture content reached 1.8%.

3.4. Determination of frying conditions for instant noodles

From the above results, the frying conditions for raw noodle strands were established as following:

- Noodle had infinite cylindrical shape with the radius r = 0.001m;
- Frying time was 90s;
- Temperature of frying oil was 150°C.

After frying at these conditions, the temperature of product reached 150 °C and moisture content after frying was satisfactorily 1.8%. The resultant instant noodles were crispy and had a fine yellow color (***Figure 6***).

Nomenclature

W_o = 0.548 = 54.8%: Initial moisture of instant noodles

c_p = 2930.79 ($J.kg^{-1}.K^{-1}$): Specific heat of instant noodles

ρ = 1100 ($kg.m^{-3}$): Density of instant noodles

λ = 0.45 ($W.m^{-1}.K^{-1}$): Thermal conductivity coefficient of instant noodles

α = 100 ($W.m^{-2}.K^{-1}$): heat emission coefficient of frying environment

T_M (°C): temperature of modelling

T_E (°C): experimental temperature.

τ (s) : frying time

$a = 1.395x10^{-7}$ ($m^2.s^{-1}$), thermal diffusivity instant noodle strands

Bi: Biot number of instant noodles

Fo: Fourier number of instant noodles

4. Conclusions

- This study modeled the instant noodle frying process by using mathematical models from (1) to (5). This was an unstable heat transfer model which was suitable for instant noodle frying process in practical.
- Solving the mathematical models from (1) to (5) would obtain the solutions for the mathematical models represented by equations (6), (7), (8) and (9), in which model (9) was the general solution of the mathematical modelsfrom (1) to (5). Experiments also proved that model (9) were compatible with experimental data. Therefore, model (9) can be used to calculate the frying conditions for instant noodles as well as to design the deep frying equipment.

- This study had established the conditions for frying instant noodles as follows: noodle strands had infinite cylindrical shape, with radius of 0.001 m; frying time was 90 seconds; temperature of frying oil was 150°C. After frying at these conditions, the temperature of product reached 150°C and moisture content after frying was satisfactorily 1.8%. The product of instant noodles was crispy and had a beautiful yellow color (See ***Figure 6***).

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EFFECT OF PARAPROTEX AND THE MIXTURE OF GRAPE MARC, CINNAMON AND CLOVE ON THE CARP (*Cyprinus carpio*) GROWN IN RAS - RECIRCULATING AQUAPONIC SYSTEM

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Aquaponic system;
Cyprinus carpio;
Grape Marc;
Cinnamon;
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ABSTRACT

Aquaponics, a complex cultivation method obtained by combining two different systems, hydroponics and aquaculture, operates on the principle that fish waste becomes a resource for plants. Recirculating aquaponic systems are mostly used in research, being a less polluting alternative and being more effective from the cost points of view. RAS used for the research was placed into an ICDIMPH-HORTING Bucharest greenhouse, using three plants callus tunnels for supporting two sections: the fish breeding section represented by *Cyprinus carpio*, and the plant growth represented by *L. sativa*. The biological material was placed into 8 tanks where phytobiotics represented by *Paraprotex* and *MCC* mixture were added into the daily fish feed to monitor the fish health and the effect that the two types of mixture have on the fish health The fish health status was monitored by performing blood tests in the first day of experiment, in the middle and at the end of the experiment. The values of hematological indicators showed variations during the experiment period, some of them being within the range values for *C. carpio*, while others fell within the normal limits. The aim of the study is to evaluate the fish health status and their development under the influence of natural phytocomplexes administrated in the feed.

1. Introduction

Climate change, which affects most natural ecosystems, can have several impacts on extensive and semi-controlled aquaculture systems by decreasing the water quality, leading to increased stress and diseases outbreaks (Handisyde *et al*., 2017). Aquaponia is a combined method of vegetable farming and fish farming that does not require any soil.

Aquaponics can be defined as a system that combines hydroponicagriculture with aquaculture, and it is an excellent way of reducing water usage and disposing of aquaculture effluents (Nuwansi *et al.,* 2017).

In this system, aquaculture wastewater is recycled and reused for the hydroponically grown plants instead of discharging to neighbouring water bodies (Shete *et al.,* 2015). These nutrients are absorbed by plants from wastewater before returning to the fish tank. Further, if remediated water is applied to an aquaponic system, the whole system becomes cost-effective, economically

viable, and offer additional benefits like supplementation of extra nutrients, minimizing the establishment period of the hydroponic unit, and facilitates hosting decomposers (Nuwansi *et al*., 2019).

The Aquaponics is the symbiosis between hydroponics (growing plants without soil) and aquaculture (growing fish or other aquatic organisms), in which fish and plants grow together in a single integrated system (Rakocy *et al*., 2006).

Aquaponics can be useful, regardless of a country development degree which has limited resources of agricultural production, but also a large population (Mchunu *et al*., 2018). It can provide a variety of good quality food (animal protein and vegetal) for both rural and urban areas (Liang *et al*., 2013).

Aquaponic systems are a less polluting alternative to the environment compared to traditional food production techniques. This technological method has the potential to produce proteins with less labor, less soil, fewer chemicals and fewer water consumption (Specht *et al*., 2014).

The most recommended aquapocic systems are recirculating aquaponic systems because the nutrients in the water can be maintained at optimal concentrations for both fish and aquaponic cultures (Nair *et al*., 2005).

According to literature, this aquaponic technique consists in mounting floating supports directly on the surface of the water gloss related to the fish breeding units. In this case, polystyrene plates are used as supports for the plants whose roots are in permanent contact with the technological water loaded with nutrients (Jensen *et al*., 1985).

Medicinal plants, which harbour a myriad of bioactive metabolites, have arose in the last decade as an extremely attractive alternative in aquaculture (Reverter *et al*., 2014; Sutili *et al*., 2018; Zhu, 2020). The use of plant-enriched diets has shown to be able to improve growth, digestive enzymes, anti-oxidant activities and immune parameters of fish (Awad *et al*., 2013; Yousefi *et al*., 2020b; Zemheri-Navruz *et al.*, 2020). However, as shown before some plants also contain anti-nutritional compounds or toxic metabolites, and therefore careful examination of plant species and dosage are required before their broad use in aquaculture systems (Reverter *et al.*, 2020b).

Recent research showed that the introduction of relatively small concentrations of vitamins, probiotics, prebiotics and newest phytobiotics in animal diet can ensure some specific demands or influence in a positive way (directly or indirectly) the growth performances and the health status (Maurilio 2011; Kasiri *et al*., 2011; Antache *et al*., 2013).

Phytobiotics are natural compounds that lead to an optimization of animal productivity which, when incorporated into diets (Cristea *et al*., 2012). Increasing number of recent studies present the positive aspects of phytobiotics administration, in diets of different fish species: the immunostimulator (Khalil *et al*., 2009), bioproductive (El-Dakaret *et al*., 2008), antioxidant and antimicrobial effect.

In aquaculture, prophylactic administration of immunostimulants is one of the most effective method of strengthening the defense mechanism (Raa *et al*., 1992). In fish, the immunostimulants are known to increase immunity (Harikrishnan *et al*., 2010). Some nutrients are linked to the immunological status of fish. This has drawn the attention of fish nutritionists to the immunoprotection of fish, besides the growth. Sustainable aquaculture depends on the balance between health and growth condition of fish (Kumar *et al*., 2005).

The paper presents the monitoring and evaluation of the effect of the two types of mixtures of phytocomplex *ParaProteX* and *MCC* administered in different concentrations together with food, on the health of carp raised in the recirculating aquaponic system.

2. Materials and methods

2.1. Materials

The recirculating aquaponic system used for the research described in this paper, was placed into a ICDIMPH-HORTING Bucharest greenhouse, using three plants callus tunnels for supporting two sections: the fish breeding section represented by

Cyprinus carpio, Linnaeus 1758 and the plant growth represented by *L. sativa* L.

Two tunnels were reconditioned by modifying the supporting pedestals and the height, for the growth of the plants (*Lactuca sativa*). The 2 plant growth enclosures have a size of 7.4x1.8x0.3 m, and for carp growth a tunnel with dimensions 7.4x1.8x0.4 m was used, being divided into 8 pools of equal dimensions.The aquaponic system works on the principle of communicating vessels. The water from the aquaponic system circulates through the polypropylene pipe, from where it is taken with the help of a CPM self-priming pump with a flow rate of 7.2 m3 / h, placed at ground level and passed through a mechanical filter.

The carp used in the experiments came from the Brateș Research and Development Base - Research and from Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galati.

Figure 1. Recirculating aquaponic system (RAS).

For populating the aquaponic growth system with biological material, dimensions of the experimental tank and the volume of recirculated water were taken into consideration. Thus, the optimal population density was 5 kg of 2-year-old carp for each experimental tank. A total of 40 kg (172 fish) of carp evenly distributed in 8 experimental tanks, with an average weight of 232.5 g/fish were monitored (Figure 1).

In the last stage of the experiment, to ensure the health of reared fish species in aquaponic systems and to comply with their physiological requirements, feed additives were added to fish feed.

Juvenile carp (biological material) used for this experiments was represented of 40 kg fish biomass being equally distributed into the eight rearing units (M1 and M2) - control variant and three different concentrations of mixture natural phytocomplexes: B1.1. with Paraprotex 2g/100g feed dry and B2.1. with 2g/100g feed dry MCC; B1.2. with Paraprotex 4g/100g feed dry and B2.2. with 4g/100g feed dry MCC; B1.3. with Paraprotex 6g/100g feed dry and B2.3. with 6g/100g feed dry MC.

The natural phytocomplexes were incorporated directly in 100g of standard feed NC 60 II, the daily ration for each experimental tank, varying the concentrations.

The fish from the control tanks M1 and M2 were fed only with 100g of standard feed NC 60 II / day, with a crude protein concentration of 32-34%. The amount of daily food was 2% of the body weight of the fish in each experimental tank.

In order to monitor the health of fish reared in the aquaponic recirculating system (RAS), two experiments were performed by using for over 42 days *ParaProteX* phytobiotics and *MCC mixture* (grape marc, cinnamon and cloves) introduced classical as powder into the fish feed (Figure 2; Figure 3). The used phytocomplexes were purchased as powder, of trade.

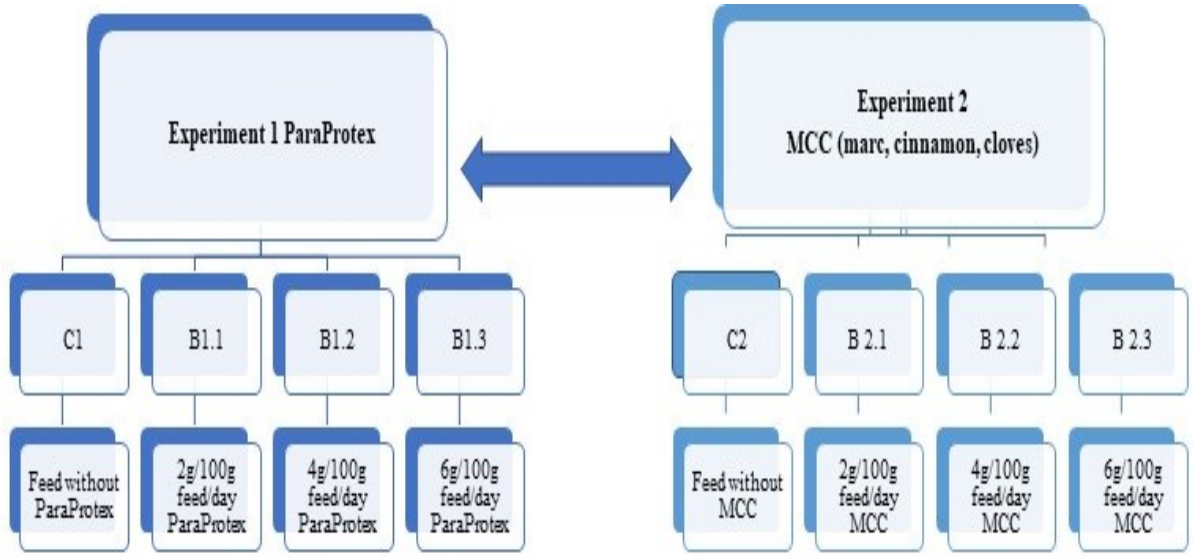

Figure 2. Experiment scheme.

Figure 3. MCC mixture (grape marc(a), cinnamon (b) and cloves (c)

2.2. Blood collection

Blood collection was performed using the cardiac puncture method, without the need to slaughter the fish.

Blood samples were collected at the beginning of the experiments (day 1), in the middle of the experiments (day 21) and at the end of the experiments (day 42), to determine the hematological and biochemical parameters.

Before collecting blood samples, the fish were anesthetized by soaking them in 0.5 ‰ 2-phenoxyethanol solution for 60 seconds.

From each experimental, 5 fish/experimental variants were analyzed, representing 25% of the total population. Disposable sterile syringes were used to collect blood. The needle penetration point is halfway between the base of the pectoral fins and the ventral line (Figure 4).

Two blood vacutainers were collected from each analyzed specimen: a vacutainer with EDTA anticoagulant for hematological examination and a vacutainer without additives for biochemical examination.

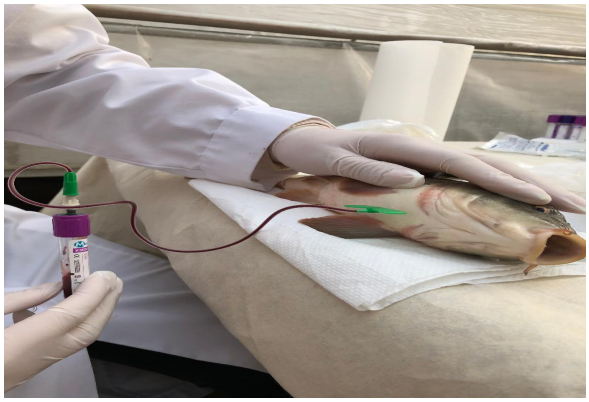

Figure 4. Blood collection by cardiac puncture method.

2.3. Methods of hematological and biochemical parameters investigation

After harvest, the blood samples were transported with a refrigerated box to the veterinary analysis laboratory.

Blood vacuum cleaners were introduced into the hematology analyzer for veterinary use Abacus Vet5, Figure 5. The following parameters were determined: no. of erythrocytes (E), hemoglobin (Hb), hematocrit (Ht) and erythrocyte indices MCV, MCH, MCHC.

The biochemical parameters determined were: glucose, total proteins, calcium, phosphorus, cholesterol, urea, creatinine. Biochemistry analyzes were performed with the Skyla Vb1 Veterinary Chemistry Analyzer, Figure 6.

Biochemical analyzes were determined from serum. After harvesting, the blood is allowed to coagulate at room temperature. By retracting the clot followed by centrifugation for 10 minutes at 3000 rpm, the serum is obtained. For the statistical analysis the Program PRIMER 7 was used.

Figure 5. Hematology analyzer used to determine hematological parameters.

Figure 6. Skyla VB1 veterinary chemistry analyzer used to determine biochemical parameters.

3. Results and discusions

The use of plant supplements to boost immune parameters and disease resistance in fish has recently attracted much attention as a sustainable, low-cost and effective alternative in the prevention and treatment of aquaculture disease outbreaks (Van Hai, 2015; Reverter *et al.*, 2020a). Multiple studies have exposed the beneficial effects of using medicinal plant-based supplements in aquaculture, such as better growth, enhanced immune defenses and decreased pathogen and stress susceptibility (Hoseinifar *et al.*, 2020a; Reverter *et al.*, 2020b; Yousefi *et al.*, 2020a; Yousefi *et al.*, 2021).

In order to see the physiological response of fish to the stressful action of technological and

physico-chemical factors in the recirculating aquaponic system, the dynamics of blood parameters were analyzed. There is a permanent dynamic balance between the fish environment, the physico-chemical parameters of the water, the application of breeding and feeding technology, so that any disturbance that occurs in this system can disrupt this balance and lead to changes in blood hematological and biochemical parameters. That is why it is very important to monitor these blood parameters in order to be able to draw a uniform conclusion about the health of the biological material.

3.1. Analysis of hematological results

The hematological parameters were used as fish health status indicators and for the detection of possible physiological changes occurred under conditions of stress caused by:

- administration of a feed with a modified composition by incorporating natural phytocomplexes of the *ParaProteX* type and *MCC mixture* was done by the direct method.
- the need for the fish organism to adapt to the water oxygenation conditions and in particular to the specific conditions of an aquaponic recirculation system.

The dynamics of hematological indicators determined in 2-year-old carp juveniles was analyzed in terms of the phytocomplexes action on fish health and in order to establish an effective feeding strategy.

3.1.1. Erythrocyte count (E)

In carp (*Cyprinus carpio*) the normal values of erythrocytes are between 1.1-2.2x10^6/µL (Ghittino, 1983; Misăilă, 2008). An increase in the number of erythrocytes is observed before reproduction and when the water has a high oxygen content. The massive decrease in the number of erythrocytes, leading to anemia is observed in various pathological conditions.

The average value of erythrocytes during experiment 1 (*ParaProteX*) decreased in the first 21 days, at the end of the experiment, the the number of erythrocytes recording a value of 1.12 µg/L in B1.3, falling within the normal values for carp (Figure 7).

Following the analysis of the average number of erythrocytes during the experiment with the MCC mixure, a slight increase of the obtained values was observed, the most significant in B2.3 (Figure 7).

There were no significant differences between the values of erythrocytes in the control tank and the values obtained in the *ParaProteX* tanks (Figure 7).

3.1.2. Hemoglobin

To assess the physiological state of fish, hemoglobin dosing is considered a rapid test to verify hematological homeostasis (Misăilă, 2008).

Physical or environmental stress causes a rapid increase in blood hemoglobin concentration, due to the recruitment of erythrocytes from the spleen and hemoconcentration installed as a result of plasma water loss.

The normal values for carp (*Cyprinus carpio*) are between 6.5-10.6 g/dL. Mean hemoglobin values ranged from one tank to another and from one concentration of administered phytocomplex to another. In Figure 7 it can be seen that in tanks B1.1 and B1.3 at the analyzes performed on the first day and on day 21, a decrease is observed, showing that the change of living environment related to water quality, and that the administration of *ParaProteX* in different concentrations, caused discomfort to the carp fish population (Figure 8).

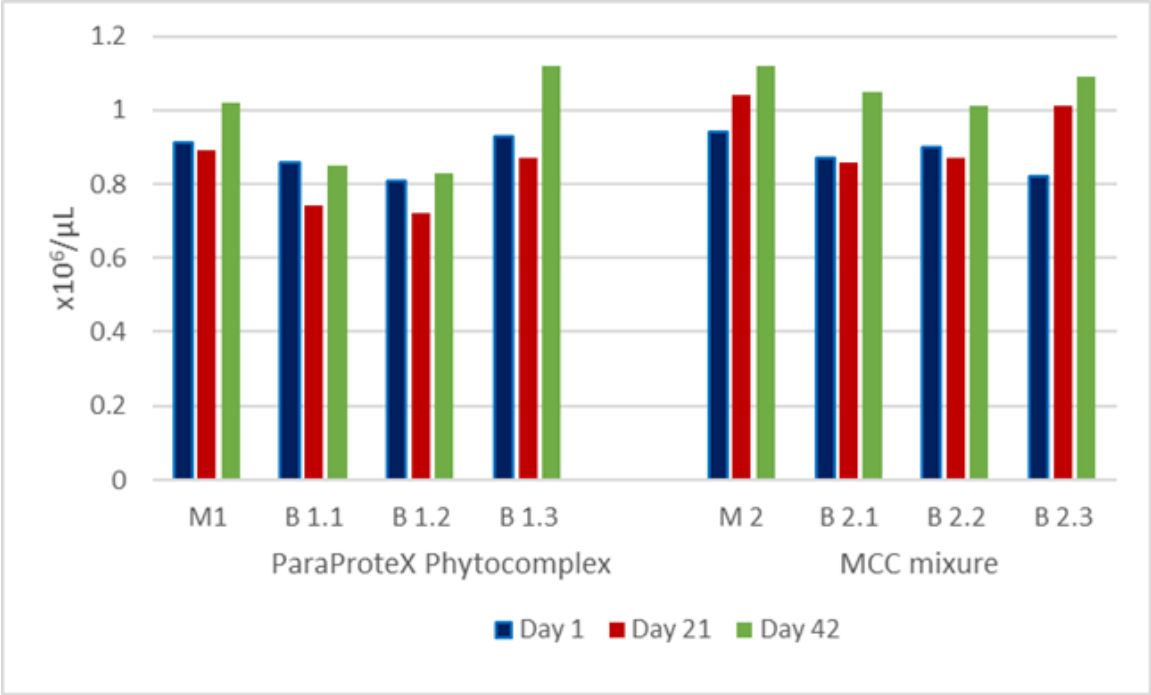

Figure 7. Variation in erythrocyte values in fish blood from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

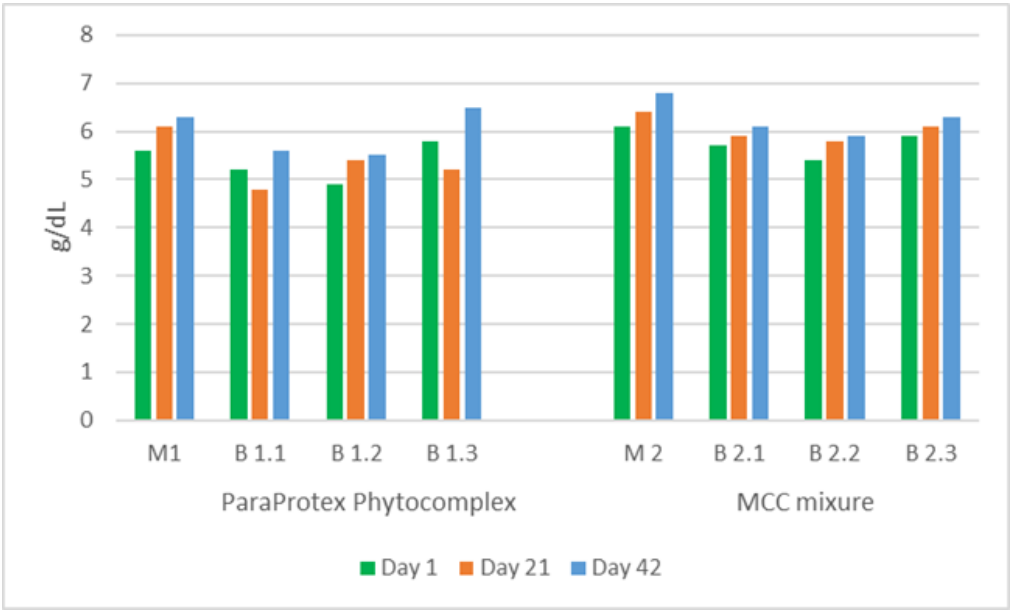

Figure 8. Variation in hemoglobin values in fish blood from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.1.3. Hematocrit

The hematocrit value, expressed as percentage, shows the volume of erythrocytes in relation to the total blood volume. In carp (*Cyprinus carpio*) the normal values of hematocrit are between 32-43.9% (Ghittino, 1983; Misăilă, 2008).

Pathological decreases in hemoglobin and hematocrit indicate anemia. During the experiments it is found that in the tanks where the

food mixed with *ParaProtex* was administered, the fish population is more difficult to accommodate, towards the end of the experiment the discomfort process showing a decrease (Figure 9).

All hematological parameters determined at the end of the experiments with *ParaProteX* mixture, were within the normal values reported in the literature for the carp species, *Cyprinus carpio*.

Compared to *PraraProtex,* the *MCC mixture* had a beneficial effect on the fish, shortening their period of adaptation to the new living environment in the recirculating aquaponic system type RAS (Figure 9).

All hematological parameters determined at the end of the experiment with *MCC mixture*, were within the normal values of the species. Increases of WBC, which are cells with immune functions, is also considered a sign of enhanced immune response and has been previously reported after plant supplementation in fish (Mbokane and Moyo, 2018; Nhu *et al*., 2019).

There were no significant differences between the values of the hematological parameters in the control tank and the values obtained in *the ParaProteX* and *MCC mixture* tanks.

3.2. Analysis of the results of biochemical parameters

The biochemical parameters determined in the *ParaProteX* and *MCC* experiments were used to establish the blood metabolic profile and to draw a unitary conclusion on the general physiological condition of the fish population used in the experiments.

The dynamics of the biochemical indicators determined in the 2-year-old carp juvenile was analyzed in the light of the action of *ParaProteX* and *MCC* mixture on the health of the fish and the establishment of an efficient feeding strategy.

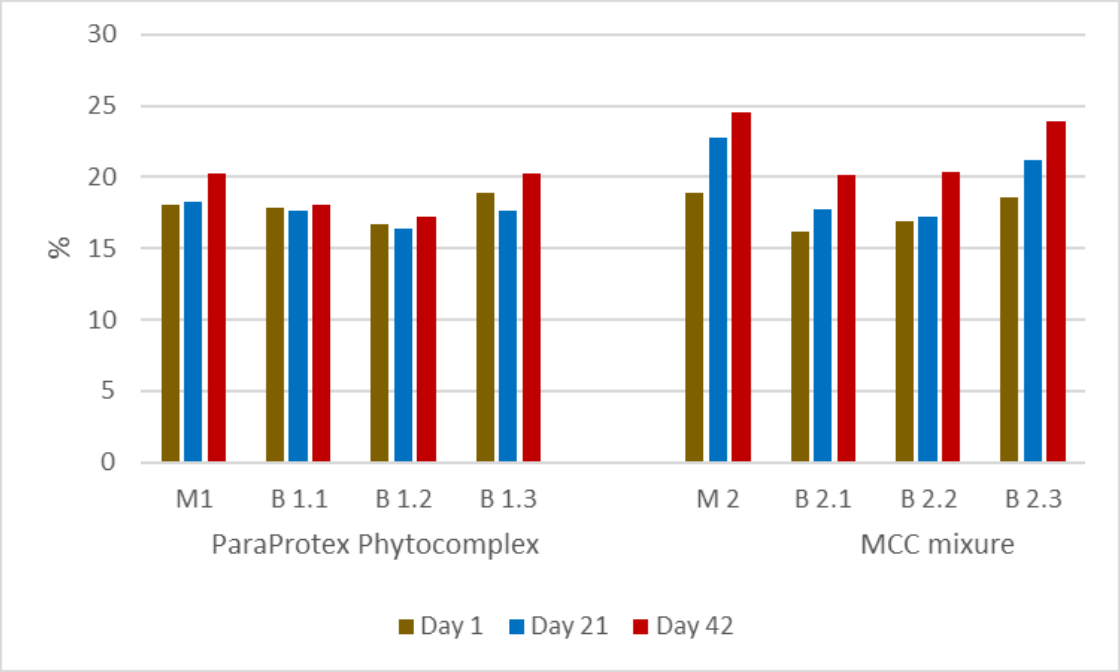

Figure 9. Variation of hematocrit values in fish blood from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.2.1. Total protein level

Knowing the total protein level is the most important biochemical indicator of the nutritional status and fish health.

Plasma protein levels in fish are influenced primarily by the protein value of the used feed, as well as by species, age, sex, physiological status, stage of sexual maturity, water temperature.

Total protein is often considered an indicator of the abundance of immune-related proteins and as such total protein increases in fish serum have been often suggested as signs of enhanced immune responses (Ghelichpour *et al*., 2017; Dehghani *et al.*, 2020).

Normal values of total serum protein in 2-year-old carp juveniles are between 3.4-4.2 g/dL (Patriche T *et al*., 2010), and in adult carp range between 3.5-5.5 g/dL (Siwieki *et al*., 1993).

Total serum proteins recorded high values at the end of the experiment in all experimental tanks with *ParaProteX,* compared to the values recorded at the beginning of the experiment. It results that the food improved with the *ParaProteX* phytocomplex was well consumed by fish, high values being registered in the experimental tank B 1.3, where the fish was fed with the highest concentration of *ParaProteX*, 6g/100g feed (Figure 10).

Total serum proteins at the end of the experiment are higher in all experimental tanks where *MCC* was used, compared to the values recorded at the beginning of the experiment.

It turns out that the improved food with the *MCC* mixture was consumed by the fish, the best values being recorded in experimental tank B 2.2 (4g/dL), where the fish were fed with feed enriched with *MCC* mixture (4g grape marc + 4g cinnamon + 4g cloves per 100g feed / day / tank) (Figure 10).

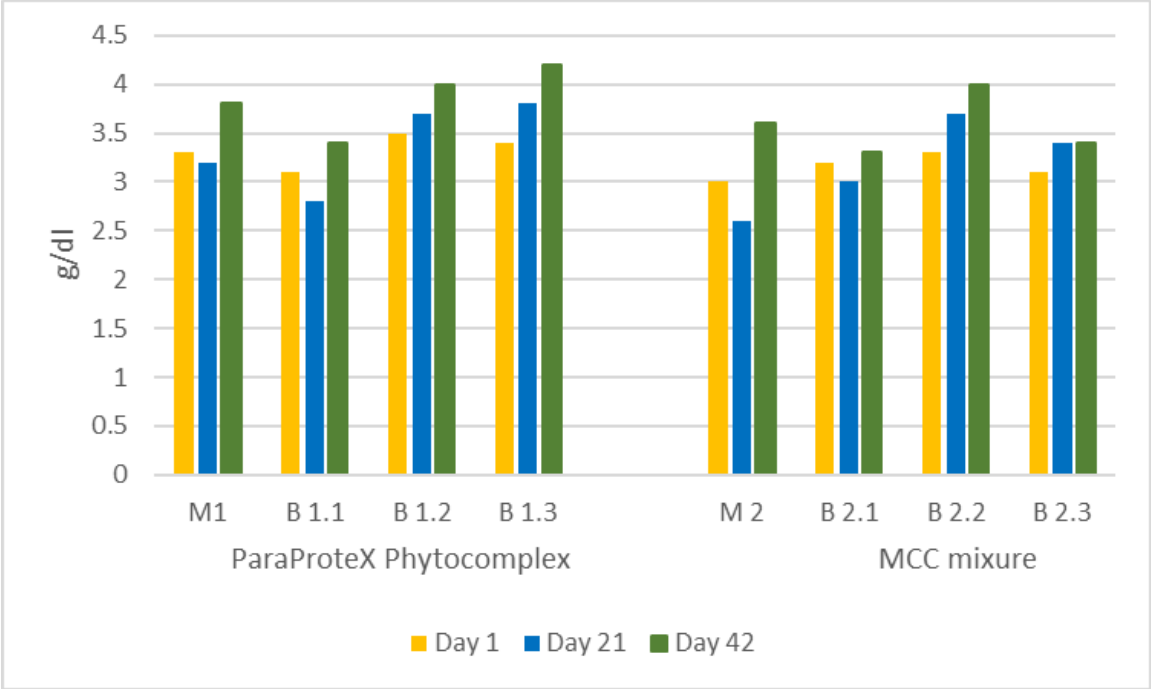

Figure 10. The evolution of total proteins in the blood of fish from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.2.2. Serum glucose

Serum glucose is the fastest method of assessing all acute or chronic stress states that occur in fish population.

In experiment 1 (*ParaProteX*) the serum glucose values recorded in the carp species, *Cyprinus carpio*, 2-year-old brood, at the beginning and end of the experiment were within normal values (V.N. = 40-90 mg/dL).

Serum glucose determined in experiment 2 (*MCC*) at the beginning and end of the experiment, recorded values that fell within the normal range indicated in the literature for the carp species, *Cyprinus carpio*, 2-year-old brood (VN = 40-90 mg/dL).

The values determined in the middle of the experiment show a slight increase, due to the stress of handling and overcrowding, as well as the

adjustment period necessary to change the diet by incorporating *MCC* mixture (grape marc, cinnamon, cloves) in the feed, indicated by the value of serum glucose, within the range at the end of the experiment (Figure 11).

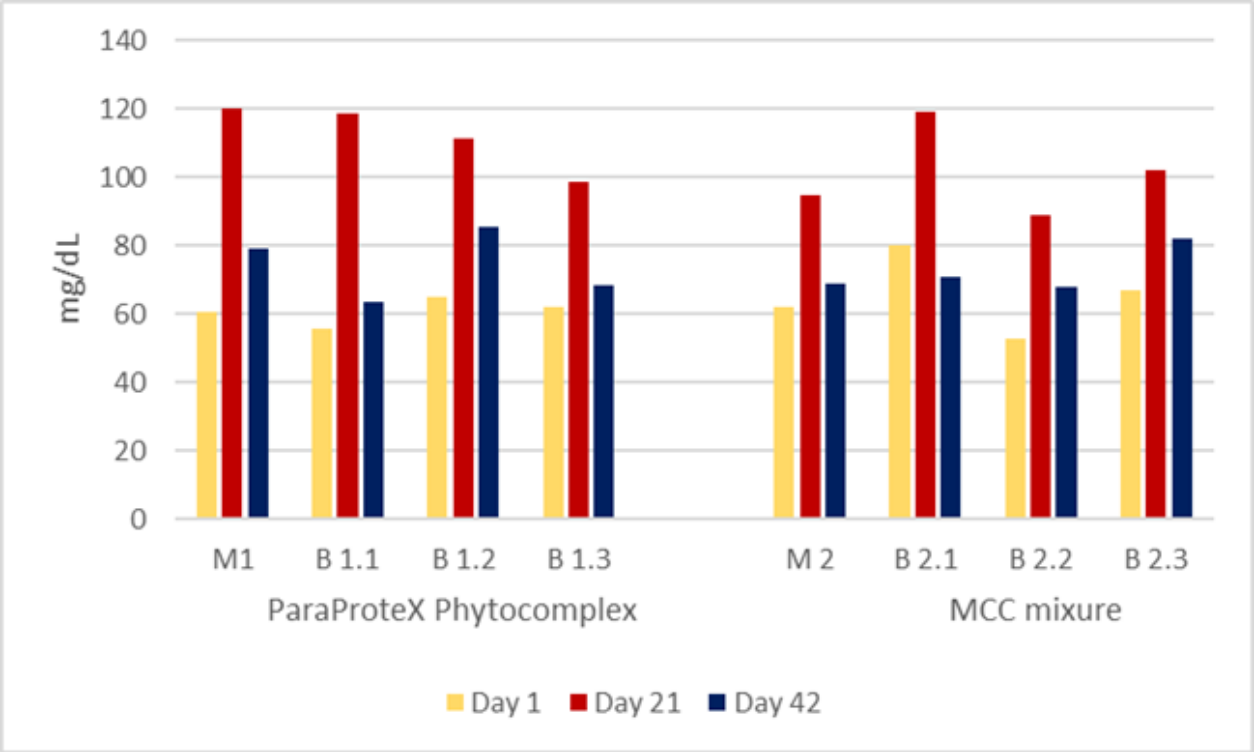

Figure 11. The evolution of serum glucose in the blood of fish from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.2.3. Calcium and serum phosphorus

Calcium (Ca) is the most important mineral component with major implications for bone metabolism. Phosphorus (P) is after calcium, the most abundant mineral element in the body. Phosphorus is found in most tissues, but the highest amount is found in bones, so the Ca/P ratio is important in ossification (Patriche *et al*., 2010).

At the beginning of the experiment with ParaProteX mixture, the fish recorded values below the lower limit given by the specialized literature for the 2-year-old carp brood (VN Ca = 12.1-13.3 mg/dL; P = 11.0-17.4 mg/dL (Patriche *et al*., 2010), especially phosphorus. Then, the values increased constantly, at the end of the experiment recording values close to the normal limit. This indicates an intense bone metabolism, specific to the growth period of the carp juvenile and which was stimulated by *ParaProteX* (Table 1).

Calcium and serum phosphorus recorded at the beginning of experiment 2 (*MCC*) values below the lower limit given by the literature, especially for phosphorus, afterwards the values increased constantly, being at the end of the experiment close to the normal limit. This indicates an intense bone metabolism, specific to the carp juveniles growth period, which was stimulated by the *MCC* mixture (grape marc, cinnamon, cloves) (Table 2).

3.2.4. Serum cholesterol

Cholesterol is an important component of cell membranes and the outer layer of plasma lipoproteins (Patriche *et al*., 2010).

At the beginning of experiment 1 with *ParaProteX*, it was within the normal limits indicated by the literature for the 2-year-old carp brood (VN = 116.5-132.1 mg/dL (Patriche *et al*., 2010), then it increased during the experiment, with

values above the upper limit at the end of the experiment.

This indicates that the state of stress caused by recirculating aquaponic fish farming technology (handling and overpopulation stress) was maintained throughout the experiment (Table 1).

Serum cholesterol at the beginning of experiment 2 (*MCC*) was within normal limits, then it increased during the experiment, with values above the upper limit at the end of the experiment. This indicates that the state of stress caused by intensive fish farming technology (handling and overcrowding stress) was maintained throughout the experiment (Table 2).

3.2.5. Urea and serum creatinine

Urea is the end product of protein catabolism. Serum urea concentration is an important indicator of liver and kidney function (Patriche *et al*., 2010).Creatinine comes only from muscle metabolism. The amount of creatinine in the blood depends only on the total muscle mass, therefore it is the least variable blood parameter (Patriche *et al*., 2010). In the experiment values that were within the normal limits given by the literature for carp

carp for 2 years were recorded (VN Urea = 8.4-11.2 mg/dL; Creatinine = 0.34-0.68 mg/dL (Patriche *et al*., 2010)), showing that the fish were properly fed (Table 1).

Urea and serum creatinine recorded values that were within normal limits, which tells us that the fish were fed with quality feed, and the addition of natural phytocomplex mixture *MCC* (grape marc, cinnamon, cloves) did not affect metabolic processes of fish and did not cause liver and kidney dysfunction (Table 2).

Table 1. The average values recorded for biochemical indicators - calcium, phosphorus, cholesterol, urea, creatinine in Experiment 1 – *ParaProteX*.

Parameter	Experiment 1 ParaProteX	Experiment 1 Day 1	Experiment 1 Day 21	Experiment 1 Day 42	Standard deviantion
Calcium [mg/dL] /SD	Control Tank 1	9.1 ±0.05	9.3 ±0.1	9.3 ±0.1	± 0.1
	Tank 1.1	9.7 ±0.1	9.6 ±0.1	9.8 ±0.2	± 0.1
	Tank 1.2	9.9 ±0.2	10.1 ±0.2	10.5 ±0.5	± 0.3
	Tank 1.3	**9.6 ± 0.7**	**10.5 ±0.9**	**11.4 ± 1.0**	**±0.9**
Phosphorus [mg/dL] /SD	Control Tank 1	5.1 ± 0.4	4.8 ±0.2	5.5 ± 0.5	±0.4
	Tank 1.1	5.9 ± 0.9	5.5 ± 0.8	7.3 ± 1.0	±0.9
	Tank 1.2	5.2 ± 0.9	6.9 ± 1.0	7.1 ± 1.2	±1.0
	Tank 1.3	**6 ± 0.9**	**6.7 ± 0.9**	**8.1± .1.0**	**±1.0**
Cholesterol [mg/dL] /SD	Control Tank 1	123 ± 24.0	136 ± 25.6	172 ± 26.0	±25.4
	Tank 1.1	116 ± 23.4	145 ± 24.0	165 ± 26.7	±24.6
	Tank 1.2	118 ± 23.9	154 ± 25.8	169 ± 28.6	±26.2
	Tank 1.3	**117 ± 12.1**	**124 ± 12**	**141 ± 13.3**	**±12.3**
Urea [mg/dL] /SD	Control Tank 1	8.0 ± 0.3	7.9 ± 0.3	7.5 ± 0.2	±0.3
	Tank 1.1	7.9 ± 0.3	8.5 ± 0.5	7.7 ± 0.3	±0.4
	Tank 1.2	7.9 ± 1.0	7.9 ± 1.0	6.2 ± 1.0	±1.0
	Tank 1.3	**8.3 ± 0.5**	**9.2 ±0.5**	**8.9±0.5**	**±0.5**
Creatinine [mg/dL] /SD	Control Tank 1	0.30 ± 0.02	0.29 ± 0.02	0.33 ± 0.03	±0.02
	Tank 1.1	0.32 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	±0.01
	Tank 1.2	0.29 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	±0.01
	Tank 1.3	**0.30 ± 0.01**	**0.28 ± 0.01**	**0.30± 0.01**	**±0.01**

Table 2. Average values recorded for biochemical indicators - calcium, phosphorus, cholesterol, urea, creatinine in Experiment 2 - phytocomplex mixture *MCC* (grape marc, cinnamon, cloves).

Parameter	Experiment 2 MCC	Experiment 2 Day 1	Experiment 2 Day 21	Experiment 2 Day 42	Standard deviantion
Calcium [mg/dL] /SD	Control Tank 2	9.5 ±0.2	9.7 ±0.3	10.1 ±0.4	±0.3
	Tank 2.1	9.3 ±0.1	9.5 ±0.1	9.5 ±0.1	±0.1
	Tank 2.2	**9.2 ±1.0**	**9.8 ±2.0**	**11.1 ±3.0**	**±2.0**
	Tank 2.3	9.4 ±0.4	10.3 ±0.2	9.75 ±0.1	±0.5
Phosphorus [mg/dL] /SD	Control Tank 2	5.0 ±0.1	4.9 ±0.1	5.4 ±0.1	±0.3
	Tank 2.1	5.7 ±0.02	5.8 ±0.03	5.6 ±0.02	±0.1
	Tank 2.2	**6.0 ±0.1**	**6.9 ±0.2**	**8.0 ±0.6**	**±1.0**
	Tank 2.3	5.5±0.2	5.7 ±0.2	5.9 ±0.2	±0.2
Cholesterol [mg/dL] /SD	Control Tank 2	119 ±14.2	131 ±15.5	149 ±16.0	±15.1
	Tank 2.1	120 ±26.4	177 ±29.3	157 ±30.0	±29.0
	Tank 2.2	**114 ±5.0**	**113 ±5**	**125 ±8.0**	**±6.7**
	Tank 2.3	112 ±18.4	143 ±21.5	156 ±23.4	±22.6
Urea [mg/dL] /SD	Control Tank 2	8.5 ±0.8	8.3 ±0.8	7.2 ±0.6	±0.7
	Tank 2.1	8.4 ±0.2	8.8±0.2	8.7 ±0.2	±0.2
	Tank 2.2	**8.1 ±0.4**	**8.5 ±0.5**	**7.8 ±0.4**	**±0.4**
	Tank 2.3	8.4 ±0.2	8.7 ±0.2	9.0 ±0.5	±0.3
Creatinine [mg/dL] /SD	Control Tank 2	0.31 ±0.01	0.34 ±0.01	0.32 ±0.01	±0.01
	Tank 2.1	0.35 ±0.01	0.36 ±0.01	0.33±0.01	±0.01
	Tank 2.2	**0.33 ±0.02**	**0.32 ±0.02**	**0.36 ±0.02**	**±0.02**
	Tank 2.3	0.40 ±0.03	0.37 ±0.02	0.34 ±0.03	±0.03

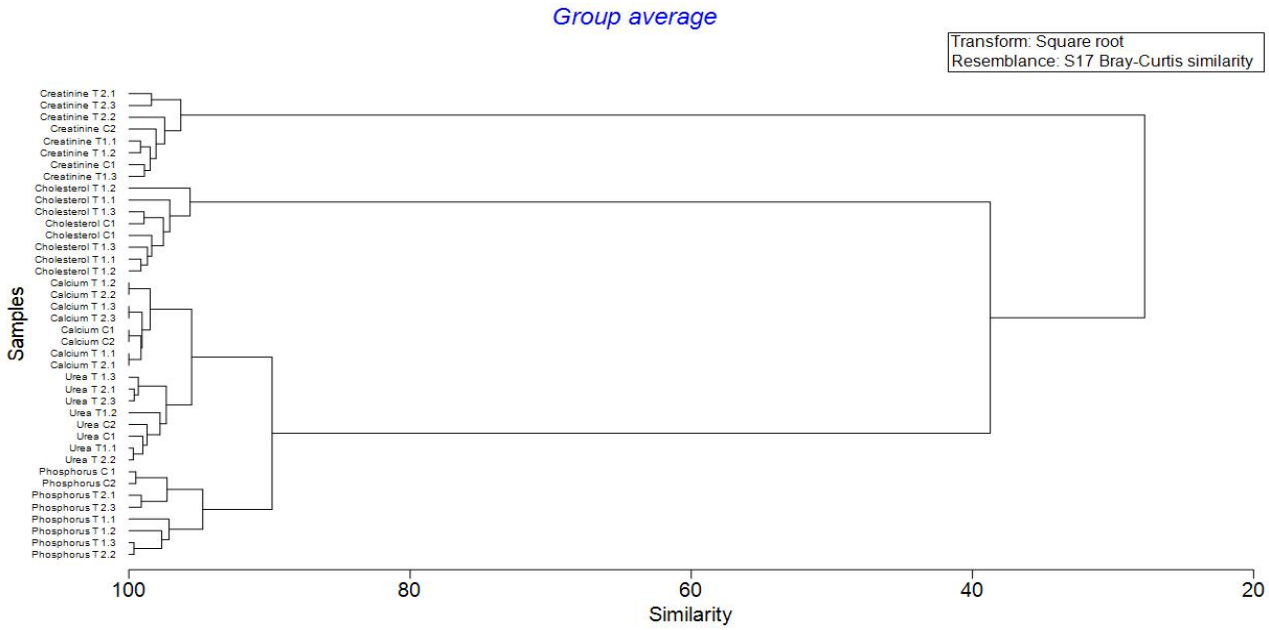

Figure 11. Bray- Curtis matrix – data obtained from the two experiments (square root transformation).

Bray-Curtis matrix showed high similarities (98-100%) between the two experiments in which the two natural phytocomplexes ParaProteX and MCC (grape marc, cinnamon, and cloves) were used (Figure 11).

At the end of the two experiments we can say that the use of the two natural phytocomplexes *ParaProteX* and *MCC* (grape marc, cinnamon and cloves) embedded in fish feed raised in an experimental recirculating aquaponic system type RAS, helped to: grow and develop a healthy biological material, as demonstrated by the values recorded by the hematological and biochemical parameters determined and the improvement of the technological comfort state by reducing the stress and the period of accommodation of the fish to the aquaponic recirculating system. Despite the rich biochemical composition of phytocomplexes used and its multiple bioactivities, the effects synergistic on fish health status are still unknown. The common carp is one of the most popular freshwater fish species cultured throughout the world. It is the fourth most cultured fish with production of more than 4.1 million tons in 2018 contributing 7.7% of the world's total fish aquaculture production (FAO, 2020).

There were no significant differences between the values of the biochemical parameters in the control tank and the values

obtained in the *ParaProteX* and *MCC mixture* tanks.

3.3. Discussions

Aquaponics is a complex cultivation method that has been obtained by combining two different systems, hydroponics and aquaculture. Aquaponic systems operates on the principle that fish waste becomes a resource for plants.

The aim of the experiments was to evaluate the synergistic influence of the combination of standard feed NC 60 II with a content of 32-34% crude protein and a natural plant phytocomplexes, *ParaProteX* mixture, respectively *MCC* mixture (grape marc, cinnamon and cloves) on the health of the 2-year-old carp juvenile for a period of over 42 days. The two mixtures of *ParaProteX* and *MCC* phytocomplexes were chosen for their special antiparasitic, antibacterial, antifungal and antiviral properties.

The dynamics of hematological and biochemical indicators determined in carp juveniles was analyzed in terms of phytocomplexes used in experiments to assess the physiological response of fish to stressful action of technological, physico-chemical factors and to establish a correct and efficient feeding strategy.

A slight decrease in the values recorded for haematological indicators on the 21st day from the beginning of the experiments compared to the values recorded at the beginning of the experiments was observed. Compared to the initial time (day 1) the biological material has undergone a period of adaptation to the new environmental conditions in the RAS type aquaponic recirculation system. Also, the incorporation of *ParaProteX* and *MCC* mixtures in different concentration caused discomfort to the fish population. All hematological parameters recorded at the end of the experiments were within the normal values reported in the literature for the carp species, *C. carpio*.

Total serum proteins recorded values that fall within the normal range of the carp species *C. carpio*, 2-year-old brood. The values recorded for serum proteins at the end of the experiments are increased in all tanks compared to the values recorded at the beginning of the experiments. It turns out that the food improved with the phytocomplexes of *ParaProteX* and *MCC* was well consumed by fish.

Calcium and serum phosphorus recorded at the beginning of the experiments, values below the lower limit given by the literature, especially phosphorus, after which the values increased constantly, registering values close to the normal limit, at the end of the experiments This indicates an intense bone metabolism, specific to the 2-year-old carp hatchery growth period.

Serum cholesterol at the beginning of the experiment was within the normal limits given by the literature, then it increased during the experiment, registering values above the upper limit at the end of the experiment. This indicates that the stress caused by intensive fish farming technology was maintained throughout the experiments.

Urea and serum creatinine recorded values that were within the normal limits given by the literature, which show that the fish were fed with

proper feed, the incorporation of the natural phytocomplexes *ParaProteX* and *MCC* in the feed did not cause digestive discomfort to the fish.

4. Conclusions

In conclusion, these variations in the blood parameters underlined the role of the hematology and biochemical parameters in assessing the homeostatic response of fish to different technological conditions. From the data analysis regarding the hematological and biochemical indicators which emphasize the changes on the physiological status assessment of carp reared in recirculating aquaponic system, it appears that natural phytocomplexes mixture can be used for health improvement and remediation, thus improving the resistance to disease of biological material. The natural phytocomplexes used in feeding the carp have contributed to its adaptation and growth in optimal conditions, specific to the recirculating aquaponic system, through the synergistic action of its components with antibacterial, antiviral, antifungal and antiparasitic effect.

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EFFECT OF HYDRO-ALCOHOL SOLVENT POLARITY ON THE ANTIOXIDANT, ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF FOUR MOROCCAN LETTUCE VARIETIES (*Lactuca sativa* L.): A COMPARATIVE STUDY

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This research work aimed to find a correlation between solvent polarity on the extraction yield, on the content of polyphenols and flavonoids as well as on the antioxidant, antibacterial and anti-inflammatory potencies of two red and two green varieties of *Lactuca sativa* L. hydroalcoholic extracts.

The results showed that extraction with polar solvent (H_2O, 100%) presented maximal yields while the alcohol alone gave the lowest yields. Furthermore, the mixture between these two solvents with different proportions (alcohol with water) showed more other interesting characters than alcohol or water taken separately. Phytochemical contents were affected by varying solvent polarity, within, the extraction with solvent polarity 5.8 (H_2O 25% /Ethanol 75%) showed the highest content of total polyphenols while the polarity of 7.3 (H_2O 50% / Ethanol 50%), was specifically richer in flavonoids. Our results further showed that the extracts of the two red varieties (*capitata* L *nidus tenerrima* and *crispa*) exhibited a broad spectrum of bioactivities more significantly than the two green varieties (*longifolia and capitate* L *nidus jaggeri*). The hydro-alcoholic extracts of polarity 5.8 were the most effective *in vitro* and *in vivo* in the evaluation of the antioxidant, antibacterial and also anti-inflammatory capacities with the best activity against DPPH was recorded for the red variety *Lactuca sativa* var. *crispa*, moreover, this same extract at 1 mg / ml showed a maximal inhibitory activity of 80.8% on the bovine serum albumin denaturation, it showed also a comparable anti-inflammatory effect to dexamethazone which is achieved at high concentrations (2 to 4 g / Kg).

1. Introduction

Lactuca sativa L. is one of the most popular plant worldwide with an increased food consumption (Liu *et al*., 2007). According to statistics of the United Nations Food and Agriculture Organization, the production of lettuce and chicory all over the world was about

26,779,564 tons in 2016, of which China alone being the major producer of about 14,933,121 tons annually.

Botanically, *Lactuca sativa* L. belongs to the family of Asteraceae, its leaves are endowed with minerals element, such as calcium, iron, potassium, magnesium, manganese, copper and zinc (Pirvulescu and Sala, 2013), in addition, it is considered as an excellent origin of phytonutrients that may impact positively human nutrition and Metabolism (López *et al*., 2014; Pinto *et al*., 2015). Nonetheless, lettuce is mentioned much less frequently for its medicinal properties (Harsha *et al*., 2013; Ahangarpour et *al*., 2014) even though previous studies carried out on its seeds have shown its potential health benefits against various conditions including its antimicrobial effects (Edziri *et al*., 2011), anxiolytics (Harsha and Anilakumar, 2013), antioxidants (Komaki *et al*., 2014), anti-inflammatory and analgesic effects as well (Soro *et al*., 2009; Harsha *et al*., 2013).

Generally, in Scientific literature, recommending the relative polarity of the solvent for an optimal depletion of plant material is somewhat scarse. According to some previous studies conducted respectively on the fruits of *Quercus coccifera* L and *Juniperus phoenicea* (El Akrem *et al.,* 2007*)* as well as on aerial part of *Limnophila aromatica (*Quy *et al.,* 2013*)*, confirmed evidence that the yield and quality of the extracted metabolites are associated with solvent polarity and biological activities, indeed using a ternary mixture of solvents help improve the extraction yield and the content of polyphenols which affects bioactivities and the antioxidant potential more particularly.

In this study, we sought to compare the contents of polyphenols according to the quality of solvent depletion with increasing polarities of the leaves of four Moroccan varieties of *Lactuca sativa* and we will further evaluate their antimicrobial, antioxidant and anti-inflammatory capacities.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

In this experimental study, the vegetable products include the four *lactuca sativa* varieties: *longifolia* (Green variety), *capitata L. nidus jaggeri* (Green variety), *capitata L nidus tenerrima* (Red variety) as well as *crispa* (Red variety). These were obtained from a farm located in Kenitra city in the Northwest of Morocco. After drying, the leaves were pulverized, and stored in food bag.

2.1.2.Animals

Male Wistar rats aged between 5 and 6 weeks and weighting 160-200g were obtained from the Emirate Center for wildlife propagation, in Misour, Morocco. The animals were allowed to adapt for a week, with water and food supplied *ad libitum*. The experimental animal Protocol approved by the Animal Ethical Committee, has been conducted in accordance with European legislation.

2.2. Methods

2.2.1. Chemicals

Ethanol was obtained from Fluka (Munich, Germany), Folin–Ciocalteu reagent and $NaNO_2$ were obtained from MERCK (Darmstadt,Germany), Gallic acid, Na_2CO_3, H_2O_2, NaOH, $AlCl_3$ were obtained from Fisher scientific (Illkirch, France), Catechin, DPPH were obtained from Sigma-Aldrich (Saint-Louis, USA), $K_3Fe(CN)_6$ was obtained from Farco chemical supplies (beijing, China), Trichloroacetic acid were obtained from LabChem (Chicago, USA), $FeCl_3$ were obtained from SD fine-chem limited (Maharashtra, India), Ascorbic acid were purshased from Solvachim (Casablanca, Morocco), Formalin were obtained from Pure chems (Tamil Nadu, India).

2.2.2. Preparation of extracts

The solvent extraction was conducted with five solvents of different polarities reported in the Table 1. Soxhlet apparatus is used for continuous extraction until eachi of the solvent

was discolored indicating extraction exhaustion. The extracts were designated as EX_{ij} where i: 1 to 4 for the four species studied and j: 1 to 5 for the five solvent polarities.

Table 1. Water/Ethanol solvents (% by weight) with their experimental and calculated polarities according to Snyder (1978) of the five extracts of each *Lactuca sativa* varieties and their respective designation

(H_2O%)		100	75	50	25	0
(Et-OH%)		0	25	50	75	100
Polarity		10.2*	8.7	7.3	5.8	4.3*
Varieties of *Lactuca sativa*	*Lactuca sativa* L. var. *longifolia*	EX_{11}	EX_{12}	EX_{13}	EX_{14}	EX_{15}
	Lactuca sativa L. var. *capitata* L *nidus jaggeri*	EX_{21}	EX_{22}	EX_{23}	EX_{24}	EX_{25}
	Lactuca sativa L. var. *capitata* L *nidus tenerima*	EX_{31}	EX_{32}	EX_{33}	EX_{34}	EX_{35}
	Lactuca sativa L. var. *crispa*	EX_{41}	EX_{42}	EX_{43}	EX_{44}	EX_{45}

*: Snyder polarity (Snyder. L.R., 1978)

2.2.3. Preliminary phytochemical screening

To determine the class of secondary metabolites, present in the plant product, a qualitative phytochemical screening of *Lactuca sativa* L. species were carried out respectively for alkaloïds, flavonoïds, saponins, tannins, sterols as well as triterpenes following the exact protocol assayed by (Soro *et al*., 2016).

2.2.4. Total phenol content (TPC)

The TPC was determined using the protocol adapted by (Chekroun *et al*., 2015) with some modifications using the known Folin–Ciocalteu reagent. A volume of 100µl of extract or gallic acid as positive control were respectively added to 3 ml of a 2 % Na_2CO_3 solution, and incubated for 5 min. 100 µl of Folin-Ciocalteu reagent (1N) was added to the mixture, the solution was left for 30 min at room temperature. After that, the absorbances were measured at 765 nm against blank solution. The results are expressed in terms of Gallic acid equivalent (mgGAE eq/mg of dry mass).

2.2.5. Total flavonoids content (TFC)

The TFC was carried out according to the protocol of Chekroun *et al*. (2015). Briefly, 0.5ml of extract or cathechin at concentration 1mg/ml were diluted in 2ml of distilled water. 0.15 ml of a 15% Sodium nitrite ($NaNO_2$) was added to the mixture, and incubated for 6 min. 0.15ml of $AlCl_3$ (10%), 2ml of NaOH (4%) and distilled water were added to bring the final volume to 5ml. after 15 min of incubation the absorbance was measured at 510 nm and the TFC are expressed as mg of catechin equivalent (mg CAE/g dry mass).

2.2.6. Antioxidant activity

2.2.6.1. FRAP (ferric-reducing antioxidant power) radical scavenging activity

FRAP assay was tested according to the following method. 1 ml of each extract solution at different concentrations (2 -1 -0.5 -0.25 -0.13 -0.06 mg/ml) received 2.5 ml of phosphate buffer (0.2mol/l, pH 6.6) and 2.5 ml of a 1% potassium hexacyanoferrate ($K_3Fe(CN)_6$) were added. The solution was incubated for 20 min at 50 ºC. After that, 2.5ml of trichloroacetic acid ($C_2HCl_3O_2$) (10%) were added to this solution which was centrifuged at 3,000 r/min for 10 min, then 2.5 ml of the supernatant were diluted into 2.5 ml of distilled water. Finally, 0.5 ml of a 0.1% iron trichloride was added to the mixture (Chekroun *et al*., 2015).

2.2.6.2. 1.1-Diphenyl-2picrylhydrazine (DPPH) free radical scavenging activity

This was assayed exactly as described by (Harsha *et al*., 2013), 50µl of the sample extract at 5 concentrations (2.5 -1.25 -0.63 -0.31 -0.16 mg/ml) were added to 2 ml of phosphate buffer (0.02 M, pH 6) and 1 mL of DPPH (0.2 mM) ($C_{18}H_{13}N_5O_6$) and left for 30 min at room temperature in the dark. Afterwards, the absorbance was measured at 517 nm and activity was expressed as percentage of radical inhibition, the IC_{50} values were determined using XLSTAT 2016 software.

2.2.6.3. Determination of the scavenging effect on hydrogen peroxide

This was performed by the method of (Saumya and Mahaboob, 2011), briefly, a solution of hydrogen peroxide (H_2O_2) (20mmol/l) was introduced in phosphate buffered saline (PBS, 0.1mol/l, pH 7.4). A volume of 1 ml of extracts or standard at concentration (1 mg/ml) were added to 0.6 ml of hydrogen peroxide solution in PBS and incubated for 10 min. The absorbance was measured at 230 nm against a blank solution.

$$\text{H2O2 activity (\%)} = \frac{\text{Abs(1)} - \text{Abs(2)}}{\text{Abs(1)}} * 100 \quad \textbf{(1)}$$

Where: Abs (1): Absorbance of the control and Abs (2): Absorbance of the extracts/standard.

2.2.7. Evaluation of antibacterial activity

The extracts were tested against *Staphylococcus aureus* (Gram+) and *Pseudomonas aeruginosa* (Gram-) bacteria. These bacteria were obtained from the Mohammed V Regional Hospital of Meknes city (Morocco). The *in vitro* antibacterial activity of the scrutinized extracts was assessed as was described by (Smania and Delle, 2006; Balouiri *et al*., 2016) using the micro-dilution method and the minimum inhibitory concentration (MIC) were determined. Bacteria inoculation were prepared and adjusted to 0.5 McFarland standard of turbidity, the extracts were at first prepared at the highest concentration and then serial twofold dilutions were performed (250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 1.9 and 1 mg/mL). 96 well-sterile micro-plates were prepared by dispensing into each well 50 µL of the diluted crude extract. These dilutions were inoculated with 50 µL of a solution containing 10^6 CFU/mL and 160 µL of Mueller-Hinton Broth. In addition, a series of dilution containing Mueller Hinton broth and the tested inoculums was used as positive control; while another series of dilution containing only Mueller Hinton, broth was used as negative control.

The micro-plate was incubated at 35-37 °C for 24 h. The MIC was considered the lowest concentration of the extract that inhibited the growth of 90 % of the microorganism being tested as detected by lack of visual turbidity, matching a negative control. However, the minimum bactericidal concentration (MBC) is the lowest concentration of extract able to kill more than 99.9 % of initial microbial inoculum.

2.2.8. Inhibition of protein denaturation

Inhibition of protein denaturation was evaluated by the method of Brindha and Arun (2014). briefly, the test solution (600 µL) consisted of 500 µL of 1 % bovine serum albumin and 100 µL (1 mg/mL) of each plant extract. The mixture was kept at room temperature for 10 minutes, followed by heating at 51 °C for 20 minutes. The resulting solution was cooled to room temperature and the absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was conducted in triplicate and percent inhibition for protein denaturation was calculated using equation 2:

$$\%\, Inhibition = 100 - \frac{A1 - A2}{A0}.100 \quad \textbf{(2)}$$

Where: A1: the absorbance of the sample, A2: the absorbance of the product control, A0: the absorbance of the positive control

2.2.9. In-vivo antioxidant activity

The rats, distributed into 3 groups of 5 animals, were treated by gavage for seven days. The distribution of animals was performed

randomly as follows: the negative control group received only 1 mL of physiological water and the second and third groups were respectively treated with 1 mL of extract of *Lactuca sativa* L. var *crispa* at the dose of 4 g/kg as well as with 1 mL of ascorbic acid at the dose 10 mg/g. After the treatment period, a blood sample was taken from each rat of the three batches and then centrifuged to recover the plasma (Soottawat *et al*., 2004). The antioxidant activity was then determined by using 100 µL of plasma with the use of the aforementioned protocol (Harsha *et al*., 2013). The inhibition percentage was calculated using equation 3:

$$IP = \frac{Abs\ (1) - Abs(2)}{Abs(2)} * 100 \quad \textbf{(3)}$$

Where: Abs (1): Absorbance of treated group and Abs (2): Absorbance of the control group

2.2.10. Formalin-induced chronic inflammation

This was assayed as described by Al-Hejjaj *et al*. (2011), anti-inflammatory effect was evaluated by formalin-induced paw edema, within 0.1ml of 2% formalin injected into the sub-plantar area of the right hind paw of the ether anaesthetized rats. The extract was administered with doses of 800 mg/kg, 2 g/kg and 4 mg/kg body weight for the batch treated with *Lactuca sativa* L. var *crispa*. The positive control group received a dose of 1 mg/kg of dexamethasone, while the negative control received only 2 mL/kg of physiological water. These treatments were given 30 min prior to formalin injection and continued for four consecutive days. All drugs were administered orally once daily using oral gavage.

2.2.11. Statistical analysis

The results are expressed as mean ± standard deviation (SD) and analyzed by ANOVA test of three determination, a p-value < 0.05 was considered statistically significant.

The heat maps were based on serval matrices containing different sets of information, Hierarchical Clustering Analysis (HCA) was carried out for columns using Euclidean distance. Circle scales were adopted for each individual case. Heat maps were created using the software (XLSTAT 2016, USA), based on their biological activities as was described by Darwish et *al*. (2018).

Principal component analysis (PCA), A multivariate analysis approach was used to reduce a large dataset of variables to a small dataset that still contains most of the information of the large dataset, the results were performed using XLSTAT 2016 software according to Saikat and Jun (2008).

3. Results and discussions

3.1. Preliminary phytochemical test

The four varieties of lettuce were found to contain tannins, flavonoids, anthocyanins, along with sterols through preliminary phytochemical screening, data are summarized in the Table 2.

Table 2. Phytochemical screening of the four varieties of *Lactuca sativa* L. studied

Secondary metabolism families	**GREEN VARIETIES**		**RED VARIETIES**	
	longifolia	*capitata* L *nidus jaggeri*	*capitata* L *nidus tenerima*	*crispa*
Tanins	P	P	P	P
Flavonoids	P	P	P	P
Anthocyans	P	P	P	P
Sterols - triterpens	P	P	P	P
Mucilage	P	P	P	P
Alcaloïdes	A	A	A	A
P : Presence				**A : Absence**

3.2.Yield of extraction

The yield of extraction depends on solvent polarity, temperature, extraction time as well as lettuce variety of the sample. Under the same extraction time and temperature, solvent and sample chemical composition remain the most important parameters. In our study, extracts of the four varieties of *Lactuca sativa* L. were obtained by using different proportions of water and ethanol (H_2O/EtOH) as solvents of extraction.

Table 3. Solvent polarity on the extraction yields expressed as % of dry leaves powder of the four varieties *Lactuca sativa* L. For each solvent, values lacking a common letter are significantly different at $p < 0.05$ (Tukey's HSD test)

SOLVENT POLARITY	Green varieties		Red varieties	
	longifolia	*capitata* L *nidus jaggeri*	*capitata* L *nidus tenerima*	*crispa*
4.3	15.33 ± 1.10^{E}	14.33 ± 1.60^{E}	13.33 ± 1.20^{E}	19.67 ± 1.30^{DE}
5.8	28.33 ± 2.50^{CD}	26.67 ± 2.60^{CD}	25.33 ± 2.50^{CD}	29.00 ± 3.00^{BC}
7.3	34.67 ± 3.70^{AB}	35.33 ± 3.40^{ABC}	38.33 ± 3.80^{AB}	41.33 ± 4.30^{A}
8.7	42.67 ± 3.70^{AB}	36.67 ± 3.70^{AB}	35.00 ± 3.40^{ABC}	34.67 ± 3.70^{AB}
10.2	43.67 ± 4.20^{A}	42.33 ± 4.10^{A}	37.00 ± 3.80^{AB}	38.67 ± 4.00^{A}

Our results are depicted in Table 3. *Extraction* yield increased with the increasing percentage of water giving rise of the respective solvent polarities 4.3, 5.8, 7.3, 8.7 and 10.2. Moreover, compared to other mixtures, the ethanolic extract showed low yields ranging from (13.33 ± 1.2^{E} % to 19.67 ± 1.3^{DE} %) and the extracts of polarity 7.3 and 8.7 presented almost the same yield for the four varieties (34.67 ± 3.70^{AB} % ≤yield≤ 42.67 ± 3.70^{AB} %). On the other hand, aqueous extracts exhibited a higher yield ranging from (37.00 ± 3.80^{AB} % to 43.67 ± 4.20^{A} %) due probably to highly glycosylated phenolics and other non-phenolic primary metabolites such as aminoacids, polypeptides and carbohydrates.

Table 4. Total polyphenol content (expressed as mg GAE/mg dry weight, means ± standard deviation in function of the solvent polarity of the four varieties. For each solvent, values lacking a common letter are significantly different at $p < 0.05$ (Tukey's HSD test)

SOLVENT POLARITY	Green varieties		Red varieties	
	longifolia	*capitata* L *nidus jaggeri*	*capitata* L *nidus tenerima*	*crispa*
4.3	0.10 ± 0.03^{FGH}	0.07 ± 0.02^{GH}	0.17 ± 0.02^{CDE}	0.28 ± 0.03^{B}
5.8	0.13 ± 0.01^{EFG}	0.15 ± 0.01^{DEF}	0.35 ± 0.04^{A}	0.39 ± 0.02^{A}
7.3	0.09 ± 0.01^{FGH}	0.11 ± 0.01^{EFGH}	0.26 ± 0.03^{B}	0.37 ± 0.01^{A}
8.7	0.09 ± 0.01^{FGH}	0.10 ± 0.01^{EFGH}	0.23 ± 0.00^{BC}	0.22 ± 0.03^{BCD}
10.2	0.06 ± 0.03^{H}	0.07 ± 0.03^{GH}	0.24 ± 0.01^{B}	0.15 ± 0.04^{DEF}

As regard of flavonoids chemical content, the obtained results revealed that the red varieties presented likewise the most important content of flavonoids mostly observable with solvents polarities of 7.3 and 8.7 respectively for *crispa* variety and at polarities 8.7 and 10.2 for *nidis tenerrima* variety. The results are presented in table 5 below.

3.3. Total phenolic content

Phenolic compounds in plants constitute one the major class of secondary plant metabolites with well-known bioactive potential attributed

in great part to their antioxidant, antiradical and antibacterial activities.

The extraction of the phenolics is influenced by both the polarity of the solvents and the variety of *Lactuca sativa* L as well. The yields of phenolics from the two red varieties were significantly superiors compared to the 2 other green varieties, this data were in agreement with the finding reported by (Llorach et *al*., 2008) who have studied five Spain lettuce varieties. We next observed that in the case of red varieties, the solvents polarities of 5.8 and 7.3 have significantly depleted more polyphenols content compared with the other ones, of these, the variety *Lactuca sativa* L. var. *crispa* had the most important polyphenols content. As regard of the green varieties, i.e. *Lactuca sativa* L. var. *longifolia* and *Lactuca sativa* L. var. *capitata nidus jaggeri*, they did show no significant difference in all extraction field Table 4.

Table 5. Total flavonoids content expressed as mg CAE/g dry weight, means ± standard deviation according to the solvent polarity of the four varieties. For each solvent, values lacking a common letter are significantly different at $p < 0.05$ (Tukey's HSD test)

SOLVENT POLARITY	Green varieties		Red varieties	
	longifolia	*capitata* L *nidus jaggeri*	*capitata* L *nidus tenerima*	*crispa*
4.3	1.41 ± 0.02^{O}	1.44 ± 0.04^{O}	3.74 ± 0.03^{G}	3.91 ± 0.01^{G}
5.8	2.58 ± 0.16^{JK}	2.34 ± 0.03^{L}	6.97 ± 0.1^{D}	6.45 ± 0.02^{E}
7.3	2.71 ± 0.01^{I}	2.98 ± 0.03^{H}	9.59 ± 0.01^{B}	10.27 ± 0.02^{A}
8.7	2.80 ± 0.02^{HI}	2.41 ± 0.16^{KL}	7.54 ± 0.01^{C}	9.45 ± 0.06^{B}
10.2	1.66 ± 0.04^{N}	2.12 ± 0.01^{M}	7.45 ± 0.07^{C}	5.87 ± 0.1^{F}

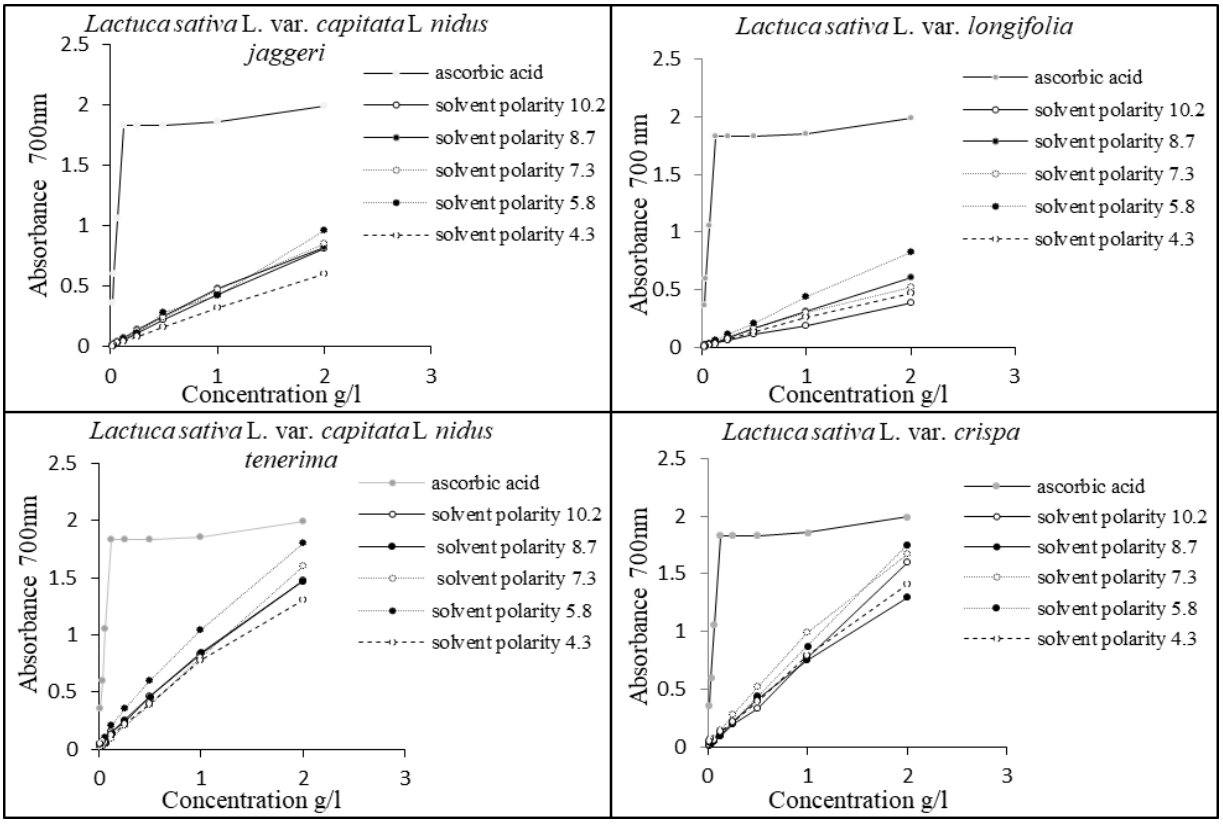

Figure 1. Reducing power of ascorbic acid and *of Lactuca sativa* L. extracts in different solvent polarities

Furthermore, previous elegant reports have been reported about characterization of the individual polyphenolics in a variety of lettuce extracts, Llorash et *al*.(2008) reported that the lettuce is a species rich particularly in hydroxycinnamic acid such as the caffeic acid derivatives along with flavonol derivatives including quercetin and a flavone such as luteolin, Moreover in our recent study we also highlighted the presence of ferulic acid with a prenyl chain (GOFA) in *Lactuca sativa* var *crispa* species (Zekkori et *al*., 2018).

3.4. Antioxidant activity

3.4.1 FRAP (ferric-reducing antioxidant power) radical scavenging activity

The FRAP activity of *Lactuca sativa* L. of 20 extracts was investigated by following reduction Fe^{3+} to Fe^{2+}. The results are depicted in the Figure. 1 and clearly showed that the red varieties exhibited an important reduction power compared to the green varieties. Moreover, at a concentration of 2 mg/mL they also clearly manifested an antioxidant activity comparable to the ascorbic acid. Moreover, all extracts showed a clear dose-response relationship. Also, the hydro-alcoholic extracts of polarity 5.8 present a significant reduction power compared to aqueous and ethanolic extracts.

3.4.2. DPPH free radical scavengingactivity

2-diphenyl-1-picrylhydrazyl (DPPH) is a radical able to take one electron or one hydrogen radical and next form a stable diamagnetic non-radical product, it changes its color from purple to yellow indicating an antioxidant potential of the sample solution. For this Assay, Ascorbic acid was used as a positive control (IC_{50}=0.03±0.00^{A}). According to the results obtained, the activity of the four *Lactuca sativa* L. subspecies with their respective IC_{50} values (mg/mL) in different solvent polarities are depicted in Table 6.

The IC_{50} values were determined to help evaluate the content of the samples required for 50% inhibition of DPPH radicals. The extracts of *Lactuca sativa* L. var. *crispa* have shown the best free radical scavenger potency for respectively the solvents extracts polarities 10.2; 8.7; 5.8 and 4.3. the IC_{50} were ranged from (1.7±0.8^{GHI} mg/mL) for extract of polarity 5.8 to (9.1±0.8^{D} mg/mL) for extract of polarity 4.3, furthermore, the extracts of *Lactuca sativa* L. var. *capitata* L nidus *tenerima* showed also an interesting result of bioactivity with an IC_{50} of (1.2±0.7^{HI} mg/mL) for the Extract of polarity 7.3.

Table 6. IC_{50} values (mg/mL) of extracts of four varieties of *Lactuca sativa* L. in different solvent polarities. For each solvent, values lacking a common letter are significantly different at $p < 0.05$ (Tukey's HSD test)

SOLVENT POLARITY	Green varieties		Red varieties	
	longifolia	*capitata* L *nidus jaggeri*	*capitata* L *nidus tenerima*	*crispa*
4.3	22.7 ± 0.7^{B}	27.1 ± 1.2^{A}	11.6 ± 0.1^{C}	9.1± 0.8^{D}
5.8	4.1 ± 1.0^{FG}	5.6 ± 0.3^{EF}	1.9 ± 0.4^{GHI}	1.7 ± 0.8^{GHI}
7.3	8.7 ± 0.2^{D}	5.5 ± 0.6^{EF}	1.2 ± 0.7^{HI}	2.1 ± 0.6^{GHI}
8.7	6.4 ± 0.1^{E}	5.3 ± 0.4^{EF}	3.7 ± 0.4^{FG}	2.3 ± 0.9^{GHI}
10.2	7.4 ± 0.6^{DE}	7.0 ± 0.3^{DE}	3.3 ± 0.1^{FGH}	2.3 ± 0.3^{GHI}

The lower the IC_{50} value, the higher the antioxidant capacity of the sample extract Table 3. *Lactuca sativa* L. var. *longifolia* and *Lactuca sativa* L. var. *capitata* L *nidus jaggeri* varieties exhibited the greatest IC_{50} values, which means that they were less active at the antioxidant level. Our results mirrored those already reported by (Gan and Azrina, 2016).

3.4.3. Determination of the scavenging effect on hydrogen peroxide

As shown in Table 7, all the four varieties of lettuce showed a positive activity on the free radicals of hydrogen peroxide, in addition, the extracts of polarity 5.8 had the most significant activity followed by the extracts of polarity 7.3, 8.7 and the aqueous extract. In contrast, the ethanolic extract possesses the lowest activity of neutralization of the above-mentioned free radicals.

Table 7. Hydrogen peroxide scavenging activity in function of the extracts of different solvent polarities. For each solvent, values lacking a common letter are significantly different at $p < 0.05$ (Tukey's HSD test)

SOLVENT POLARITY	Green varieties		Red varieties	
	longifolia	*capitata* L *nidus jaggeri*	*capitata* L *nidus tenerima*	*crispa*
4.3	0.42 ± 0.01^{I}	0.58 ± 0.03^{G}	0.42 ± 0.02^{I}	0.57 ± 0.02^{G}
5.8	0.59 ± 0.01^{FG}	0.72 ± 0.03^{D}	0.95 ± 0.02^{A}	0.91 ± 0.03^{B}
7.3	0.46 ± 0.01^{H}	0.70 ± 0.02^{DE}	0.90 ± 0.03^{B}	0.81 ± 0.02^{C}
8.7	0.46 ± 0.01^{H}	0.67 ± 0.03^{E}	0.67 ± 0.01^{E}	0.73 ± 0.03^{D}
10.2	0.43 ± 0.01^{HI}	0.62 ± 0.01^{F}	0.60 ± 0.02^{FG}	0.60 ± 0.03^{FG}

3.4. Evaluation of antibacterial activity

According to the preliminary phytochemical tests assayed, the hydroalcoholic extracts of *Lactuca sativa* subspecies were endowed with a significant content of polyphenols and flavonoids which influenced the antibacterial activity. This bioassay was evaluated by the method of microdilution and the results are summarized in Table 8 and revealed that the extracts of the four lettuce varieties tested against the *Staphylococcus aureus* strain presented a tolerance for 18 extracts from the 20 tested, however, a notable bacteriostatic effect was observed for the two extracts of polarities 4.3 and 5.8 of the plant *Lactuca sativa* L.var. *capitata* L.*nidus jaggeri*. The aqueous extract, the extract of polarity 8.7 and the ethanolic extract of the plant *Lactuca sativa* L. var. *capitata* L. *nidus tenerrima* have showed the same MIC. Our results are confirmatory to those already reported by (Edziri et *al*. 2011).

As regards to the *Pseudomonas aeruginosa* strain Table 8B, this also showed a tolerance for the majority of the extracts with the exception being observable with *Lactuca sativa* L. var. *capitata L. nidus jaggeri* variety where an evident bacteriostatic activity for the extracts of polarities respectively of 4.3, 5.8, 7.3 and 8.7 have been demonstrated.

Table 8. Antibacterial activity of *Lactuca sativa* L. extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. If MBC/MIC < 2, the extract presents a bactericidal effect, if MBC/MIC > 2, the extract is bacteriostatic and if MBC /MIC > 32, the bacterial strain presents a tolerance effect (T) **MIC* ***MBC and ****MBC/MIC*.

A: *Lactuca sativa* L. extracts against *Staphylococcus aureus* strain				
SOLVENT POLARITY	*Lactuca sativa* L. var. *longifolia*	*Lactuca sativa* L. var. *capitata* L *nidus jaggeri*	*Lactuca sativa* L. var. *capitata* L *nidus tenerima*	*Lactuca sativa* L. var. *crispa*
4.3	7.0* -** T***	1.5 43.5 29.0	3.0 - T	2.0 - T
5.8	9.0	2.0	11.0	2.0

	- T	54.0 27.0	- T	- T
7.3	9.0 - T	10.0 - T	14.0 - T	8.0 - T
8.7	1.0 - T	9.5 - T	3.0 - T	6.0 - T
10.2	9.0 - T	9.0 - T	3.0 - T	9.0 - T
B : *Lactuca sativa* L. extracts against *Pseudomonas aeruginosa* strain				
SOLVENT POLARITY	*Lactuca sativa* L. var. *longifolia*	*Lactuca sativa* L. var. *capitata* L *nidus jaggeri*	*Lactuca sativa* L. var. *capitata* L *nidus tenerima*	*Lactuca sativa* L. var. *crispa*
4.3	115.0* 119.0** 1.6***	8.0 43.5 5.4	3.0 - T	10.0 - T
5.8	140.0 - T	10.0 54.0 5.4	11.0 - T	1.0 - T
7.3	- - T	54.0 250.0 5.6	14.0 - T	8.0 - T
8.7	- - T	49.0 250.0 5.1	13.0 - T	250.0 250.0 1.0
10.2	- - T	48.0 - T	- - T	- - T

The extract of polarity 4.3 of the plant *Lactuca sativa* L. var. *longifolia* has a bactericidal character from the concentration (190 mg/mL), whereas the extract of polarity 8.7 of the plant *Lactuca sativa* L.var. *crispa* demonstrated a bacteriostatic effect at a concentration of (250 mg/mL). In general, *Lactuca sativa* L. has a remarkable inhibitory effect at high concentration, this can be explained by the low concentration of the active molecule or molecules that can exert a synergetic effect on these above-mentioned strains.

3.5.Assessment of *in-vitro* anti-inflammatory activity / Inhibition of albumin denaturation

Anti−inflammatory activity of the *Lactuca sativa* L. extracts was evaluated against heat–induced denaturation of bovine serum albumin. The outcomes of these experiments are summarized in Table 9, within, we observed an increase of the absorbance in the test samples with respect to the negative control which indicated the stabilization of the protein.

All extracts of *Lactuca* species were able to inhibit protein denaturation in a concentration-dependent manner and exhibited appreciable inhibition of heat-induced protein denaturation ranged from (25.47 ± 1.47^{EF} %) to (80.36 ± 3.6^{A} %) for *Lactuca sativa* L. var. *crispa* , (20.02 ± 0.16^{FGH} % to 66.59 ± 2.97^{B} %) for

Lactuca sativa L. var. *capitata* L *nidus tenerrima*, (15.91±2.32^{HI} %) to (50.12±1.74^{CD} %) for *Lactuca sativa* L. var. *capitata* L *nidus jaggeri* and (11.75±2.25^{I} %) to (44.77±3.38^{D} %) for *Lactuca sativa* L. var. *longifolia*.

From the result of the present study, at the solvent polarity 5.8, *Lactuca sativa* L. var. *crispa* showed (80.8 %) as the maximum inhibitory activity on protein denaturation at 1 mg/ml (Table 9).

Table 9. Percentage inhibition of the bovine serum albumin denaturation of the extracts of *Lactuca sativa* L. for each solvent, values lacking a common letter are significantly different at $p < 0.05$ (Tukey's HSD test)

	Green varieties		**Red varieties**	
SOLVENT POLARITY	*Lactuca sativa* L. var. *longifolia*	*Lactuca sativa* L. var. *capitata* L *nidus jaggeri*	*Lactuca sativa* L. var. *capitata* L *nidus tenerima*	*Lactuca sativa* L. var. *crispa*
4.3	11.75±2.25^{I}	15.91±2.32^{HI}	20.02±0.16^{FGH}	25.47±1.47^{EF}
5.8	44.77±3.38^{D}	50.12±1.74^{CD}	66.59±2.97^{B}	80.36±3.6^{A}
7.3	11.39±2.51^{I}	43.79±3.59^{D}	56.14±1.79^{C}	70.02±1.74^{B}
8.7	24.14±1.11^{EFG}	29.23±2.03^{E}	56.24±1.80^{C}	65.3±0.84^{B}
10.2	22.59±3.00^{EFGH}	17.49±3.82^{GHI}	50.55±5.14^{CD}	56.10±1.84^{C}

3.6. Data analysis

The hierarchical ascending classification of the twenty extracts from the four varieties of *Lactuca sativa* L as well as their designations are depicted in the Table 1, by the dissimilarity of the Euclidian distance based on Ward's Aggregation Algorithm, entailed the distribution of these extracts into three main group as depicted in Figure 2:

The first group includes the ethanolic extracts of polarity 4.3 of the four varieties (EX_{15}, EX_{25}, EX_{35} and EX_{45}), the aqueous as well as the hydroalcolic extracts of polarity 10.2 and 5.8 (EX_{11}, EX_{21}, EX_{14} and EX_{24}) of the two green varieties.

The second group has hydroalcolic extracts (EX_{12} and EX_{22}) of polarity 8.7 for green varieties and polarity extracts 7.3 (EX_{33} and EX_{43}) of red varieties as well.

Finally, the third group contains the extracts of polarity 7.3 (EX_{13} and EX_{23}) of the two green varieties, along with the extracts (EX_{34}, EX_{41} and EX_{44}) of polarity 5.8 and 10.2 belonging to the red varieties.

The heat map depicted in Figure 2, demonstrate that the polarity of the solvent and the type of lettuce cultivars influenced strictly the character of each extract. Indeed, the extract EX_{44} of polarity 5.8 and which belongs to the variety *Lactuca sativa* L. var. *crispa* encompass both the antioxidant, antibacterial and anti-inflammatory character due to its high content of polyphenol and flavonoids.

More particularly, statistical analysis by heat map technique shows that the extracts of the two red varieties as well as the EX_{24} extract tend to reduce ferric iron to ferrous iron, whereas, the green varieties have only low activity.

Furthermore, EX_{33}, EX_{34}, EX_{43} and EX_{44} have strong activity as regards of the inhibition of hydroxyl and DPPH radicals, while the extracts EX_{42}, EX_{32}, EX_{22}, EX_{23}, EX_{24} exhibited moderate activity.

In terms of antimicrobial activity, *Staphylococcus aureus* would be sensitive to both extracts EX_{12} (MIC = 1 g/L) and EX_{25} (MIC = 1.5 g/L), while extracts EX_{24}, EX_{44} and EX_{45} exhibited a less activity. On the other hand, the bacterium *Pseudomonas aerugiosa* tolerates all the extracts except the EX_{44} extract which exhibited an inhibition of it at a concentration of 1 g/L.

The anti-inflammatory activity tested *in vitro* showed that the hydro-alcoholic extracts of polarity 8.7 and 7.3 of the green varieties and the hydro-alcoholic extracts of the red varieties

except the ethanolic extracts of polarity 4.3 possess a notable anti-inflammatory activity. The results of the PCA analysis are given as a two-dimensional correlation bi-plot and are depicted in Figure 3, the five extracts for each of the four varieties (i.e., 20 extracts) were studied, the results are presented in Table 1. The correlation matrix among the total extractions is summarized in Figure 3, thus capturing (75.02 %) of the total data variability.

Pearson's correlation analysis was performed to assess the correlations between matrix on plant extracts based on the antioxidant, antibacterial and anti-inflammatory activities and the content of polyphenols and flavonoids, the results showed significant positive correlations were found between content of polyphenol and flavonoids, antioxidant activities and anti-inflammatory activity with Pearson's correlation coefficients of (0.810, 0.824 and 0.660), respectively for FRAP, DPPH and H_2O_2 as well as a correlation coefficient of 0.787 with flavonoid content.

The flavonoid contents showed also a higher correlation versus antioxidant activities FRAP (0.830), DPPH (0.754) and H_2O_2 (0.729).

The antioxidant activity by H_2O_2 test showed similarly a positive correlation with DPPH test (0.822) and FRAP test (0.692)

In addition, DPPH test and FRAP test showed also a positive correlation between them (0.727).

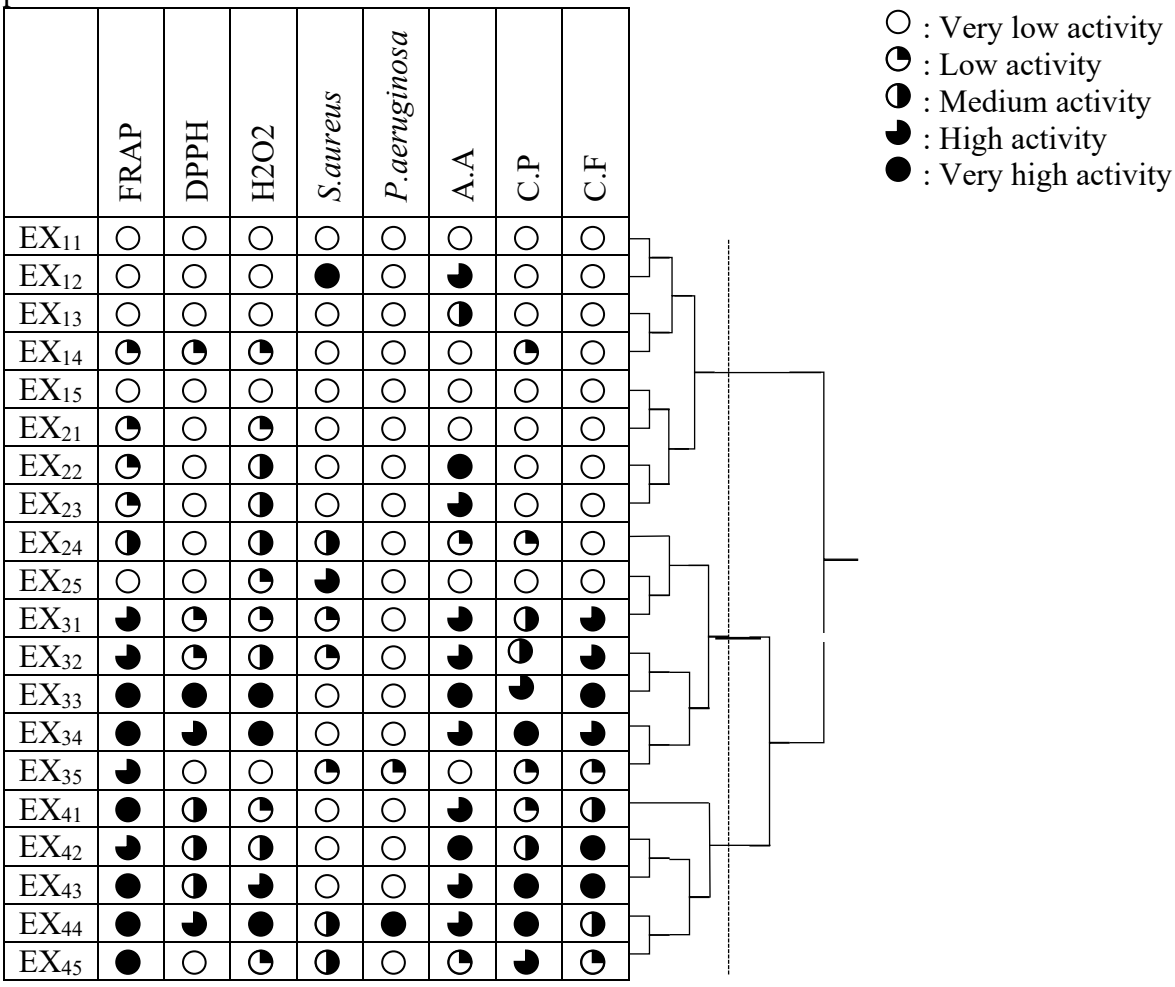

Figure 2. Heat map showing classification of 20 *Lactuca sativa* L. extracts activities. FRAP: Ferric-reducing antioxidant power, DPPH: Antioxidant activity on DPPH free radical, H_2O_2: Antioxidant activity on hydrogen peroxide, C.F: Content of flavonoid, C.P: Content of polyphenol, S.aureus: Antibacterial activity against *staphylococcus aureus,* P. aeruginosa: Antibacterial activity against *Pseudomonas aeruginosa,* AA: Anti-inflammatory activity

Table 10 recapitulates the coefficient correlations (R) obtained between biological activities and Total phenols as well as flavonoids contents, within, we evaluated anti-inflammatory activities and observed that anti-inflammatory activity presented a positive correlation between antioxidant activities FRAP (0.780), DPPH (0.824), H_2O_2 (0.837), polyphenol content (0.790) and flavonoid content (0.821), however, the antibacterial activity of *pseudomonas aeruginosa* presented a low correlation with the content of polyphenol (0.161), while the antibacterial activity of *staphylococcus aureus* exhibited a negative correlation.

Lactuca sativa L. var. *crispa* extract EX_{44} of polarity 5.8 showed the higher F1 and F2 factors respectively 4.171 and 3.223. PCA analysis confirmed that this extract presents antioxidant, anti-inflammatory and antibacterial properties since they are positively correlated with each other. This implies, solvent polarity influenced the polyphenols and flavonoids content of the extracts which in turn influenced the antioxidant, antibacterial and anti-inflammatory activities.

Table 10. Pearson's correlation coefficients of antioxidant, antibacterial((S.a: *Staphylococcus aureus*)(P.s: *Pseudomonas aeruginisa*)) and anti-inflammatory (AA) activities capacities, total polyphenol content (CP) and total flavonoid content (CF).

Parameters	FRAP	DPPH	H_2O_2	1/MIC *S.a*	1/MIC *P.s*	AA	CF	CP
FRAP	**1**							
DPPH	**0.727**	**1**						
H_2O_2	**0.692**	**0.822**	**1**					
1/MIC *S.aureus*	-0.099	-0.269	-0.198	**1**				
1/MIC *P.aeruginosa*	0.400	0.310	0.371	0.264	**1**			
Anti-inflammatory	**0.780**	**0.824**	**0.837**	-0.145	0.400	**1**		
CF	**0.830**	**0.754**	**0.729**	-0.118	0.426	**0.821**	**1**	
CP	**0.810**	**0.824**	**0.660**	-0.209	0.161	**0.790**	**0.787**	**1**

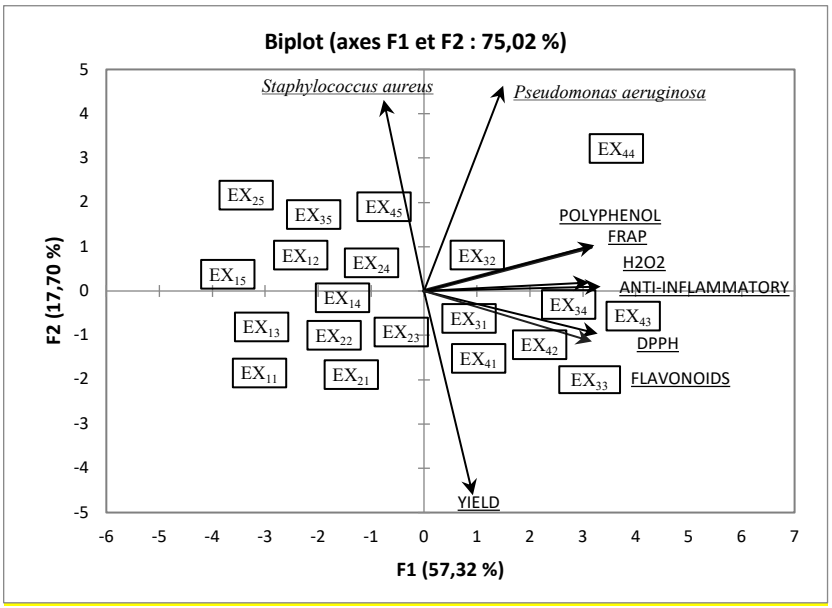

Figure 3. Bi-plot representation on the factor-plane (PC1–PC2) showing vector distribution of phytochemical contents, antioxidant and anti-bacterial activities within score plot of 20 extracts of *Lactuca sativa* L. varieties

3.7. In-vivo antioxidant activity

In vitro experiments may be a useful indicator of a potential in vivo bioactivity, the method was conducted to evaluate the antioxidant capacity of plasma against the DPPH radicals, and the activity was performed more particularly to assess in-vivo antioxidant activity of the extract EX_{44}, our results are depicted in Table 11 and showed that the administration of EX_{44} at 4 g/kg body weight dose, exhibited a maximum absorption at the wavelength of 517 nm as opposed to the negative control. Moreover, no significant difference using Tukey's HSD test was observed between ascorbic acid (IP=29.94%±1.00^{A} %) and the EX_{44} extract (IP=29.12%±3.00^{A} %).

Table 11. Plasma absorbance of rats treated by extract EX_{44} and ascorbic acid as a positive control, compared with a negative control, Different letters in superscript indicate significant difference at $p < 0.05$ (Means and SD as error bars, Tukey's HSD test).

Treatment	Dose	**Antioxidant activity of plasma (Means ± SD)**
Negative Control	1 mL/kg	22.09%±2.00^{B}
Ascorbic acid	10 mg/kg	29.94%±1.00^{A}
Extract EX_{44}	4 g/kg	29.12%±3.00^{A}

3.8. In-vivo anti-inflammatory Activity

Formalin administrated to the paw of rats causes inflammatory pain by inducing capillary permeability and liberating endogenous substances which excite the pain nerve ending thus producing swelling of the paw (Viswanatha, et *al*., 2011). In this test, all the doses (0.8, 2 and 4 g/kg body weight) of the extract EX_{44} have shown significant inhibition of formalin - induced paw edema as compared to negative controls Table 12. On the other hand, the positive control carried out with dexamethasone, showed no significant difference compared to the batch treated by this extract at the doses of 2 and 4 g/kg body weight, these results supported the results recently reported by (Gyawali et *al*., 2020).

Table 12. Kinetics of anti-inflammatory activity of the EX_{44} extract and Dexamethasone compared to a negative control, Different letters in superscript indicate significant difference at $p < 0.05$ (Means and SD as error bars, Tukey's HSD test.

Treatment	**Dose**	**Development of volume paw (mL) (Means ± SD)**			
		Day 1	**Day 2**	**Day 3**	**Day 4**
Negative Control	1 mL/kg	1.59 ± 0.06^{B}	1.68 ± 0.08^{A}	1.36 ± 0.06^{A}	1.15 ± 0.06^{A}
Dexametasone	10 mg/kg	1.08 ± 0.04^{A} 32.5%	0.76 ± 0.08^{C} 49.4%	0.70 ±0.20^{C} 43.0%	0.75 ± 0.09^{C} 39.2%
Extract EX_{44}	0,8 g/kg	1.15 ± 0.09^{C} 29.4%	1.01 ± 0.07^{B} 33.1%	0.97 ± 0.07^{B} 26.0%	0.90 ± 0.05^{B} 25.0%

	2 g/kg	1.03 ± 0.06^{D} 34.4%	0.95 ± 0.07^{B} 43.8%	0.77 ± 0.05^{C} 42.2%	0.76 ± 0.05^{C} 35.0%
	4 g/kg	1.05 ± 0.03^{D} 35.6%	0.78 ± 0.07^{C} 50.0%	0.79 ± 0.05^{C} 41.5%	0.72 ± 0.07^{C} 36.7%

4. Conclusions

Overall, this study reported comparative chemico-biological investigations of activities of 20 extracts from four varieties of *Lactuca sativa* L. More particularly, the polarity of the extraction solvent significantly affected extraction yield, polyphenol and flavonoid contents as well as antioxidant, anti-inflammatory and antibacterial activities. Extraction yield growed with increasing polarity, hence the content of polyphenol and flavonoid among other metabolites showed an important value in solvent polarity comprised between 5.8 and 8.7. These results gave also proofs that consumption of red varieties of *Lactuca* species may bring more health beneficial effects than the green ones, indeed, content of flavonoids and polyphenolics compounds are more significant in the variety *crispa*. Mathematical statistics showed that polyphenols and flavonoids content were positively correlated with antioxidant and anti-inflammatory activities. Furthermore, Principal Components Analysis showed that the extract EX_{44} of polarity 5.8 had the most important antioxidant and anti-inflammatory activities *in vitro* and *in vivo* as compared with the other extracts from the other varieties of *Lactuca sativa* species.

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ECOFRIENDLY UTILIZATION OF BY PRODUCTS FROM BANANA PEEL IN FOOD PRODUCTION AND OTHER INDUSTRIAL APPLICATIONS. A REVIEW

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ABSTRACT

The banana (*Musa Sapientum*) is a member of the Musaceae family. The nutritional value of this fruit and its peels is significant. Every year, 36 million tonnes of banana peel are produced. Starch, bioactive substances, anti-oxidants, pectin, cellulose, minerals, phenolic acids, flavonoids, carotenoids, biogenic amines, and other phytosterols are all found in banana skin. Lipids, carbs, protein, dietary fibers, and a variety of other essential components are also present. It contains anti-nutritional elements such as hydrogen cyanide, oxalate, phytate, and saponin, but only in trace amounts, with the exception of saponin. Banana peel can be used to make buns, bread, pasta, confectionaries, and gluten-free items in the culinary sector. It's also utilized as bio substrate, as well as for medical uses, livestock feed, fertilizer, and bio substrate. Peel can also be used for various reasons, such as removing Cr (IV) and producing bio-Ethane and bio-methane.

1. Introduction

Musa Sapientum, also known as banana, is a member of the Musaceae family. It originated in the tropical reign of southern Asia and is currently farmed across the tropics. *Musa Sapientum* can reach a height of 2-8 meters and has leaves that are around 3.5 meters long. Its pseudostem produces the single bunch of bananas before it dried and replaced by fresh ones. Fruits grow in a cluster that hangs from the ceiling, with twenty fruits per tier and three to twenty tiers each bunch. The skin protects the fruit (Anhwange and colleagues, 2009).

The banana is a popular fruit that is well-known for its nutritional value. (Aurorae and colleagues, 2009). Climacteric fruit is a type of fruit. This fruit is consumed fresh and processed into various items on a small and industrial scale. (Vu *et al* 2016.). Banana carbohydrate (both starch and non-starch) has a poor digestion, making it a great ingredient for food preparations. A total of 102 million tonnes of bananas are produced each year. Because banana peel accounts for 35% of the total weight of the fruit, about 36 million tonnes of banana peel are produced each year. (Vu *et al* 2016.). The banana is said to have originated in Southern Asia's tropical regions, and it is currently grown all over the world. Anhwange (2008, Anhwange, Anhwange, Anhwange, Anhwang described that banana is the most popular fruit, its world fruit production is accounting for 16.8%, with in apple, orange, and each accounting for 11.4

percent.(Pereira and Maraschin 2015). Banana production has been increased dramatically in last 20 years, in 2013. (Vu *et al* 2018). Asia is the world's largest banana. From 46 million tonnes in 1993 to 105 million tonnes in 2013, there has been a steady increase. (Vu and colleagues, 2018). Asia is the world's top banana producer, accounting for over 57.3 percent of worldwide output, with America, Africa, Oceania, and Europe following closely after. They generate the least, accounting for less than 0.3 percent of world output. Production has increased as a result of increased demand, population growth, expansion of agricultural areas, and productivity. (Vu *et al* 2018).

The major player of the banana industry is Pakistan, containing a land area of 349000 ha, with a total output and productivity of 29.7 million tons/ha, respectively. In Sindh province, 90 percent of the land is in the country's South East Asia. (Memon *et al.,* 2015). According to FAO figures, Asia is the world's greatest banana producer, accounting for 54.4 percent of global banana production, with an average banana intake per capita of 12 kg. Banana is one of the world's most significant food crops, behind rice, wheat, and maize. (Khoozani *et al* 2019.)

Banana peel is the most common byproduct, accounting for about 40% of the fruit total weight. (Agama-Acevedo *et al.* 2016, Agama-Acevedo *et al*. Until today, banana peels were discarded as waste, contributing a significant amount of organic material to landfills. Since the banana peel has been mostly focused by researcher, he has begun extracting and isolating key components for use in food enrichment and other applications. (Agama-Acevedo et al. 2016, Agama-Acevedo *et al*. Peel contains starch, bioactive substances, pectin, cellulose, and a variety of vital minerals and components. (Singh *et al.,* 2016). Several substances, including phenolics, flavonoids, phytosterols, carotenoids, biogenic amines, and other phytochemicals are found in in peel (Pereira and Maraschin 2015). The peel of a banana has higher nutritional fibre. Dietary fiber is a non-digestible carbohydrate polymer, which can be divided into two categories according to its water solubility: soluble fiber (pectin and some hemicellulose) and insoluble fiber (cellulose, lignin, and resistant starch). (Khoozani *et al* 2019). Burns, diarrhea, ulcers, inflammation, diabetes, cough, snake bites, and excessive menstruation are just a few of the problems that banana peel is used to treat. (Pereira & Maraschin 2015) Banana peels also include more dietary fibre and phenolic compounds, as well as anti-oxidants, anti-microbial, and antibacterial capabilities. Anjum, Sundaram, and Rai (Anjum, Sundaram, & Rai, 2014). The content of phenolic compounds in banana peel was 4.95-47 mg garlic acid equivalent/g dry matter (mg GAE/g DM). These chemicals have been associated to health advantages such as the prevention of cardiovascular illnesses, diabetes, cancer, and obesity (Hernández-Carranza *et al*., 2016). (Vu and colleagues, 2018). Gallocatechin, an antioxidant found in banana peel enriched with natural antioxidants. Gallocatechin is found in higher concentrations in banana peel (about 158 mg per 10 g dry weight) and has the strongest antioxidant effect against lipid auto oxidation. Someya and colleagues (Someya *et al*., 2002). Peel contains 0.9 percent, 1.7 percent, 59 percent, and 31.70 percent protein, fat, carbs, and crude fibre, respectively. The findings showed that banana peels are a rich source of carbs and fibre. Because it contains more fiber, it aids in the relief of constipation and enhances overall health. The banana peel moisture content is 6.7 percent. This is low value, and it could be because of the harvesting time (Vu and colleagues, 2018). The low score indicates that banana peel has a long shelf life without mold growth. The amount of ash in the product is 8.50 percent. The amount of organic matter in the soil is approximately 91.50 percent. Organic matter is a metric for a plant's nutritional worth (lipids, proteins, and carbohydrates).Vu and colleagues, 2018). Banana peels have a high nutritional value, indicating that they are a good source of nutrients. (Anhwange and colleagues, 2009)

The potassium content of *Musa Sapientum* peel is higher than that of other minerals (78.10 mg/g). The concentrations of peel calcium, sodium, manganese, iron, rubidium, bromine, strontium, zirconium, and niobium 19.20, 24.30, 76.20, 0.61, 0.21, 0.04, 0.03, 0.02, and 0.02, respectively. If the peels are properly developed and processed, they can be a good source of high-quality carbohydrates and minerals at a low cost. Vu and colleagues, 2018). Because of its high potassium concentration, peel can assist to maintain blood pressure and balance bodily fluids, as well as control kidney failure, cardiac difficulties, and respiratory difficulties. Because iron transports oxygen to cells, it is required for energy production, collagen formation, and the normal functioning of the immune system. Manganese aids in the development of skeletal and cartilage tissue. (Khoozani *et al* 2019.) Bromine, rubidium, strontium, zirconium, and niobium are non-essential minerals with concentrations ranging from 0.21 to 0.02 mg/100g. The results show that banana peel has relatively low non-essential mineral concentrations. (Anhwange and colleagues, 2009). The peel of a banana has more dopamine than the pulp. Dopamine is a bioactive molecule that regulates hormones in glycogen metabolism. (Khoozani *et al* 2019.)

Anti-nutrients are also detected in banana peel, indicating that hydrogen cyanide concentrations are 1.33 mg/g. Acids react with metal cyanides to produce hydrogen cyanide, which is exceedingly deadly. A large quantity of hydrogen cyanide can kill you in a matter of minutes, while smaller doses produce throat and chest stiffness, palpitation, and muscle weakness. However, they are present in the range of 0.5 to 3.5 mg/g and are listed as a safety limit. The oxalate content was nearly 0.51 milligrams per gram. (Kumar *et al*., 2012). Oxalate consumption is linked to kidney illness, which can lead to death, as well as a reduction in the availability of important minerals like calcium. When compared to the 0.7 mg/g reported for cocoyam, the result obtained is low. The amount of phytate in each gram was found to be 0.28 mg/g. (Anhwange and colleagues, 2009). This finding is low when compared to the maize and sorghum results of 146–353, 206–208mg/g, respectively. Saponin content was found to be 24 percent. Consumption of saponin can cause sensory system paralysis. It has been discovered that it inhibits pig and poultry growth while increasing cholesterol excretion in the body. When compared to the 3.00 percent described as the lowest safe amount for animals, particularly cattle, the result achieved is significantly high. Except for saponin, the anti-nutrient content of the peel has been studied and found to be minimal. This suggests that, if properly handled, the peels could be a useful source of feed for cattle. (Anhwange and colleagues, 2009).

Banana peel is used in traditional medicine.
For decades, the peel of a banana has been used as a herbal treatment to treat a number of ailments including burns, ulcers, coughs, and diarrhea. (Pereira & Maraschin, 2015).

For instance, burn wound healing has been healed by wrapping a ripe banana peel around the area to lessen pain as well as swelling (Pereira & Maraschin, 2015). Banana peel has been proved to be an excellent cure for reducing inflammation and swelling after mosquito bites. (Kumar et al., 2012)The banana peel's antibacterial and antibiotic characteristics, and further research into these biological activities and bioactive components is required. Unripe banana peels have also been shown to be useful in the treatment of diarrhea. The peel works as an antacid against stomach ulcers. (Kumar et al., 2012).

Flavonoid component leucocyanindin found in banana peel has been shown to thicken the stomach's mucous membrane layer.(Imam & Akter, 2011). Furthermore, the peel is used to prevent and treat a variety of illnesses, including depression (linked to the tryptophan concentration in bananas), anemia (high iron concentration increases hemoglobin production), and high blood pressure (peel high amount of potassium and low amount of salt). (Kumar *et al*., 2012; Kumar *et al*., 2012). Furthermore, the banana skin contains high fructose oligosaccharides, which are prebiotics

that feed healthy bacteria in the colon. (Kurtolu and Yildiz 2011) by creating vitamins and digesting enzymes, these beneficial bacteria boost the human body's ability to absorb nutrition. (Walker and Duffy 1998). As a result, banana peel has a lot of potential as a traditional medicine. (Vu *et al* 2018).

2. Livestock feed

Major nutritional components such as carbohydrates, proteins and lipids at higher concentration in banana peel and account for 91.50% of dry weight. Indigestible fiber is also present in material at high level. (Anhwange, 2008). More than 9 minerals are also present some anti-nutritive component is also present such as hydrogen cyanide but present in minor than safe limit (0.5-3.5mg/g). Banana peel is used as livestock feed and it contains similar quality of some ingredients with in soybean, cassava used for feeding pigs (Tartrakoon, Chalearmsan, Vearasilp, & ter Meulen, 1999).

3. Manure

Traditionally banana skin was used as manure by replenish the soil nutrients simply by decomposing. Banana skin produces many types of organic fertilizer because of higher demand of bio fertilizers and biological advances. (Kalemelawa *et al.,* 2012; Pangnakorn, 2006). Under aerobic and anaerobic conditions when banana peel is dumped with cow dung, poultry litter and earth worms then composed. This organic fertilizer contains high potassium (> 100g / kg) and nitrogen (> 2%), which is effective for all kinds of plants. (Pangnakorn, 2006).

4. Bio-substrate

By using polymer enzyme and acids cellulose can be hydrolyzed and has been used to produce sugars, organic acids, fuels and enzymes. As banana peel consists of high level of cellulose so it has been used as bio-substrate (Oberoi *et al.,* 2011). For edible mushrooms cultivation and wine production banana peel has been used as substrate. (Padam *et al.,* 2014). Banana skin has been used as substrate to produce xylitol, a sugar that has beneficial properties and used as alternative source of conventional sweetners. (Rehman *et al.,* 2013).

5. Utilization of banana peel in food industry. Production of buns

Substitution of refined wheat flour with banana peel fibers with, buns were prepared. The supplementation of refined wheat flour (30 g) with banana peel fibre up to 40 and 10% blend were prepared. Other ingredients that are include were sugar 5g, yeast 5 g, milk powder 5 g, water 20 ml and oil 10 ml or 5 g butter, 50 mg salt. (Budhalakoti, 2019). Refined wheat flour and banana peel fibre were sieved and a uniform blend was made. Luke warm water were used to dissolve yeast and little amount of blend mixture was added to it. (Budhalakoti, 2019) Proofing of this mixture is done at 30°C for 1 or 1/2 hours. Sugar was also added and remaining mixture was added to it and kneaded it to till soft smooth dough. Dough was again punched for few minutes. Dough was sheeted, rolled and molded. Then it was placed in greased sheet, covered with a wet cloth and allowed to rise in tin under 30°C.Water was sprayed on the bun surface before putting it on oven. Bun was baked at 200°C for 10 minutes. After 10 minutes, bun was taken out from oven and allowed to cool. Buns that contain 10% fiber were texturally acceptable and more palatable. (Budhalakoti, 2019)

5.1. Bread production from banana and banana peel flour

Processing of flour from banana and banana peel has similar steps. Due to high dietary fiber and bioactive compounds, these flours are used in food stuffs for remarkable functionalities. Bread that are produced from banana of banana peel flour has high value of starch, ash, protein and TDF. (Khoozani *et al.,* 2019). In term of minerals the bread has more value of Mg, K, Na, and Ca. and increase inTDF and RS were also shown to have increased by 9% and 5%, respectively, in the mucous membrane layer of the stomach. Adding GBPF and gluten to bread has the unfavorable effect of making it harder and stickier. Because of

the deficiency of stability in gluten structure, cohesion, elasticity, and chewiness decrease when supplementing is done at 30%. (Khoozani *et al.,* 2019).

5.2. Production of pasta

Pasta products, like other foods, play a significant role in people's diets. In comparison to white bread or rice, pasta has a low glycemic index, a long shelf life, and is simple to prepare. (Nilsson *et al.,* 2010)

In 2009 spaghetti made with semolina flour that was enriched with different substitution of GBPF. Textural results indicate an increase in adhesiveness and chewiness that was because of release of amylose from starch during cooking. Pasta made with GBPF consists of 42.54% RS2 and high percentage of polyphenols and antioxidants. (Agama-Acevado *et al.,* 2009). A combination of 15% sprouted flour and 15% GBPF provided best nutritional and technological attributes. (Krishnan and Parhabashankar 2010).

5.3. Confectionaries production

By addition of 60% GBPF in cake premix instead of wheat flour increases its shelf life over 4 months. The pH of the Premixture does not change. As well as harmful development, such as fungus and yeast. (Borges et al., 2010; Borges et al., 2010; Borges et al., 2010 Cakes prepared with fine GBPF particle sizes have superior nutritional characteristics without having a negative impact. (Segundo *et al.,* 2017). In biscuits manufacturing a prior treatment of mashed peel together with DF increases softness. Even at 75% substitution there were not significantly change in organoleptic properties such as color, flavor, after taste and mouth feel. (Joshi 2007).

5.4. As functional ingredient in yellow noodles

Banana peel noodles are prepared by substitution of wheat flour and green Cavendish banana peel flour which were characterized for physiochemical properties and in vitro starch hydrolysis. Cooked noodles were assessed for estimated glycemic index, vitro hydrolysis index, color, pH, and tensile strength. (Ramli *et al.,* 2009). Banana peel noodles has higher elasticity but same tensile strength as of control. GI of glycemic index of banana peel noodles was lower than the control. Partial substitution of banana peel into noodles may be useful to control starch hydrolysis of yellow noodles. (Ramli *et al.,* 2009)

5.5. Gluten free products

As the prevalence of gluten-related illnesses including celiac disease and dermatitis herpetiformis rises, so does the need for gluten-free products. If left untreated, it can lead to intestinal cancer, food shortages, and oxidative stress, thus choosing gluten-free goods with added nutritional value is crucial. (Wang *et al.,* 2017; Wang *et al*., 2018). The addition of 47 percent banana peel flour to a 100g pasta recipe resulted in pasta with more egg white and hydrocolloids. (Zandonadi and colleagues, 2012). Gluten-free bread and pastries are also available; however, their limited qualities necessitate further research into GF starch-based items. Bioactive substances are prevalent in GF diets; as most starchy foodstuffs lack technical features. Torres *et al*. (Torres et al., 2017).

5.6. Natural Preservative

Because of its antibacterial and antioxidant qualities, banana peel could be used as a natural preservative in food products. To improve the quality and shelf life of poultry meat and fish oil, for example, extracts were added. (Anal and colleagues, 2012)

Banana extracts were found to have preservation properties comparable to synthetic preservatives such as Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) (BHA). (Devatkal *et al*., 2014)

5.7. Other uses of banana peel

Heavy metals such as lead, chromium, copper, cadmium, and zinc are always a concern to humans, animals, and the environment. The skin of a banana has been reported to be an efficient heavy metal absorber. Synthetic colors are also

absorbed by banana peel (Castro *et al*., 2011). (Osma and colleagues, 2011)

Banana peel is a good source of extractable pectin, which can be used in the food sector for a variety of purposes. (Oliveria and colleagues, 2015) The main aromatic ingredient used in banana taste is Isoamyl acetate, which is also derived from banana peel. (2015, Ji et al.) In the food sector, banana flour is used as a carbohydrate source or a thickening ingredient. Alkarkhi et al. (Alkarkhi *et al*., 2011). Because banana starch is regarded superior to maize starch, it has a higher market value. Padam *et al*. (Padam et al. 2014) Selective removal of Cr (VI) from industrial wastewater:

Banana peel a commonly produced fruit waste used for the removal of Cr (VI) from industrial waste water. Chromium is waste water from metal fishing and chromo plating industries. (Torres *et al.,* 2017) By ingestion of high level of Cr (IV) causes glomuler damage, tubular and kidney damage. So its removal is essential from waste water. Maximum acceptable limit for drinking water by WHO is 0.05mg/l. Banana peel is used as economical sorbent. (Memon *et al.,* 2008) Initial metal ion concentration, parameters pH, contact time and temperature were investigated and efficient absorption 95% within 10 min were determined. (Torres *et al.,* 2017) Optimal absorption occurs at pH 2 and binding metal ion was pH dependent. By using 5ml of 2M H_2SO_4 all retained species were eluted and by using flame atomic absorption and ultraviolet visible spectroscopy technique total amount of chromium and Cr (IV) were analyzed. (Itelima *et al*., 2013). Partitioning behavior for the system at different temperature were used to describe by Langmuir and dubinin-redushkevich isotherms. By using banana peel kinetics and thermodynamics of Cr (VI) removal were also studied. (Memon *et al.,* 2008)

5.7.1. Bio-Ethanol production from banana peel

Banana peels that are waste of fruit industry and present in abundance and are used to produce Bio-Ethanol. Peels were subjected to scarification and fermentation simultaneously for 7 days by using co-culture of Aspergillus Niger and Saccharomyces Cerevisea. (Itelima *et al*., 2013) Ethanol yield, reducing sugar concentration, cell dry weight and biomass yield were determined after 24 hours' interval. After 7 days of fermentation was banana peel had biomass yield of 1.60 OD. 0.20-0.82 mg/cm^3 and ethanol yield were about 7.45% v/v. fruit waste that contain fermented sugars such as banana should be used for alternative source of energy. (Itelima *et al*., 2013)

5.7.2. Bio methane production from banana peel

Raw banana peel is also used for bio methane production. Physical treatment by grinding the peel into small pieces prior to anaerobic fermentation was used. Batch reactor was used under mesophilic conditions pH 7 and different concentration of total solids used. (Pisutpaisal *et al.,* 2014) Air and liquid samples were collected at 12 hours' interval for gas composition and volatile fatty acids analysis. At 7.5% concentration of TS maximum yield and production of bio methane were 439 Ml g^{-1}TVS and 5.31 Ml hr^{-1}. Size reduction of banana peel and fungal pretreatment might improve the methane yield from banana peel fermentation in future work. (Pisutpaisal *et al.,* 2014).

6. Conclusions

This assessment overlooks the effective use of banana peels, which are frequently discarded. The conversion of banana peel into numerous valuable applications in the food sector, for medicinal purposes, livestock feed, and the creation of bio ethane and bio methane was reviewed in this paper. The peel of a banana gives nutritious value to a variety of foods. The use of banana peels as a cheap and rich source of antioxidants, phenolic compounds, and minerals points to a future research direction of cost-effective and efficient nutrient recovery, usage, and enrichment.

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VALORIZATION of HAZELNUT and SESAME PROTEIN ISOLATES in SUSTAINABLE MEATBALL MANUFACTURE

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Hazelnuts;
Sesame;
Meatballs;
Sustainability.

ABSTRACT

The ever-increasing global demand for proteins necessitates the generation of sustainable plant protein products. The aim of the current study is the utilization of cold press cakes for the generation of hazelnut and sesame protein isolates and their valorization in meatball manufacture. Protein isolates were generated from cold press cakes using an alkaline extraction-isoelectric precipitation (AE-IP) method. The functional properties (solubility, emulsion and foam formation capacity, and oil and water holding capacity) of hazelnut and sesame protein isolates were examined. Furthermore, physicochemical and sensory properties (texture, size, color, and sensory attributes) of the meatball samples fortified by these isolates were investigated. Protein fortification altered color of the meatballs and increased the firmness of meatballs at elevated protein contents. However, toughness or meatball size were unaffected by fortification. The differences between treatments were attributed to the molecular size characteristics of proteins and fiber content in the isolates. Sensory data confirmed that the acceptance for meatballs were maximum for samples fortified with 5% protein and sesame protein isolate was more preferable over hazelnut counterparts. Since commercial meatballs contain approx.20% protein, ≥5% plant protein fortification could be a significant development in protein content and sustainability in meatball production.

1.Introduction

The trend towards the consumption of protein-rich food products have increased over the recent decades along with the world population and a keen research interest is especially present in novel protein resources. Millions of tons of agricultural or industrial food by-products or waste are discarded every year globally. Although the majority of such streams are mostly biodegradable, their disposal leads to serious environmental problems such as water pollution and generation of unwanted odors. These by-product streams can potentially be utilized as low-cost resources of proteins in human nutrition (Ogunwolu et al., 2009). However, inclusion of novel proteins should not lead to impaired sensory, functional or nutritional properties in the final products. In this context, many research studies have been conducted on plant protein resources including canola, soy and various pulses, all of which contain significantly high amounts of protein (Moure et al., 2001).

Currently, the demand for ready-to-eat foods is also increasing all over the world. In this product category, ready-to-cook meatball varieties represent an essential group (Yılmaz, 2015). Global meat industry aims to increase

the product quality, and nutritional attributes in their products while generating safe products at reduced production costs. In this context, there is a need for protein components that have the potential to increase the protein content in ready-to-eat meatballs while sustaining the organoleptic properties of products, and reducing the overall cost. Furthermore, utilization of plant proteins in meat products could potentially lower their environmental footprint (Saget et al., 2021). The conversion efficiency of plant proteins to animal proteins is known to be in the order of 15% (Day, 2013).

In conventional oil processing, vegetable oils are generally extracted from ground seeds by treating them with organic solvents such as hexane, which is followed by solvent evaporation. Cold press technology is an alternative method during which solvent extraction or heat application procedures are not necessary (Parker et al., 2003). Consequently, this gentle approach enables the preservation of residual materials in cold press cakes. Since seeds contain significant amounts of proteins, their corresponding cold press cakes are further concentrated in proteins (Coşkun et al., 2019) and can be utilized in food products (Coşkun et al., 2020).

Hazelnuts (*Corylus avellana* L.) are highly sought after in pastries and chocolate industries due to their desirable organoleptic properties. Hazelnut components are also widely used as a flavor and aroma additive in the bakery and dairy industries, salad dressing products due to their rich protein, fat, vitamin and mineral contents (Fallico et al., 2003). The protein content in the deoiled hazelnut cakes is in the range of 35-41% (Yağcı and Göğüş, 2008). Some of the bioactive attributes of hazelnut proteins and peptides have been recently reviewed (Aydemir et al., 2014; Çağlar et al., 2021).

Sesame seeds (*Sesamum indicum* L.) have been cultivated for nearly 4000 years and are characterized with a high-energy value and fat content. Potential health benefits of sesame include anti-oxidative, anticancer, anti-hypersensitivity, and anti-immunoregulatory activities. The seeds are used in oil manufacture, salad making and various food formulations. Sesame seeds contain 50-60% oil, 18-25% protein, 13.5% carbohydrate and 5% ash. Oxidative stability of sesame oil can be attributed to endogenous antioxidant lignans along with tocopherols. Sesame seeds have long been considered in the Eastern regions of the world as a healthy food that prevents aging, and serve as a rich source of calcium (approx. 1%) and phosphorus (approx. 0.7%) (Prasad et al., 2012). Based on these data, supplementation of foods with hazelnut and sesame protein products could enhance various nutritional components of foods.

Protein molecules have the capabilities to hold oil and water and improve the structural qualities of foods. Through water holding, proteins swell and affect the rheological and textural properties of foods (Seena and Sridhar, 2005). Water and oil holding capacities of proteins are functions of shape, size, hydrophobic and hydrophilic interactions of the protein molecules. The interactions between proteins and fats/oils affect the sensory quality of many foods. Proteins with low solubility and high hydrophobicity can hold large amounts of fats and oils (Guerra et al., 2011). Consequently, the influence of protein fortification on food properties including color, texture, water and oil holding capacities, and the ability to stabilize emulsions and foams need to be well-understood.

In this study, based on appropriate aqueous extraction techniques, protein isolates were manufactured from cold press cakes of hazelnut and sesame seeds. In order to determine the suitability of these protein isolates to meatball manufacture, functional properties of the protein ingredients and final products were evaluated. In this context, solubility, foam and emulsion stabilization characteristics as well as fat and water holding capacities were investigated. Furthermore, their influence on plant protein fortified meatball characteristics including sensory, visual and textural attributes were studied in an effort to reduce the environmental footprint of meatball products and increase their protein contents. Meatballs are characterized by an approximate protein

content of 20% (Serdaroğlu et al., 2005). In the current study, both protein fortification and sustainable production of meatballs have been targeted.

2. Materials and methods

2.1. Materials

Cold press cakes were obtained under gentle processing conditions such as low processing temperatures (≤40^{0}C). Hazelnut (Mecidefendi, İzmir, Turkey) and sesame (Vitalling, Adana, Turkey) press cakes were obtained from the domestic producers of cold press oils and the cakes were stored at +4ºC until further use.

All chemicals used in the analyses were purchased from Sigma (Schnelldorf, Germany). Beef samples (M. gluteus medius), spices, bread crumbs and onion powder were used as raw materials in meatball production. The fat content of ground beef used for meatballs was approx. 15%. All materials used in meatball production were supplied from local supermarkets. Meatball production and their corresponding analyses were carried out at Tekirdağ Namık Kemal University (NKU), Turkey, Microbial Biotechnology Laboratory of Dept. of Agricultural Biotechnology and Eksun Food Company R&D Center, Tekirdağ, Turkey. Meatball texture was studied at NKU Central Research Laboratories.

2.2. Methods

2.2.1 Manufacture of protein isolates

Alkali extraction-isoelectric precipitation (AE-IP) method was applied for the production of protein isolates from cold press cakes (Coşkun et al., 2019). In order to prevent protein denaturation, solvent extraction was not administered. Firstly, the cake samples were ground down to approx. 2 mm. The ground samples were mixed with ultra-pure water (Millipore, Simplicity, USA) at a ratio of 1:15 (sample:water). The mixture was kept stirred at 500 rpm using a magnetic stirrer for 1 h. Immediately afterwards, pH value of the mixture was brought to pH 9.5. Insoluble materials were separated by centrifuging the mixture at 4200xg for 15 minutes. Isoelectric precipitation of the proteins (pH 4.5) was promoted using 1 N HCl and the mixture was centrifuged again under the same conditions as before. The precipitated portion was lyophilized (Teknosem, Toros TDS 2/2V, İstanbul, Turkey) and stored in the freezer (-20°C) until subsequent procedures. Representative freeze-dried protein isolates were shown on Figure 1.

Figure 1. Freeze dried protein isolates. HPI (left) and SPI (right).

2.2.2. Analysis of the composition and functional properties of protein isolates

Firstly, hazelnut or sesame protein solubility was determined using Sigma-Aldrich Total Protein Kit, Micro Lowry, Petterson's Modification (TP0300). Total protein content of the isolates was assayed using the Kjeldahl method (Da Silva et al., 2021). The moisture contents of hazelnut and sesame protein isolates (HPI and SPI, respectively) were

determined by the gravimetric method (Da Silva et al., 2021).

For this purpose, 3 grams of samples were weighed into the stainless steel containers and the samples were kept for 4 hours in a 105±2 ºC incubator (Memmert, unb400, Germany). When constant weight was achieved, the amount of moisture removed from the samples (%) was calculated. Ash content was analyzed after an incubation period of 8 hours at 550±15ºC (Magmatherm, mt1105, Turkey) (Da Silva et al., 2021).

The water and oil holding capacity of protein isolates were determined by adding 1 g protein isolate samples to 10 ml of pure
water or 8 ml of soy oil (Sanchez-Vioque et al., 1999). The mixture was kept vortexed (Vortex Genie 2, Scientific Industries, USA) for 30 seconds every 5 minutes for 30 minutes. Thus prepared sample was kept at room temperature and centrifuged for 30 minutes at 3000xg (Selecta, Myxtasel BL., Cham, Switzerland). After this procedure, the water or oil holding capacity was determined in terms of g absorbed water or oil /g protein.

The emulsion activity index (EAI) and emulsion stability index (ESI) values of the protein isolates were determined as presented by Zhang et al. (2021). Aqueous protein dispersions (6 ml, 0.1%) and 2 ml of soy oil (Sigma, S7381) were homogenized for 3 minutes at 4000 rpm using a shear mixer (Wisd, H6-15A, Ireland). Immediately after preparation, an aliquot (50 µl) was taken from the emulsion and diluted with a 10 ml 0.1% SDS solution. The absorbance of the diluted sample was determined at 500 nm (i.e., turbidity) (Optima, SP 3000 UV VIS, Japan) and the corresponding EAI and ESI values were calculated.

The foaming capacity and foam stability of protein isolates were measured according to the method published by Chabanon et al. (2007). Foaming capacities of samples were based on volume change (%) at the time of preparation and the stability of foams (i.e., foam volume) were monitored for 90 minutes after preparation.

2.2.3. Preparation and analysis of protein enriched meatballs

Meatball samples were prepared using 0.3% black pepper, 0.2% red pepper, 0.5% cumin, 5% onion powder, 2% salt, 7% breadcrumbs and ground meat to complete a 1 kg formulation. Hazelnut protein isolate (HPI) or sesame protein isolate (SPI) (5, 10, 15, or 20%) samples were added to a 200 g sub-sample from the 1 kg mix. The ingredients were mixed by kneading, and the mixture was portioned into round meatballs with an approximate diameter of 8 cm. The cooking process was carried out on a non-stick pan without the addition of any further ingredients at medium heat and kept reversed on the pan after 3 minutes in the first run, 1 minute in the second run and finally, after 15 seconds for each sides. Consequently, the complete heating duration was 8.5 min.

2.2.4. Textural analysis

Texture analysis device (TA-XT Plus, Texture Analyzer, UK) was used with a maximum load cell of 50 kg. Compression tests were performed on meatball samples and thus the texture analysis profiles (TPA) were determined. Slices with constant thicknesses (approx. 1.5 cm) were cut from meatball samples in each different group and the analyses were performed under ambient conditions (22±1°C). In these assays, 50% compression was applied to the meatball samples and the recovery rate was monitored (Crehan et al., 2000; Herrero et al., 2007; Bozkurt and Bayram, 2006). Exponent 32 software provided by the instrument manufacturer was used in the data analysis.

2.2.5. Reduction in size

Once meatball samples were weighed and portioned, their diameters were also measured as raw and cooked. The extent of size reduction (%) due to cooking was determined quantitatively using a ruler.

2.2.6. Sensory analysis

Sensory analysis of meatballs was based on appearance, hardness, juiciness, aroma and overall acceptance parameters. The panelists evaluated these parameters using a hedonic scale of 0-9, where 1-2-3 (poor), 4-5-

6 (moderate), 7-8 (good), and 9 (very good) could be rated (Barrett et al., 2010).

Seven panelists were selected among NKU Agricultural Biotechnology faculty members, undergraduate and graduate students (3 males and 4 females between the ages of 20-30). Cooked meatballs were randomly coded using numbers 1-9. Panelists evaluated a total of 9 samples in one session, including 1 control, 4 hazelnut protein bearing (5, 10, 15, and 20%) and 4 sesame protein bearing (5, 10, 15, and 20%) samples. Panelists were provided with bread and water at room temperature to clean and rinse the palate during sensory analysis.

2.2.7. Color analysis

Color analysis was conducted using a desktop spectrophotometer (Konica Minolta, CM-5, Japan) and *L*a*b** values (L, lightness; *a: redness, b: yellowness)* were determined. The total color difference (ΔE*) between control meatball and protein isolate added meatball samples was calculated by the following formula:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*) + (\Delta b^*)^2]^{1/2} \quad (1)$$

2.2.8. Statistical analysis

Data on the physical, chemical and sensory properties of the samples were evaluated by one-way ANOVA tests using the JMP PRO software. The differences between the sample means of treatments were tested for significance at $p<0.05$ level with Tukey multiple comparison tests (Abdi and Williams, 2010). In all data presentations, upper index letters such as A, B, AB etc. represented significant differences between treatments at $p<0.05$ level when the upper index letters were not identical.

3. Results and discussions

3.1. Composition of protein isolates

Firstly, in order to determine the compositional attributes of hazelnut and sesame protein isolates, their corresponding moisture, ash and protein contents (%) were analyzed (Table 1). The protein content of hazelnut protein isolates (HPI) was found to be approximately 89.3%, whereas sesame protein isolates (SPI) contained approx. 81% protein. The ease of preparation and high protein content of HPI and SPI demonstrated their potential for being utilized in protein fortification of industrial food products. In addition, ash and moisture contents of the samples were in accordance with the Turkish Food Codex and EU regulation on whey protein isolate (Turkish Department of Food, Agriculture and Livestock, 2017; European Commission, 2018). Based on a modified Lowry test, protein solubility for SPI and HPI dispersions (15%) was 26% and 26.7%, respectively. The solubility of a protein isolate largely depends on the environmental pH, ionic strength, and medium temperature. The solubility of pumpkin seed proteins, for example, was reviewed to widely vary with medium pH and ionic strength (Bučko et al., 2015) while a relatively less soluble protein source could still be utilized in low moisture foods. For example, while initial moisture of various meatball formulations could be anticipated to be in the order of approx. 58-66% (Yılmaz, 2005), after cooking, meatballs had slightly lower extent of moisture, ranging mostly between 50-58% (Ulu, 2004; Serdaroğlu and Değirmencioğlu, 2004).

Table 1. % Moisture, % ash and% protein content, water holding capacity (WHC) and oil holding capacity (OHC) values for hazelnut protein isolates (HPI) or sesame protein isolates (SPI).

Samples	%Moisture	% Ash	% Protein	WHC (g/g)	OHC (g/g)
HPI	2.16±0.1	1.3±0.1	89.30±0.1	2.06±0. 1^{A}	2.59±0.1^{A}
SPI	1.65±0.1	1.43±0.1	80.95±0.1	1.96±0. 1^{B}	2.37±0.1^{B}

3.2. Water and oil holding capacities of the protein isolates

Water and oil holding capacities of HPI and SPI were presented on Table 1. The water holding capacity (WHC) of protein isolates was 2.06 and 1.96 (g/g), respectively, for HPI and SPI. Similarly, oil holding capacity (OHC) was 2.59 and 2.37 (g/g) for the same samples. According to these findings, OHC values of the isolates were higher than their WHC counterparts, which could improve the duration of aroma in food products such as baked goods including cakes and biscuits (Khalil et al., 2001). Relatively high OHC values could also prove instrumental upon grilling of meat products. In addition, these protein products could extend the shelf life and improve the flavor of the products by reducing water and fat loss in meat products (Guerrero et al., 2002). Consequently, an attempt was made here for their usage in meatballs. Compared to the previous works of our group, WHC and OHC values of the current samples were higher than that of black cumin protein isolates (Coşkun et al., 2019). These differences could be influenced by the processing or extraction methods, final protein composition and concentration in the samples as well as the nature of the impurities present. While the current OHC value for SPI was higher than that obtained by Khalid et al. (2003), no hexane treatment was administered in this study, which could preserve the native structure of proteins and affect the oil and/or water holding characteristics.

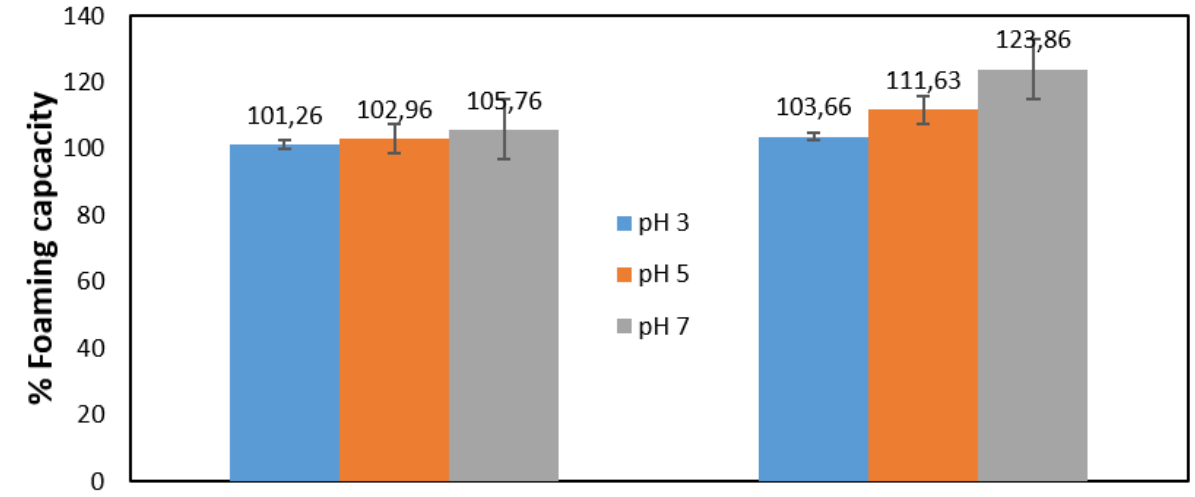
3.3. Foam formation and stabilization capacities of the protein isolates

Foams are defined as 2-phase dispersions consisting of air cells separated by a thin layer of liquid. In the stabilization of foams, surface active molecules including proteins are needed to reduce the surface tension at the air/water surface (Makri et al., 2005). Foam formation and stability are strongly dependent on foam preparation method, composition and concentration of proteins, medium pH and ionic strength, and hydrophobic interactions (Massoura et al., 1998). While globular proteins demonstrate low foaming ability due to limited surface denaturation, flexible protein molecules tend to perform highly in foam formation (Kaur and Singh, 2007).

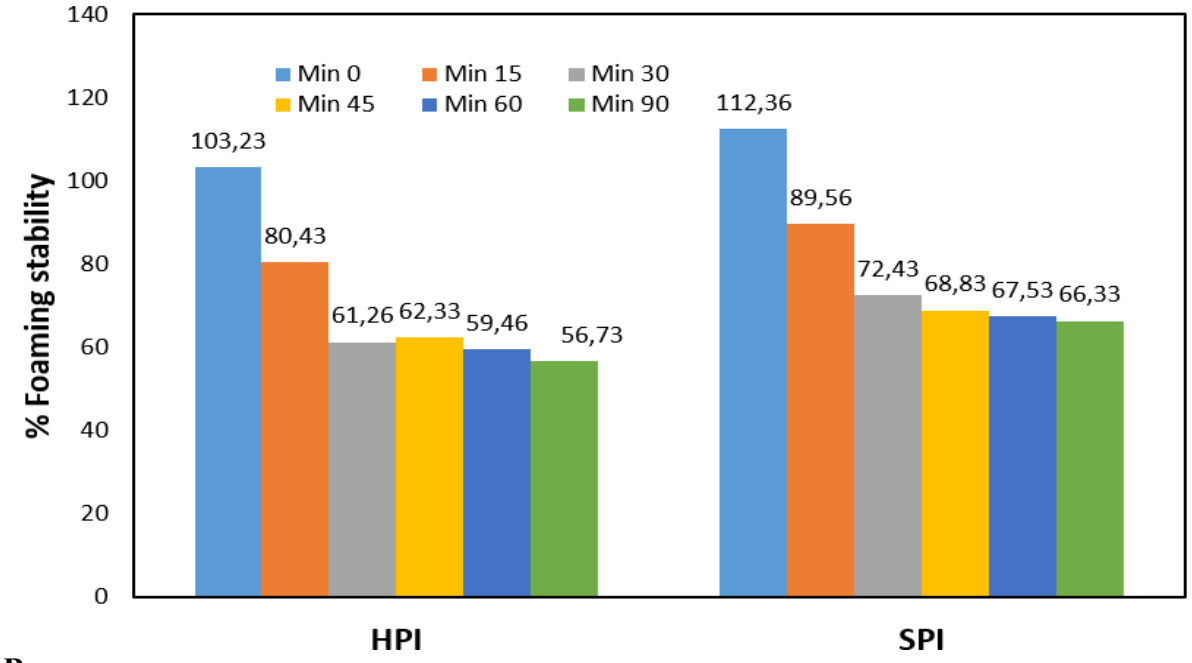
Due to the average pH value range of food products (primarily pH 3 to 7), the capacity of protein isolate dispersions (5%, 50 ml) to generate foams was considered in this pH range (Figure 2A). Generally, SPI was found to be a better foaming agent compared to HPI ($p< 0.05$). In the current pH range, the foaming capacity of both hazelnut and sesame proteins increased with pH as the overall negative charge density increased ($p<0.05$). While charge density increases, hydrophobic interactions in protein molecules may be anticipated to weaken and consequently, proteins gain a relatively more flexible structure that could facilitate foam formation (Guerrero et al., 2002; Aluko, 2004). SPI dispersions at pH 7 generated the highest foaming capacity. In most cases, however, there were no statistically significant differences between the stability values of SPI or HPI stabilized foams ($p>0.05$). In both cases, foam volume decreased from approx. A volume of 110 ml to approx. 55-65 ml range during a 90-minute storage process (Figure 2B).

3.4. Emulsion formation and stabilization capacities of the protein isolates

Surface hydrophobicity and protein concentration are the main characteristics that determine the properties of protein stabilized emulsions. Emulsification activity index (EAI) reflects the ability of a certain protein to adsorb to the oil/water interface during emulsion formation. The emulsion stability index (ESI) is a measure of the ability of a certain protein to provide stable emulsions for a certain period of time (Subagio, 2006). The emulsification activity and emulsion stability of the current samples were shown on Table 2. EAI values were determined as approximately $175 m^2.g^{-1}$ for sesame protein isolates. EAI values of sesame protein stabilized emulsions have decreased slightly over time. EAI values for hazelnut samples were found to be significantly higher (approximately 294 $m^2. g^{-1}$). The decrease in ESI and EAI values has been limited in all cases. While further optimization could enhance the foaming and emulsification attributes, the current findings were coherent with the findings of Cano-Medina et al. (2011) on sesame proteins.



A



B

Figure 2. (A) Foam formation capacities of HPI or SPI (5%) as a function of pH. **(B)** Foam stability as a function of time (0-90 min, pH 7).

Table 2. (A) Emulsion formation activities and stability index values for the HPI and SPI dispersions (5%, 50 ml) as a function of time (0-120 min).

	HPI		SPI	
t (min)	**EAI ($m^2.g^{-1}$)**	**ESI (%)**	**EAI ($m^2.g^{-1}$)**	**ESI (%)**
0	294.46 ± 0.1^{A}	49.16 ± 0.1^{A}	175.56 ± 0.1^{A}	49.33 ± 0.1^{A}
30	294.16 ± 0.1^{B}	49.03 ± 0.1^{A}	175.53 ± 0.1^{A}	49.26 ± 0.1^{A}
45	293.4 ± 0.1^{C}	48.76 ± 0.1^{B}	175.33 ± 0.1^{A}	48.83 ± 0.1^{B}
60	293.42 ± 0.1^{C}	48.53 ± 0.1^{C}	174.80 ± 0.1^{B}	48.66 ± 0.1^{C}
120	293.23 ± 0.1^{C}	48.16 ± 0.1^{D}	174.53 ± 0.1^{C}	48.53 ± 0.1^{C}

To summarize the findings, it must be noted that protein concentration in HPI samples were higher than their sesame counterparts. While solubility (%) values were comparable, WHC and OHC values of HPI samples were slightly higher. Foaming attributes of SPI samples were more significant, whereas emulsion formation capabilities of HPI were higher. The differences in the performances of HPI and SPI could be attributed to their structural differences and molecular sizes. For hazelnut proteins, the majority of 1D and 2D electrophoretic bands were found to lie between approx. 18 to 25 kDa, while their corresponding isoelectric points primarily were between pH 5-8.5 (Aydemir et al., 2014). Mostly comparable molecular weight results were obtained by Saricaoglu et al. (2018). The major bands for sesame proteins were identified around 35 and 75 kDa (Singharaj and Onsaard, 2015). Native-PAGE analysis indicated much larger aggregates in sesame proteins (11S), while the corresponding isolates were predominantly alpha-helical (Achouri et al., 2012). The authors commented that while the WHC, OHC and solubility attributes of sesame proteins were relatively less pronounced, emulsifying and foaming characteristics were relatively higher than soy protein isolate (Achouri et al., 2012). Under various extraction conditions, isoelectric points of the sesame proteins were mostly between pH 4-5 (Achouri et al., 2012). The molecular sizes and other structural attributes might lead to the differences in the performances of the current samples. In addition, composition of impurities in the isolates could also affect the results. Since the ash and moisture contents of the current samples were similar, fiber composition of the protein isolates may also be considered. Hazelnuts were found to be characterized with a total fiber content of approx. 12.9%, most (10.67%) of which was insoluble fiber (Alasalvar et al., 2003). In most studies, the crude fiber content of sesame seeds was expressed as <5-6% (Kryuchkova et al., 2021). Consequently, molecular size characteristics and fiber content of the two samples can be anticipated to be significantly different.

While physico-chemical properties of sesame proteins and to a lesser extent that of hazelnut proteins, were known, the current findings on current isolates were comparable to the previous literature. Based on these data, in the next stages of the study, sustainable plant protein fortification in meatball manufacture was investigated.

3.5. Analysis of meatballs fortified with protein isolates

In this section, the influence of HPI or SPI fortification on raw and/or cooked meatball characteristics are being summarized.

3.5.1. Reduction in size

Since the characteristic sizes of foods change during processing, the diameter values of the meatball samples were determined before and after preparation and the extent of size reduction (%) was calculated (Figure 1, Table 3). Similar extents of size reduction were observed at different protein inclusion rates for SPI, while the size changes were non-identical at various HPI concentrations. The highest extent of size reduction in meatball samples was generated by 10% hazelnut protein inclusion, whereas the least extent of size reduction took place for 5% hazelnut protein bearing meatball samples. There was no clear relationship between samples with different concentrations of protein isolates and in most cases, reduction in size was approx. in the order of 20% and the extent of reduction was mostly comparable to the control sample (i.e., 17.55%). However, in all cases including the controls, diameter reduction was more significant compared to low-fat meatballs fortified with blackeye bean flour, chickpea flour, lentil flour or rusk, where diameter reduction was <11% (Serdaroğlu et al., 2005).

3.5.2. Textural analysis

Textural profile analysis (TPA) was performed in order to detect changes in the textural attributes of the meatballs due to fortification with HPI or SPI (Table 4). Primarily, firmness and toughness parameters were examined. The firmness values for all samples were found to range between 2135 and 3375 g. The firmness value was determined to be the highest in 15% HPI bearing samples, while the lowest value was determined in the 10% HPI bearing counterpart (Table 4). In

general, the firmness values of meatball samples with varying concentrations of SPI or HPI were higher than the control and there were statistically significant differences between the samples ($p<0.05$). Previously, bacterial cellulose addition was shown to lower firmness of Chinese-style cooked meatballs as investigated by sensory analysis (Lin and Lin, 2004). In the current studies, the addition of protein isolates did not cause any statistically significant differences in toughness values ($p>0.05$).

Table 3. Cooking related size reduction (%) in meatball diameter as a function of HPI or SPI fortification (5-20%).

Sample	% Size Reduction
Control	17.55 ± 1.3^{B}
%5 SPI	20 ± 0.1^{AB}
%10 SPI	21.9 ± 0.6^{A}
%15 SPI	20 ± 0.01^{AB}
%20 SPI	21.9 ± 0.6^{A}
%5 HPI	23.15 ± 0.6^{A}
%10 HPI	20.65 ± 0.6^{AB}
%15 HPI	21.9 ± 0.6^{A}
%20 HPI	20.65 ± 0.6^{AB}

Table 4. Textural parameters (firmness and toughness) of cooked meatballs as a function of HPI or SPI fortification (5-20%).

	Firmness	Toughness
Control	2964 ± 130^{AB}	27874 ± 2831^{A}
%5 SPI	2416 ± 146^{BC}	31437 ± 2986^{A}
%10 SPI	2136 ± 174^{C}	24103 ± 3507^{A}
%15 SPI	3375 ± 36^{A}	30709 ± 312^{A}
%20 SPI	2517 ± 62^{BC}	23082 ± 653^{A}
%5 HPI	2388 ± 128^{BC}	24617 ± 2690^{A}
%10 HPI	2550 ± 49^{BC}	27127 ± 513^{A}
%15 HPI	2714 ± 70^{BC}	23083 ± 882^{A}
%20 HPI	2875 ± 39^{AB}	26564 ± 2071^{A}

3.5.3. Color analysis

The color attributes (L*, a*, b*) of meatball products fortified with HPI or SPI were given on Table 5. L* values decreased and the sample colors become visibly darker upon the addition of SPI. The L* value slightly increased with the addition of 10% HPI, and beyond that L* value decreased again. Most of the changes related to L* were not statistically significant. ΔE values express the overall color change, where color differences corresponding to $\Delta E^*>3$ are clearly visible to the naked eye (Jarpa-Parra et al.,

2017). In a number of cases, ΔE values were found to be >3 (Table 5). In terms of total color change, there was a significant difference between SPI and HPI treatments ($p<0.05$). Therefore, the influence of each protein isolate should be evaluated separately for the products.

Table 5. Color parameters for cooked meatballs as a function of HPI or SPI fortification (5-20%).

Sample	*L**	*a**	*b**	*ΔE*
Control	38.77 ± 0.2^{BC}	4.07 ± 0.2^{AB}	4 ± 0.2^{B}	N/A
%5 SPI	43.19 ± 0.2^{A}	3.87 ± 0.2^{ABC}	7.2 ± 0.1^{A}	5.46
%10 SPI	41.69 ± 0.3^{ABC}	3.28 ± 0.3^{BCD}	6.23 ± 0.4^{AB}	3.76
%15 SPI	38.77 ± 0.1^{BC}	2.75 ± 0.1^{D}	4.34 ± 1.3^{B}	1.36
%20 SPI	40.87 ± 0.2^{ABC}	4.21 ± 0.2^{A}	5.84 ± 0.5^{AB}	2.80
%5 HPI	38.15 ± 0.1^{C}	3.42 ± 0.1^{ABCD}	3.6 ± 0.1^{B}	0.99
%10 HPI	42.17 ± 0.2^{AB}	3.13 ± 0.2^{CD}	6.44 ± 0.3^{AB}	4.29
%15 HPI	39.07 ± 0.1^{BC}	2.78 ± 0.1^{D}	4.16 ± 0.1^{B}	1.34
%20 HPI	39.37 ± 0.1^{ABC}	3.52 ± 0.1^{ABCD}	4.35 ± 0.1^{AB}	0.89

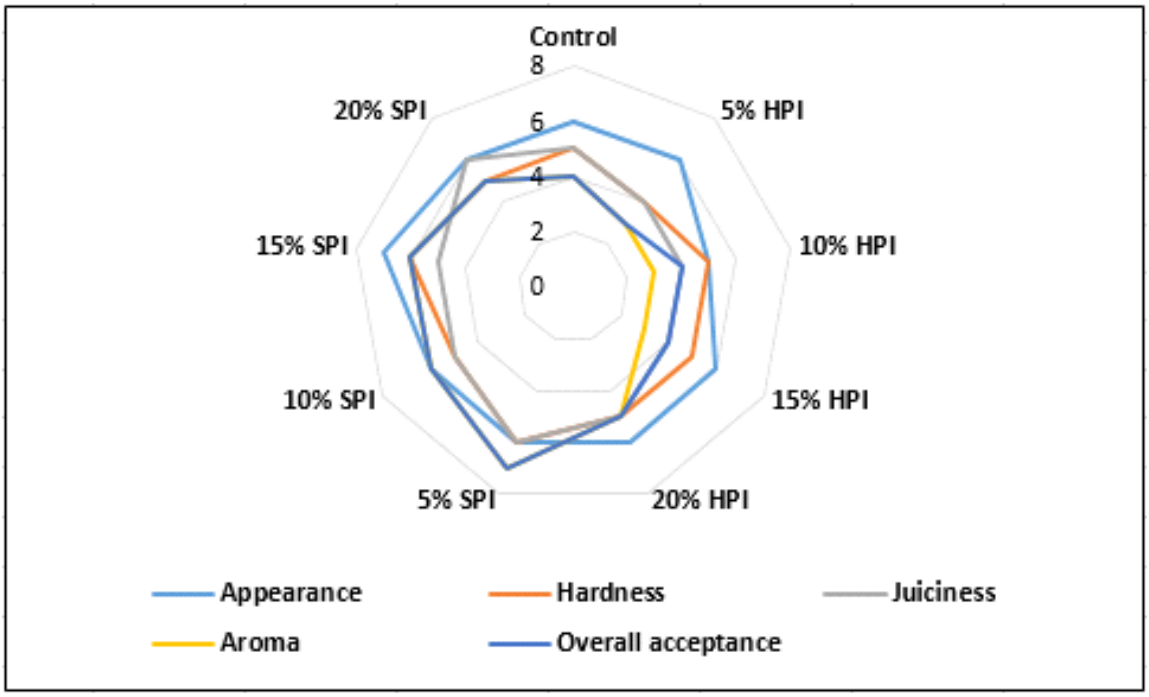


Figure 3. Sensory analysis of the meatball products after protein fortification with HPI or SPI (5-20%).

3.5.4. Sensory analysis

Meatballs fortified with different concentrations of HPI and SPI were analyzed by sensory methods and the results were presented on Figure 3. Sensory analysis was based on a number of sensory characteristics including appearance, hardness, juiciness, aroma and overall acceptance. The changes in appearance, hardness, juiciness, aroma and general acceptance values were statistically significant to the panelists upon the addition of different concentrations of HPI or SPI (5-20%) ($p<0.05$).

The panelists rated the samples that contained 5% SPI with the highest scores. Panelists have indicated that they would also rate meatballs containing 10% and 15% SPI as acceptable, whereas 5% or 15% HPI

samples and control samples were preferred at a lower rate. The acceptability of 5% SPI fortified meatballs were attributed primarily to their aroma characteristics. While bran addition to meatballs up to 10% did not alter sensory analysis results significantly, the authors emphasized the influence of particle size on sensory attributes (Huang et al., 2005). Consequently, the current results could be further enhanced based on the optimization of fortification procedures.

4. Conclusions

Cold press oil production has become increasingly popular over the recent years, which in turn lead to the generation of high quality (i.e., low oil content, low peroxide value etc.) and high-protein content press cakes. Consequently, the valorization of seed cakes has gained importance. In this study, protein isolates were generated from sesame and hazelnut press cakes and utilized in meatball manufacture. While the differences between two protein isolates were attributed to differences in molecular sizes and potential differences in fiber content, a certain protein fortification level (mostly 5%) was acceptable. Proteins generate molecular interactions amongst each other, hold significant amounts of water and fat, consequently protein fortification of meat products could easily generate an undesirable texture. In the current study, however, while protein fortification altered the color of the meatballs and increased firmness, toughness or size reduction attributes were mostly unaffected. Plant protein isolates can be utilized in either partial replacement of meat protein or in fortification of protein content and generate more sustainable products.

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FUNCTIONAL CHARACTERISTICS OF BIOACTIVE PHYTOCHEMICALS IN *BETA VULGARIS L.* ROOT AND THEIR APPLICATION AS ENCAPSULATED ADDITIVES IN MEAT PRODUCTS

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ABSTRACT

Beetroot ethanolic extract contains active compounds and valuable elements such as phenols, carotenoids, alkaloids, tannin, flavonoids, and vitamins B_3, B_9, B_6, and C. Quality characteristics and microbiological activity, texture, and colour were examined in the storage process at refrigerated temperature (up to 9 days at 4±2 °C) of beef burger pads made directly and encapsulated in alginate beads of *Beta vulgaris subsp.* (BVE). Over time, the Encaps-SDW and SDW (Control Samples) total mesophile bacteria counts peaked at CFU 8.61±0.22 and 8.74±0.17 log CFU/g, respectively, during storage (9 days). The lowest values ($p < 0.05$) were shown in the Encaps-BVE and BVE samples, with 7.23±0.12 and 6.58±0.09 log CFU/g, respectively. However, the differences between all samples were significant ($p<0.05$), the BVE extract strongly inhibited *Enterobacterial* growth, with values on average two log units lower in BVE and Encaps-BVE than SDW and Encaps-SDW samples (control samples). Also, the addition of BVE extract kept the pH of beef minced nearly constant during storage; however, the pH value of control samples increased significantly ($p<0.05$). Furthermore, samples containing Encaps-BVE showed a more consistent trend in terms of texture and colour characteristics during the storage period than the other treatments, indicating the importance of using it as a natural preservative in meat product formulations to preserve quality standards and preservation.

1. Introduction

Consumers appear to be wary of chemical additives in these cases. Thus, attention is paid to discovering natural antimicrobials occurring to preserve food due to consumer knowledge and understanding of edible food products and an increasing concern around microbial resistance to traditional preservatives (Chouhan et al., 2017). However, some conventional or regulatory licensed antimicrobials have several limitations. In these cases, customers are likely to be wary of chemical additives. Growing market demand has led to high-quality, long-life storage foods and ready-to-eat foods that are only moderately reserved and retain a natural and fresh look as much as possible (Panchal, 2020). This has resulted in the quest for new anti-microbial compounds from natural sources. It is the potential to extract natural compounds and other natural products to contain bacteria in foods. Aromatic plants have considerable commercial value in foods, pharmaceutical industries, and cosmetics (Jain et al., 2019). Since ancient times, their use has taken place, and despite many of them being replaced by synthetic ones, demand for natural products is increasing. As an antimicrobial agent for food preservation, numerous extracts have emerged on the market in recent years. Scaling up antimicrobials' activity across the permitted regulatory substances is the primary

incentive to identify effective antimicrobials among natural compounds. Medicinal plant extracts are now emerging as alternatives to traditional natural preservatives to control the growth of foodborne pathogens and food spoilage bacteria, as they are generally healthy for humans and environmentally friendly. Microbe contamination, which impairs food quality and results in financial losses, is one of the most severe issues in the food sector processing. (Priyadarshani & Rath, 2012; Aneja et al., 2014; Flores & Toldrá, 2020; Fortunati et al., 2019; Ni et al., 2021).

Beta vulgaris L. subsp. vulgaris is a *Chenopodiaceae* (*Angiosperm*) family member and is commonly referred to as beets or garden beetroot. Beetroot varies in colour from yellow to purple red depending on the variety. Red beets are consumed by humans worldwide. Beetroot is a biannual herbaceous agricultural plant that is farmed for its edible roots and leaves. Salads, soups, jams, and juice are made with beetroots (Goldman & Navazio, 2003). Moreover, because the leaves are high in vitamins and antioxidants, they can be consumed raw or cooked as a spinach alternative. Due to betalains pigments in red beets, they have pharmaceutical and commercial applications, including food product coloring, medicinal formulations, cosmetics, and artwork (Neelwarne & Halagur, 2013; De-Ancos et al., 2015; Celli & Brooks, 2017; Kumar & Brooks, 2018; Miguel, 2018;). As a result, using effective antimicrobial agents found in fruits and vegetables to guard against microorganisms (bacteria and fungi) is a crucial strategy to solving this problem. Antibacterial, anti-inflammatory, antioxidant, antithrombotic, antiatherogenic, cardioprotective, antiallergenic, and vasodilatory effects have been found in red beets (Gliszczynska & Anna, 2013; De-Ancos et al., 2015).

One of the main aspects in enhancing study the chemical composition of red beetroots quality, prolonging shelf-life, maintaining consumer protection, product safety, and minimizing waste was the microbial control in minced beef. The primary purpose of this investigation was to improve the overall stability and quality of beef meat by using two different techniques to incorporate *Beta Vulgaris* L. root: direct processing and encapsulation in sodium alginate and encapsulation in sodium alginate. During refrigerated storage at 4±2 °C, the microbial characteristics, texture, colour, and pH of beef patties were investigated.

2. Materials and methods

2.1. Extraction Technique

The roots of *Beta vulgaris* subsp. *Vulgaris* var. Plano (sugar beet-red beetroot) was collected from the local market in Egypt and transported to the meat products laboratory in the Food Sci. and Tech. Dept., Faculty of Agri., Menoufia Univ. within 1-2 hours at room temperature into plastic boxes. The roots were manually peeled after being rinsed and cleansed with tap water, and then the roots were freeze-dried and ground into a soft powder (a crude extract) using an electric blender. The crud extract was dried in an oven at 35-40 °C for 24- 30 hr. The crud extract (500 mg) was separated for 24 hours using a shaker in 100 ml ethanol or distilled water, and then the solution was filtered and refrigerated at 4 °C until use.

2.1.1. Total Polyphenol

The Folin-Ciocalteu reagent assay was estimated to stain the extract's total phenolic content (Ozsoy et al., 2008). 0.4g dry sample obtained with 20 ml ethanol 80%, soaked in brawn bottle for 24 hours at room temperature, centrifuged for 5 minutes, volume adapted to 25 ml by ethanol 80%, filtered via Whatman no.1 filter paper, 10 ml of the solution evaporated to dryness, dissolved in 5 ml HPLC grade methanol 50%, filtered through PTFE filter with pore size 0.2 µm. Subsequently, the mixture was incubated for 30 minutes at room temperature (22 ºC ± 2), and the absorbance was measured with a spectrophotometer at 760 nm (School instrument, UV line 9400, EU). For the calibration curve, gallic acid was used as a standard material. Total phenolic content expressed as gallic acid equivalent. Both experiments were carried out in triplicate.

2.1.2. Total Flavonoids

The total flavonoids content was measured using the (Sakanaka et al., 2005) process. A 0.5 ml of extract was placed in a 10 ml volumetric flask. Distilled water was added to make an even volume of 5 ml, followed by 0.3 ml $NaNO_2$ (1:20). 5 min later, 3 ml $AlCl_3$ (1:10) were added. After 6 min, 2 ml of NaOH (4%) were added, and then by using distilled water, the total volume increased to 10 ml. The solution was mixed well again, and the absorbance was measured against a blank at 510 nm using a spectrophotometer (Schoot instrument, UV line 9400, EU). The findings were expressed as mg of sample quercetin/g. All measurements were collected in triplicate.

Condition of the instrument measuring total phenolic and flavonoid compounds: Agilent 1260 infinite HPLC Series (Agilent, USA) with Quaternary pump and a Kinetex XB-C18 (Phenomenex, USA) column 100 mm x 4.6 mm running at 35 °C. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H_3PO_4 (v/v), (B) methanol, and (C) acetonitrile. The total volume injected was 20 µL. VWD detector calibrated at 284 nm for detection. The total volume injected was 20 µL to measuring total flavonoids compounds. UV detector tuned at 273 nm for detection and database management using claritychrom@ software. This methodology was modified from Mattila et al. (1989) and Goupy et al. (1999). For fractionating flavonoids and polyphenols.

2.1.3. Water-soluble vitamins (WSV)

The WSV was evaluated by HPLC analysis following sample extraction as suggested by Albalá-Hurtado et al. (1997). A sample of dried beets (0.2 g) was weighed to a centrifuge tube together with 15 mL deionized water. Centrifuge at 4000 rpm for 5 minutes after 15 minutes of extraction, then transfer quantitatively to a 25 mL volumetric flask adding more water to the mark: before treatment, filter over a 0.2µm nylon membrane.

Condition of the instrument measuring Water-soluble vitamins: Agilent 1260 infinite HPLC Series (Agilent, USA) with Quaternary pump and a Kinetex XB-C18 (Phenomenex, USA) column 100 mm x 4.6 mm running at 35 °C. A double linear elution gradient using (A) 25 mM NaH_2PO_4 at pH = 2.5 and (B) methanol is used to separate the samples. The total volume injected was 20 µL. Ascorbic acids are detected at 254 nm, while vitamins B6, B3, B9, and B12 are detected at 220 nm (Mattila et al., 1989).

2.1.4. Total Tannin

Folin-Denis's reagent is used to determine the tannin concentration in red beetroots, as suggested by (Saxena et al., 2016). A spectrophotometer (Schoot instrument, UV line 9400, EU) was used to determine the absorbance at 700 nm.

2.1.5. Total Alkaloid

The alkaloids were determined using Adham's technique (Adham et al., 1998). The % alkaloid was defined as follows:

$$\% \text{ Total Alkaloid} = \frac{\text{Weight of remain}}{\text{Weight of Sample}} \; x\; 100$$

2.1.6. Total Anthocyanins

In methanol containing 1% HCl (v/v), fresh beetroot was homogenized and then filtrated. A spectrophotometer (Schoot instrument, UV line 9400, EU) was used to read the filtration at 530 and 657 nm, as Mancinelli et al. (2006) suggested.

2.1.7. Total carotenoid

Total carotenoids of beetroot were obtained using a mixture of acetone: hexane (1:1 v/v), as Jeyanthi et al. (2014) presented. And to use a spectrophotometer (Schoot instrument, UV line 9400, EU), the absorbance of carotenoids was determined at 630 nm.

2.2. Preparation of roots of *Beta vulgaris* in Berger:

Red-colored extract (dry crud extract) was diluted in an equivalent volume of (1:3) sterile distilled water (SDW) was used to add directly or encapsulated to minced beef.

2.2.1. Encapsulation of BVE

Encapsulation of *Beta vulgaris* subsp. extract (BVE) in sodium alginate was administered as designed by (Ribeiro & Veloso, 2020). In summary, according to the

method described above, a defined volume with diluted BVE was blended with sodium alginate 0.5 % (w/v) and allowed the solution to dissolve homogeneously. Once the BVE-alginate solution was homogeneous, the weight was recorded, and the solution was injected into a calcium chloride solution of 1.5% (w/v) using a syringe (0.80 mm × 25 mm). The beads were cleaned and filtered using sterile Whatman® class I paper, then allowed to settle for 20 minutes in the air before being weighed to ensure any BVE during the pelleting process, and there is a loss. According to (Aguirre & Santagapita, 2016), the projected pill size, which might include SDW or BVE, was estimated by scanning digital images of beads (taken with a brother MFC-7360N scanner) the free license program ImageJ1. The standard deviation is an expression of the size of the beads with an average diameter of the ferret that agrees with the total distance (mm) between any two or more point's places and the bead limits.

2.2.2. Beef Burger Patty Preparation

Beef mince from two different batches (3 kg x 2 kg) was purchased at a local market in Shebin El-Kom (Egypt), brought to the meat products lab in a transportable refrigerator at 4 ± 2 °C (within 30min), and rapidly utilized for further meat combinations. To achieve a homogeneous mixture, each lot (1 kg) of minced beef was mixed in a bowl with 0.8 % (w/w) of NaCl for 3 minutes, which had been categorized into four 250g experimental groups; each module was added with sterile distilled water (SDW 5%, v/w), encapsulated sterile distilled water (Encaps-SDW 5%, v/w), *Beta vulgaris* subsp. extract (BVE 5%, v/w), encapsulated *Beta vulgaris* subsp. extract (Encaps-BVE 5%, v/w). The 5% (v/w) concentration of *Beta vulgaris subsp*. the extract was chosen based on previous research on beef slices (Aminzare et al., 2015); Parafiti et al., 2018). Several concentrations of *Beta vulgaris* extract were tested to see which one type of most inhibited microbial growth while refrigerated storage. Burger samples were made with a burger patty builder to achieve a comparable weight and size (about 50 g, with a diameter ranging of 5 cm and a thickness of 2 cm) and labeled as shown in Table 1. All samples were mobilized in an aerobic environment on a food tray with a sealed plastic polyethylene membrane and kept at 4±2 °C in a refrigerator. Prepared burger samples were used to add SDW, each encapsulated with or without sodium alginate, to ground beef as controls. Each sample from every batch was investigated for pH values, microbial parameters, colour characteristics (CIE *a*, L*, and b**), and profile of texture properties after 0, 3, 6, and 9 days of storage period at 4±2 °C.

Table 1. The study's beef burger formulation.

Treatment	Ingredients in burger patties
SDW	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) sterile distilled water
Encaps-SDW	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) of sterile distilled water encapsulated in alginate beads
BVE	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) encapsulated *Beta vulgaris* subsp. extract
Encaps-BVE	Minced meat + 0.8% (w/w) NaCl + 5% (v/w) *Beta vulgaris* subsp. extract encapsulated in alginate beads

2.3. Microbiological Analysis

The antibacterial activity of potential microbial evaluation of the *Beta vulgaris subsp*. extract (BVE) added to the preparation of pies burgers, both coated and non-coated in sodium alginate granules, was predestined by observing the increase of the microbial population after 0, 3, 6, and 9 days of storage at 4±2°C. In summary, a part of every sample (10 g) was aseptically placed in a stomacher filter package containing 90 ml of sterile Ringer's solution, homogenized for 5 minutes, then serially diluted afterward. To observe the growth of total *Pseudomonas*, total mesophilic bacteria, and *Enterobacteriaceae*, suitable dilutions were plated in Petri dishes plate Count Agar with

Violet Red Bile Glucose Agar, cycloheximide 0.1% solution, and *Pseudomonas* Agar Base, supplemented with *Pseudomonas* CFC selective agar supplement (SR-0103). At 35 or 25 °C, the dishes were incubated for 24–48 hours (*Pseudomonas spp*. count). Microorganisms were calculated using three replicates, with the mean represented as log CFU/g of burger ± standard deviation.

2.4. pH Values

The pH values of samples were measured by using a digital pH meter (Model 3510, Jenway Technology, Italy). With the help of two buffer solutions of pH 4 and 7, the pH meter electrode was calibrated. In a Cyclo-Mixer, 15 g of finely powdered sample was mixed in 50 ml of distilled water in a test tube (CM- Model 3000 USA). It was obtained using Whitman filter paper No. 1 as a filter. The pH meter's electrode was immersed in the filtrate, and the sample pH was measured. All values were calculated as the average of three replicates ± standard deviation (Khandil *et al.,* 2020).

2.5. Instrumental colour

The beef samples patty was pressed on a flat surface. The Hunter (*a*, b*,* and *L**) scale colour difference was determined using a scale colour spectrophotometer (machine colors Tristimulus) and a CIE Lab colorimeter (Hunter, Lab Scan XE - Reston, VA, USA) in reflection mode, with the colorimeters held over the samples at room temperature at 25°C (Tatli et al., 2020; Muge et al., 2020).

2.6. Instrumental texture

The texture characteristics of the burger samples were determined using Mercadante et al. (2010) methodologies with slight modifications. The burger samples were first taken from the casings and sliced into 2.5 cm length pieces before being compressed twice with a Texture Analyzer (Texture Analyzer TA-HD plus, 750 kg. (7.5 kN), England). Textural characteristics were evaluated at room temperature with the following processing parameters: 5.0 mm/s crosshead speed, 99.0 g surface sensing force, and 30.0 g threshold using a diameter cylindrical aluminum probe (5cm×4cm) 1*s* time gap between the first and second compressions. The Texture Professional version 1.0 program collected and analyzed the data (Stable Micro Systems, Surrey, England). The force-time curves were used to determine each sample's hardness, gumminess, springiness, cohesiveness, and chewiness (Bourne, 1978; Muge et al., 2020). The results were based on the average and standard deviation (±) of three replicates with one burger for each measurement.

2.7. Statistical Analysis

All experimental data were represented as mean values with ± standard deviation. Data from various tests were examined separately and compared using a one-way analysis of variance (ANOVA). Based on (Artimage & Berry, 1987; Kowalczewski & Andreani, 2015) (Laleg et al., 2019) protocols with minor adjustments, the significance of differences between the means was compared using Fisher's test ($p<0.05$). The data is displayed as mean SD (+). According to Fisher's least significant difference test ($p<0.05$), results followed by different letters within the same storage duration (0, 3, 6, and 9 days) are significantly different in each row.

3.Results and discussions

3.1. Red beet root's chemical components.

Which is shown in Table 2, total phenolics (130.44±0.11 mg/g DW), total flavonoids (1.22±0.251 mg/g DW), total tannin (4.89±0.114 mg/g DW), total anthocyanins (59.89±0.082 g/100g FW), total alkaloid (2.54 ±0.018 g/100g DW), and carotenoids (1.55±0.221 mg/100g FW) are present in the ethanolic extract.

Table 2. The Quantitative chemical composition, Phenolic and Flavonoid compounds, and Water-soluble Vitamins of *Beta vulgaris* root

Components	Composition
Total phenolic (mg Gallic acid /g DW)	130.44±0.11
Total flavonoid (mg Quercetin /g DW)	1.22±0.251
Total tannin (mg Tannic acid /g DW)	4.89±0.114
Total alkaloid (g/100g DW)	2.54± 0.018
Total anthocyanin (µg/100g FW)	59.89±0.082
Carotenoids (mg/100g FW)	1.55±0.221
Phenolic compounds (Conc. mg/100g DW)	
Gallic acid	10.22±0.02
Catechol	6.88±0.11
p-Comuaric acid	0.58±0.14
Ferulic acid	0.88±0.08
o-Coumaric acid	1.47±0.24
Cinnamic acid	0.74±0.43
Flavonoid compounds (Conc. mg/100g DW)	
Myricetin	20.22±0.11
Naringenin	19.42±0.24
Kaempferol	2.98±0.47
Apigenin	2.78±0.42
Vitamins contents (Water-soluble Vit.: mg/100g DW)	
Ascorbic acid (Vit. C)	25.87±0.17
Niacin (Vit. B3)	1.47±0.10
Pyridoxine (Vit. B6)	5.879±0.08
Folic acid (Vit. B9)	2.59±0.31
Note: **Values are mean ± SD of three replicate analyses**	

Soluble vitamins are identified using HPLC. The ethanolic extract of red beetroot includes vitamin C (25.87±0.17 mg/100g DW), vitamin B3 (1.47±0.10 mg/100g DW), vitamin B6 (5.879±0.08 mg/100g DW), and vitamin B9 (2.60 mg/100g DW, according to the data in Table 1. Phenolic compounds are separated by using HPLC). Table 1 shows that catechol (6.88±0.11 mg/100g DW), ferulic acid (0.88±0.08 mg/100g DW), gallic acid (10.22±0.02 mg/100g DW), *p*-Comuaric acid (0.58±0.14 mg/100g), and o-Cinnamic acid (0.74±0.43 mg/100g) are all present in the ethanolic extract of red beetroot. Furthermore, the ethanolic extract of red beetroot includes a variety of flavonoids, including naringenin (19.42±0.24 mg/100g DW), myricetin (20.22±0.11 mg/100g DW), apigenin (2.78±0.42 mg/100g DW), and kaempferol (2.98±0.47 mg/100g DW).

3.2. pH Determination

The pH values are presented in Fig. 1 correspond to burger patties containing BVE, either encapsulated or/not encapsulated in sodium alginate, and to Encaps-SDW or SDW samples control stored at 4±2°C for 9 days. The samples Encaps-SDW, SDW, BVE, and Encaps-BVE, had comparable beginning pH values of 5.97±0.13, 5.94±0.11, 6.03±0.11, and 5.98±0.14 for Encaps-SDW, SDW, BVE, and Encaps-BVE extract, respectively, right after treatment (0 times). As compared to the SDW (control samples), the BVE sample had a significantly ($p < 0.05$) higher pH value of 6.03±0.11. While the pH of the Encaps-SDW and SDW control samples remained stable (approximately at 5.96) after three days of

storage, the pH of the Encaps-BVE and BVE samples significantly decreased ($p<0.05$), reaching 5.31±0.15 and 5.49±0.11, respectively. The pH value in the SDW sample increased significantly ($p<0.05$) after six days of storage (Figure1), reaching 6.61±0.15. This was nearly constant (6.03±0.12) in Encaps- SDW, whereas sample BVE had the lowest values of 5.17±0.09, substantially ($p<0.05$), confirmed by Encaps-BVE at 5.13±0.05. After 9 days of storage, the variation in pH values was significantly greater. Indeed, the pH of BVE and Encaps-BVE samples was 5.16±0.12 and 5.54±0.10, respectively. In contrast, the pH of SDW and Encaps-SDW samples was significantly higher at 7.11±0.14 and 6.89±0.05, respectively.

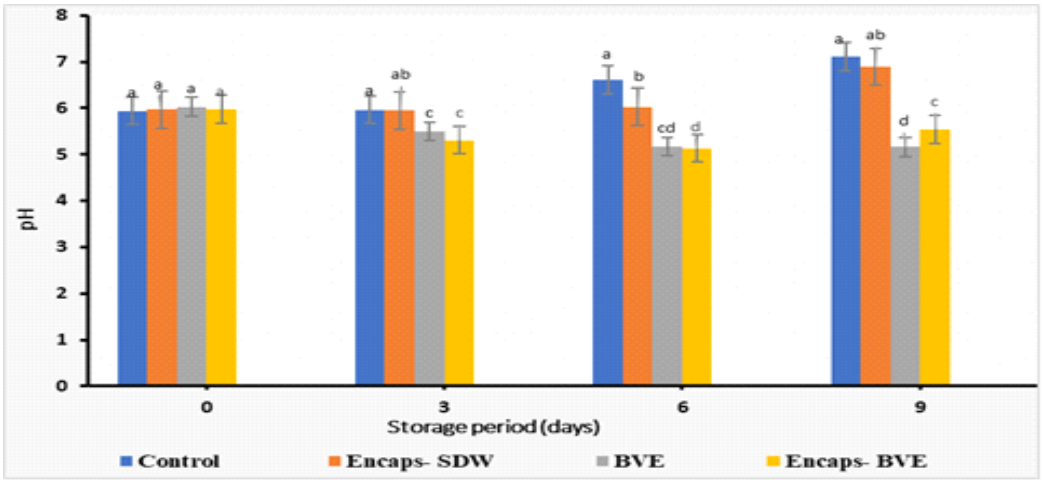

Figure 1. pH values of beef burger patties incorporating *Beta vulgaris* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

3.3. Microbiological Analysis

Figures 2, 3 and 4 show the microbiological counts on burger patties with or without *Beta vulgaris* extract, each encapsulated or/not encapsulated in sodium alginate, after 9-days of storage (4±2 °C). In furthermore, vitamins A, B, and C, as well as folic acid, can help with motor function. On plate count agar, the initial estimate of Total Mesophilic Bacteria has been 4.8 log CFU/g throughout all treatment (BVE and SDW). Despite this, it immediately rose after three days of storage, exceeding the European regulation's limit of 7.14 to 7.32 log CFU/g (5×10^6 CFU/g) for total aerobic bacterial counts (Regulation EC-2073, 2005). Although Total Mesophilic Bacteria count increased during six days of storage in Encaps-SDW and control samples SDW, it stayed relatively constant in Encaps- BVE and BVE samples. Total Mesophilic Bacteria count achieved peak values of 8.61±0.22 and 8.74±0.17 log CFU/g in Encaps-SDW and SDW (control samples), respectively, at the end of storage (9 days). Encaps-BVE and BVE samples, on the other hand, had the lowest values ($p<0.05$) at 7.23±0.12 and 6.58±0.09 log CFU/g, respectively, even though the Encaps-BVE was inside the microbiological limit (Fig. 2). The count of *Enterobacteriaceae* on burger patties processed with Encaps-SDW, Encaps-BVE, and BVE samples (Fig. 3), were not statistically different ($p>0.05$) from the SDW sample (5.41±0.13 log CFU/g) initially after procedures (0 days). The BVE and Encaps-BVE samples had the lowest enterobacteria counts ($p<0.05$) after 3, 6, and 9 days of storage when evaluated to Encaps-SDW and control samples SDW. Although there were some differences between samples, the BVE strongly inhibited enterobacterial growth, with values averaged 2 log units lesser in measurements of Encaps-BVE and BVE (6.45±0.15 and 6.01±0.09 log CFU/g, respectively) than SDW

and Encaps-SDW samples at the end of storage (9 days).

Figure 4, *Pseudomonas* spp. counts began at 4.12±0.11 log CFU/g and grew in all tests performed after 9-days of storage, although significant differences probably depend on treatments and experimental conditions. During 6 and 9 days, all samples containing BVE had considerably lower values ($p<0.05$) than SDW and Encaps-SDW, which had the highest values of 8.77±0.13 and 8.12±0.11 log CFU/g, respectively. Immediate addition of BVE (6.44±0.16 log CFU/g) produced the most significant review ($p<0.05$) at the end of storage (9 days), followed by encapsulated SDW (8.12±0.05 log CFU/g) and encapsulated BVE (7.55±0.09 log CFU/g).

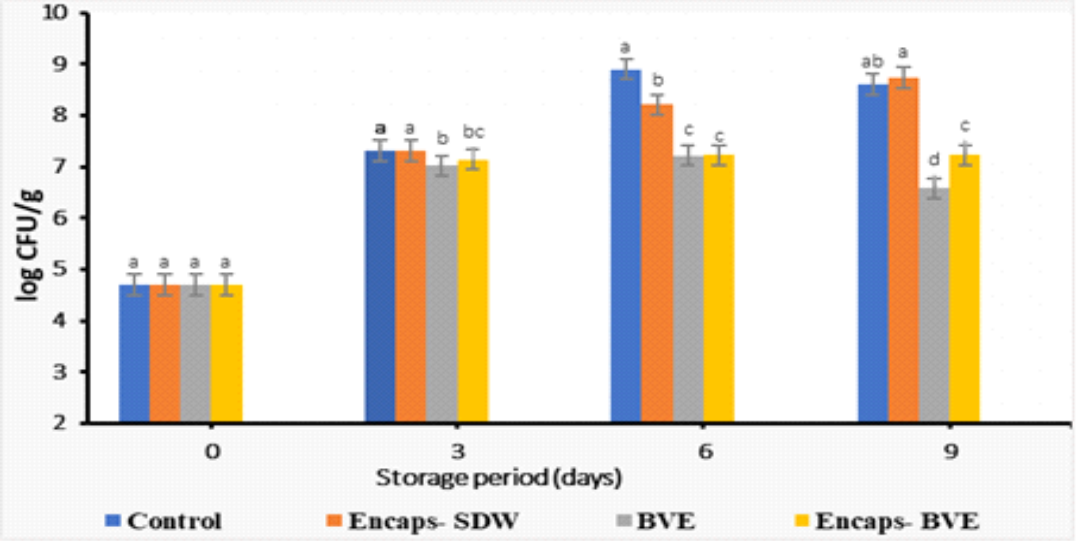

Figure 2. Total mesophilic bacteria (TMB) of beef burger patties incorporating *Beta vulgaris L.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

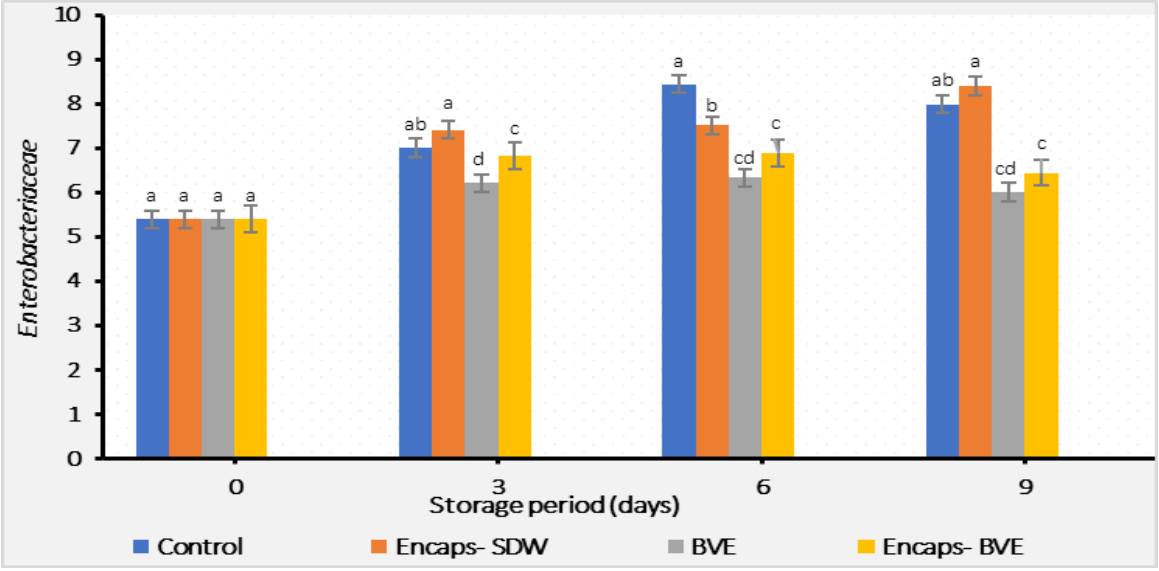

Figure 3. Growth of *Enterobacteriaceae* of beef burger patties incorporating *Beta vulgaris L.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

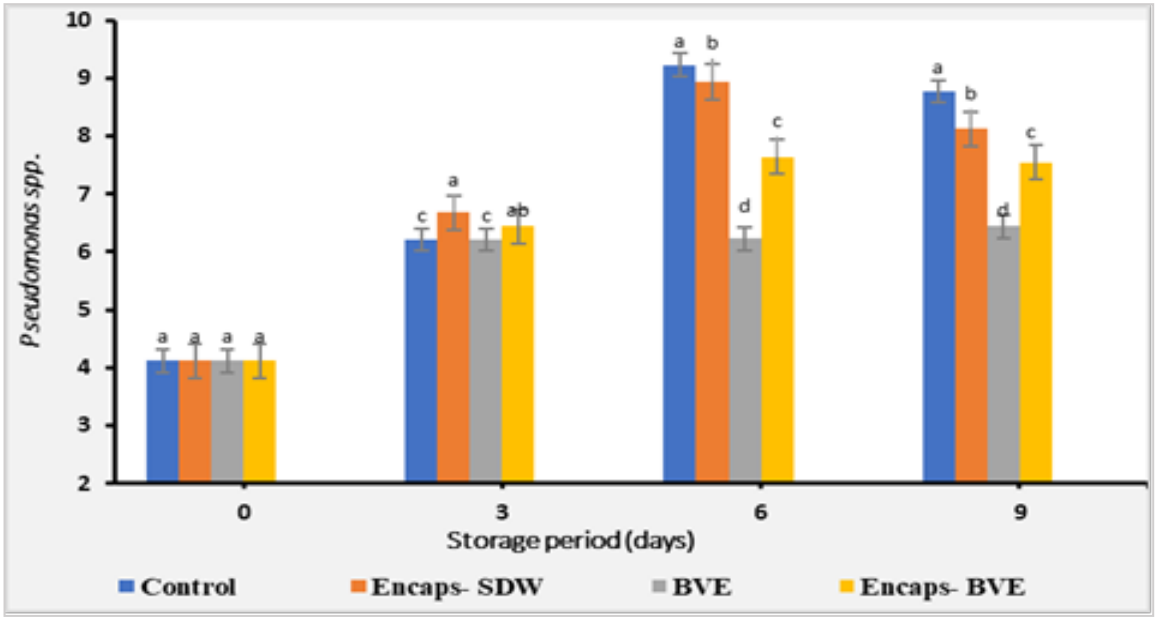

Figure 4. Growth of *Pseudomonas* spp. of beef burger patties incorporating Beta vulgaris L. root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C

3.4. Estimation of Color Characteristics

The addition of BVE, including direct and encapsulated, significantly ($p<0.05$) affected the initial *L*, a*,* and *b** characteristics of beef patties compared to the comparable controls of SDW and Encaps-SDW. While BVE was directly added to minced beef, *L** revealed the significantly lowest value ($p<0.05$) of 41.21±0.23, followed by Encaps-BVE and Encaps-SDW samples (42.18±0.14 and 46.25±0.11, respectively). After six days of storage, the lightness levels of all different treatments increased (Fig. 5). After 9 days, they were 53.87±0.12, 54.13±0.11, 46.15±0.18, and 46.01±0.17, respectively, for Encaps-SDW, SDW, BVE, and Encaps-BVE. As illustrated in Figure 6, Encaps-BVE and BVE beef samples showed significantly higher ($p<0.05$) *a** values than Encaps-SDW and SDW samples control (13.57±0.12 and 13.45±0.11, respectively). During 9 days of storage, Encaps-SDW and SDW control samples had *a** values at 9.15±0.17 and 7.99±0.14, respectively, whereas Encaps-BVE and BVE samples had *a** values of 15.33±0.12 and 15.47±0.14, respectively. Furthermore, the sample Encaps-BVE had the lowest *a** value variations over the storage period. Compared to sample Encaps-BVE, sample BVE showed an unusual trend, declining *a** value of nearly twice after 9 days. The *b** values observed in Encaps-BVE and BVE beef samples were significantly affected ($p<0.05$), with initial levels of 15.24±0.13 and 15.82±0.14, respectively, when compared to Encaps-SDW and SDW samples, which had values of 13.34±0.24 and 11.08±0.18, respectively (Fig. 7). After 3 days of storage, the SDW and Encaps-SDW were significantly lower (7.32±0.15 and 7.99±0.12, respectively); around the same time, samples of BVE and Encaps-BVE were less likely (11.20±0.21 and 10.54±0.09) to have a relationship with previously identified antimicrobial effect (Figures 5, 6, and 7). In comparison to BVE, Encaps-BVE had no significant impact ($p>0.05$) on the beginning of colour parameters; when particularly in contrast to Encaps-SDW and SDW, samples BVE and Encaps-BVE had one of the most constant trends up to 9 days of storage.

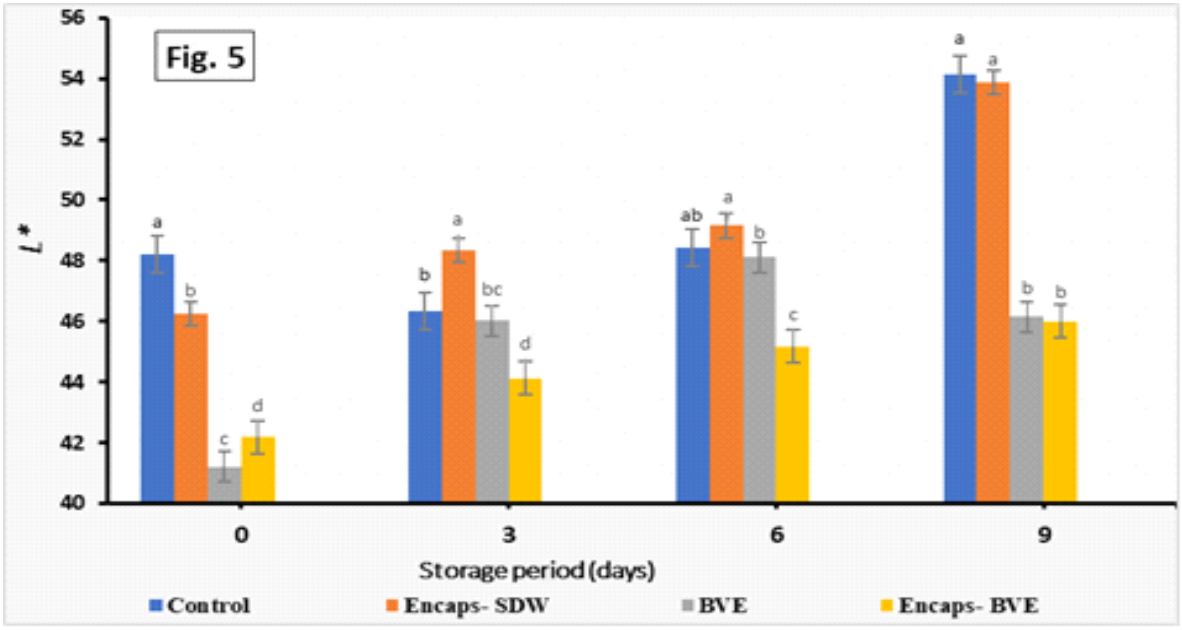

Figure 5. Color values L* of burger patties incorporating *Beta vulgaris* root extract (BVE) and encapsulated *Beta vulgaris l.* root extract (Encaps-BVE). Sterile distilled water (SDW) or encapsulated sterile distilled water (Encaps-SDW) were used as controls. Color characteristics of burger surfaces were measured during 9 days of storage at 4± 2°C. The standard deviation (±) of the mean is represented by vertical bars.

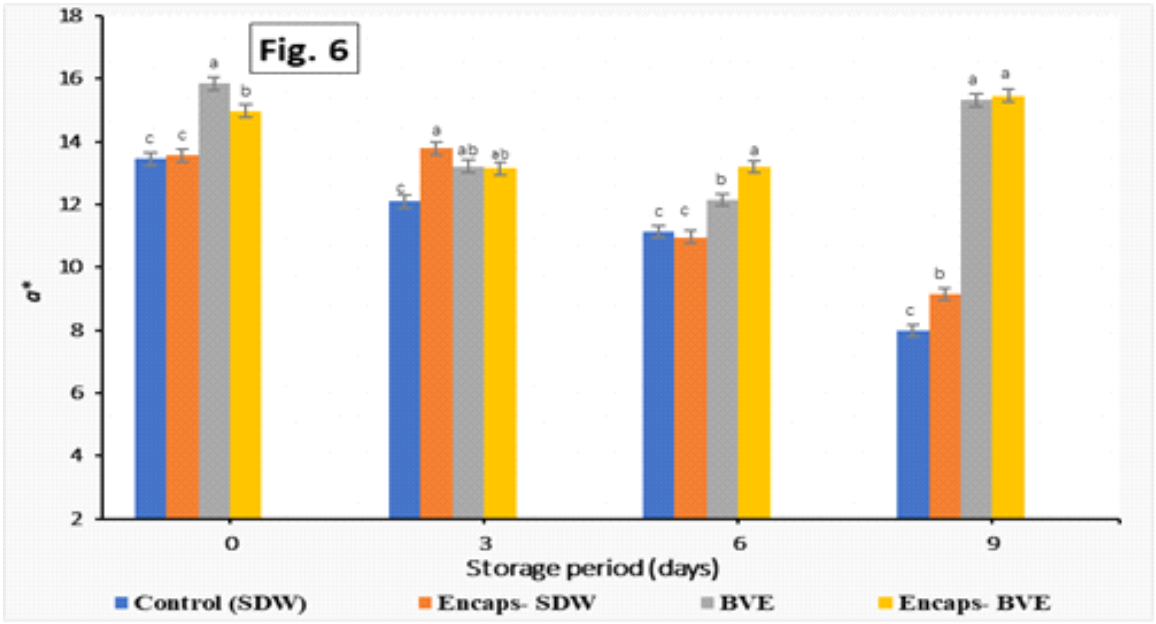

Figure 6. Color values a* of burger patties incorporating *Beta vulgaris* root extract (BVE) and encapsulated *Beta vulgaris l.* root extract (Encaps-BVE). Sterile distilled water (SDW) or encapsulated sterile distilled water (Encaps-SDW) were used as controls. Color characteristics of burger surfaces were measured during 9 days of storage at 4± 2°C. The standard deviation (±) of the mean is represented by vertical bars.

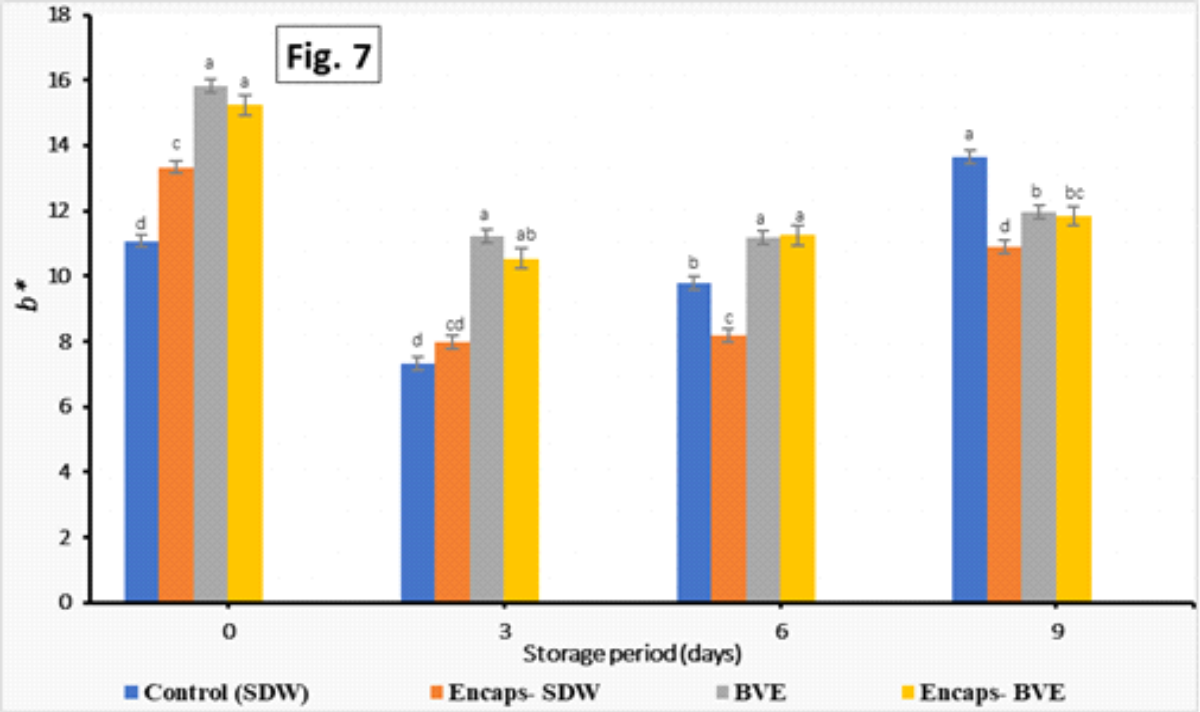

Figure 7. Color values b* of burger patties incorporating *Beta vulgaris* root extract (BVE) and encapsulated *Beta vulgaris l.* root extract (Encaps-BVE). Sterile distilled water (SDW) or encapsulated sterile distilled water (Encaps-SDW) were used as controls. Color characteristics of burger surfaces were measured during 9 days of storage at 4± 2°C. The standard deviation (±) of the mean is represented by vertical bars.

3.5. Texture profile

According to the storage period and considered parameters, the addition of BVE and Encaps-BVE considerably altered the textural characteristics of beef patties during storage (springiness, hardness, and cohesiveness) shown in Table 8, 9, and 10. Shortly after treatment (time 0), it was hardness comparable amongst treatments ($p>0.05$). After three and six days of storage at 4 ±2 °C, all except one of the samples, the SDW control samples, had increased hardness values. After nine days of storage (Fig. 8), samples of BVE extract had the highest levels ($p<0.05$), followed by Encaps-BVE extract; there was no significant difference ($p>0.05$) between the Encaps-SDW and SDW samples, which had the lowest hardness values. This impact was less apparent in sample Encaps-BVE extract due to the encapsulation of BVE extract. In addition to SDW and Encaps- SDW samples, cohesiveness and springiness improved in BVE and Encaps-BVE extract samples with storage period, achieving significantly affected ($p<0.05$) a higher value during 3, 6, and 9 days (Fig. 9 and 10).

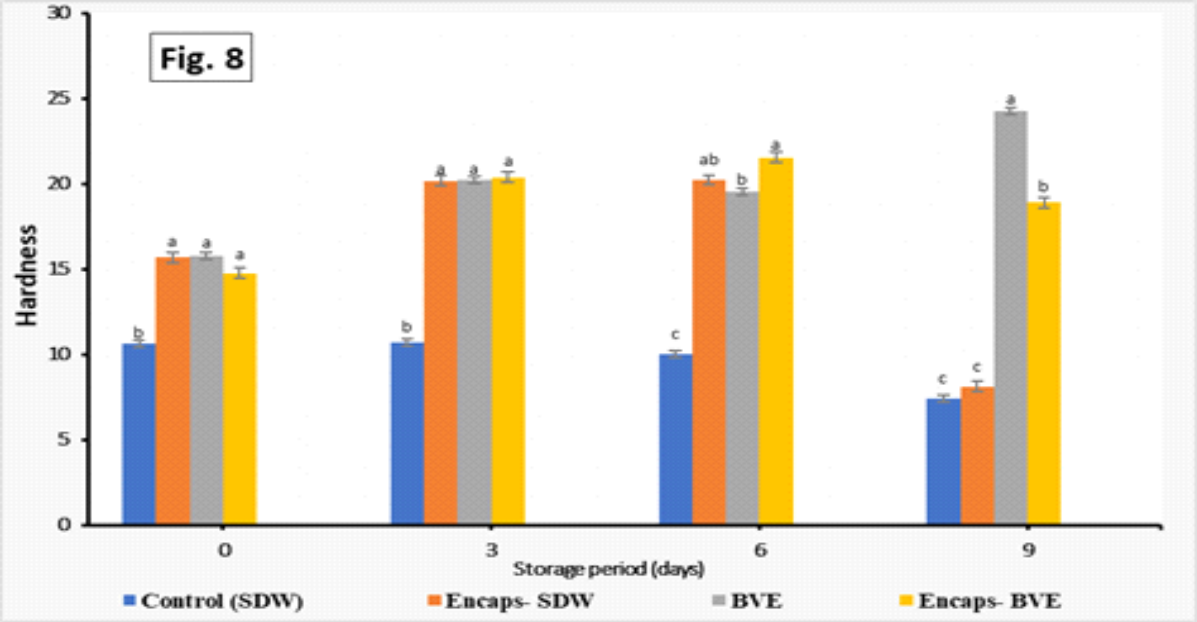

Figure 8. Hardness of beef burger patties incorporating *Beta vulgaris l.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

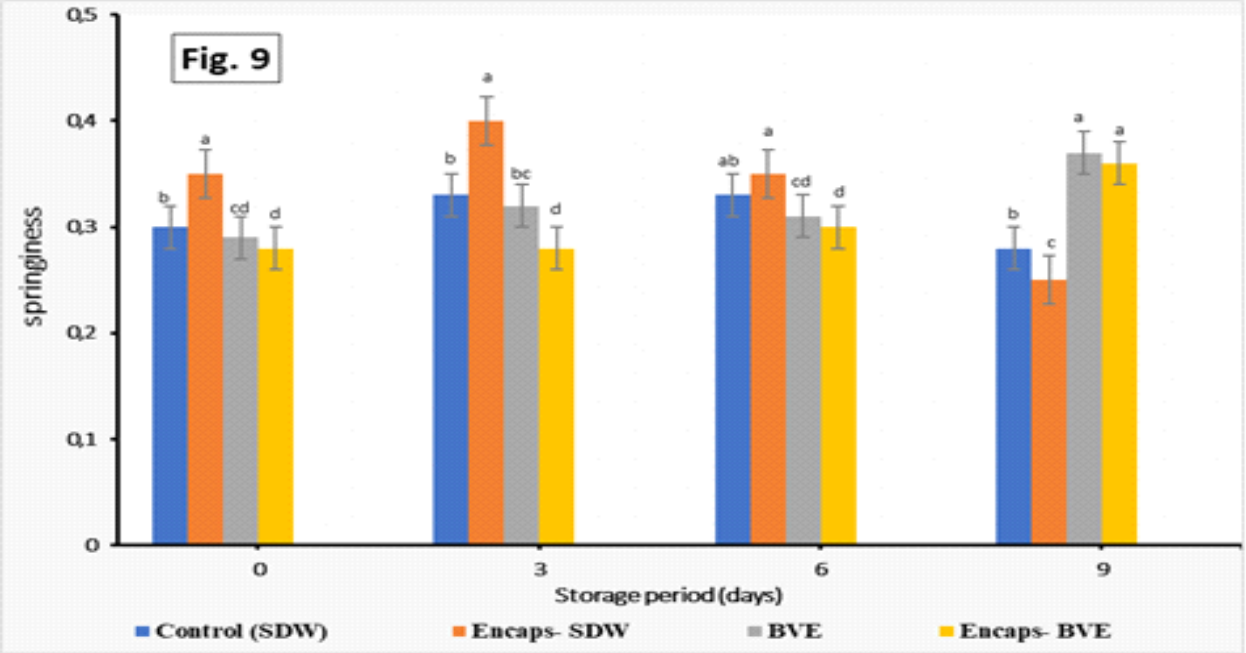

Figure 9. Springiness of beef burger patties incorporating *Beta vulgaris l.* root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

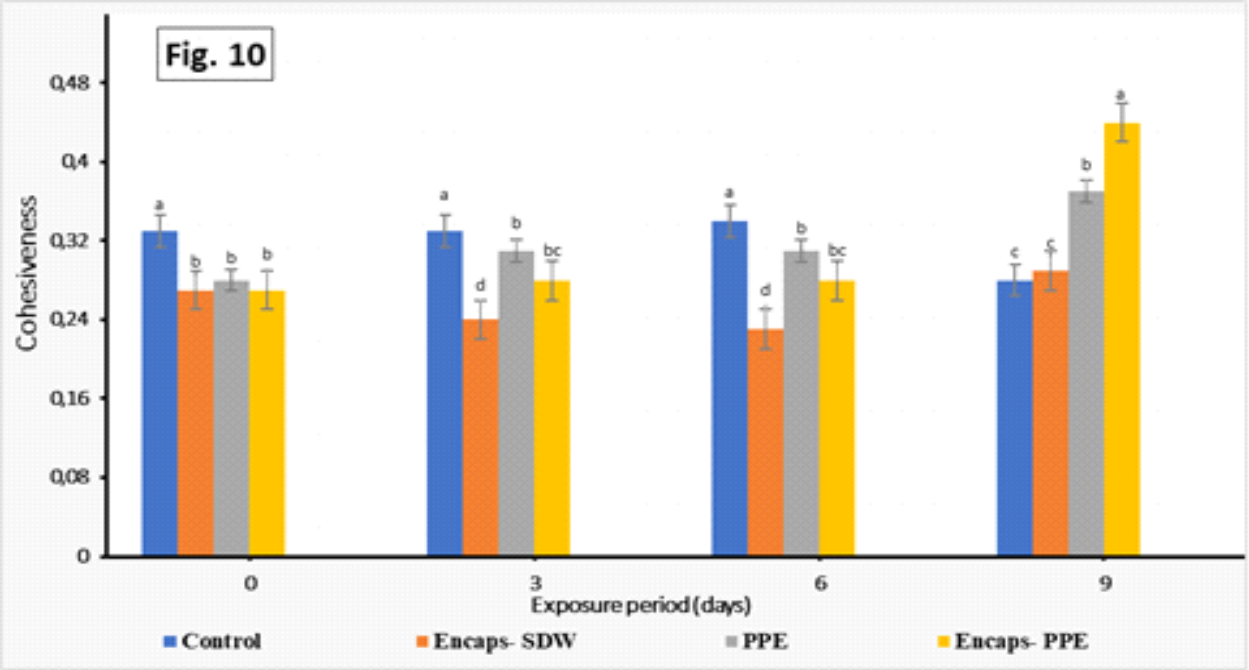

Figure 10. Cohesiveness of beef burger patties incorporating *Beta vulgaris l*. root extract (BVE) or sterile distilled water (SDW) during 9 days of storage at 4±2 °C.

3.6. Discussion

The existence of bioactive compounds in red beetroot has influenced its pharmacological and physiological properties. Polyphenols, flavonoids, alkaloids, folic acid, tannins, ascorbic acid, and reducing sugars were identified as the primary ingredients of the red beetroot extract in earlier investigations (Azmir et al., 2013; Castro-Enríquez *et al.,* 2020; Rashmi & Negi, 2020). Flavonoids, for example, have been demonstrated to have antibacterial, antineoplastic, antiviral, anti-allergic, and antioxidant, and antimicrobial properties (Sameeh et al., 2016; Karak, 2019; López-Lázaro, 2009;), acting as scavenging free radicals and metal chelators. Alkaloids have biological activities used in food industries, recreational drugs, and medicinal (Gülçin, 2011; El-Beltagi et al., 2018; Akanni et al., 2019). Tannins have been shown to protect carbohydrates and proteins against microbial breakdown in meat products (Jakobek, 2015; Bunglavan & Dutta, 2013; Allam & Dolgonova, 2017[b]).

Furthermore, carotenoids protect against cancer, inflammatory processes, cardiovascular disease, and age-related dystrophy muscular (Ahmed et al., 2014) and act as an antibacterial and antioxidant (Allam & Dolgonova, 2017[a]; El-Beltagi *et al.,* 2018; Kandil et al., 2020). Odoh and Okoro (2012) observed that beetroot contains considerable amounts of vitamins, particularly Vit. C (4.36 mg/100 g), and their findings are identical to ours. Red beetroot has a high content of ascorbic acid, according to the investigation. This vitamin is essential for human food and nutrition, especially tissue maintenance and growth, neurotransmitter synthesis, hormone production, and immune responses. These findings are comparable to those of Vuli et. al., (2010) who found vanillic, ferulic, caffeic, *p*-hydroxybenzoic, and protocatechuic acids in beetroot. Pyo *et. al.*, (2004) reported similar findings, identifying the following compounds: myricetin (2.2 mg/100 g FW), catechin (6.7 mg/100 g FW), quercetin (7.5 mg/100 g FW), and kaempferol (9.2 mg/100 g FW).

Quality characteristics and Microbiological activity, texture, and colour were examined in the storage process at refrigerated temperature (up to 9 days at 4±2 °C) of beef burger pads made directly and encapsulated in alginate beads of BVE extract. Microbial results showed

that both immediately added and or/not encapsulated BVE extract was conservative, which reduced significantly ($p<0.05$) *Enterobacteriaceae*, *Pseudomonas* ssp. counts and total mesophilic bacteria, compared to samples addicted with control samples SDW or SDW encapsulated. The commonly added BVE extract to burger formulations, on the other hand, appeared to be more capable of limiting the growth of approximated microbial populations of organisms, especially after 6 days; justifications for this must be attributed to the reality that almost all of the additional extracts interacted instantly with microorganisms and bacterial cells, decreasing their feasibility and effectiveness, or even with meat tissue and so limiting its degradation, resulting in the formation of smaller molecules that microorganisms can consume (Hassan et al., 2018). Parafati *et al*. (2019) previously published similar results on sliced beef, demonstrating the potential of BVE to decrease microbial activity while significantly at 4 °C of storage.

Furthermore, Kharrat et al. (2018) found that using BVE as a natural additive and preservative increases the microbial stability of salami, owing to the high content of betalains, phenolic, and flavonoids compounds in BVE extract. Various alternative strategies and preservation techniques were investigated because burgers are hazardous food from a microbiological or quality standpoint. Compared to control burger samples, Özvural *et al*. (2016) found that using encapsulated extract of green tea on burger pie preparation significantly decreased the coliform, total Mesophilic Bacteria, mold, and yeast count. According to observations from the last day of storage (6-8 days), the impacts of various preparations of chitosan and chitosan/sodium tripolyphosphate composite alternatives which include β carotene as edible coatings and preservatives in hamburgers on bacteriological activity, oxidative, and quality characteristics, features were recently investigated by the same authors (Özvural et al., 2016; Özvural and Huang, 2018; Parafati et al., 2019; Hemmatkhah et al., 2020; Chaudhary et al., 2020). The findings demonstrate that using the solution as a functional ingredient and edible coating was improved and much more successful microbiological characteristics and lipid oxidation. Chemical agents extracted from animals, plants, bacteria, and their metabolites that avoid the decomposition of different food items are natural preservatives. They work by inhibiting the growth of microbial, oxidation, and particular food enzymatic reactions. A preservative is a natural or synthetic derived chemical compound that protects finished products from decomposing due to microbial growth or other undesired chemical changes. They are applied to different foods to prevent them from spoiling, discoloration, or infection by microorganisms, and they improve their texture, colour, taste, and nutrition (Adham et al., 1998; Meyer et al., 2002; Kabak et al., 2006; Mei et al., 2019).

After storage, the pH of Encaps-SDW and SDW (control samples) increased (Biswas et al., 2004), most commonly due to the development of metabolites generated or primary microbial metabolites from protein beef deamination. The application of BVE extract had no discernible impact ($p>0.05$) on the pH of beef at the beginning of the storage period studied, so excluding that, it had observed effectiveness on microbial development. But even so, samples were treated with BVE extract (including both encapsulated and bulk) had the lowest of pH levels throughout the storage, implying an antibacterial activity of the bioactive extract components over time, a preventive role of meat tissue, and/or heterofermentative fungi and bacteria producing organic acids from BVE sugars (Sánchez et al., 2003; Del-Río et al., 2007; Hemmatkhah et al., 2020). When BVE and Encaps-BVE were compared, the latter had a relatively increased pH value because the extract was encapsulated in alginate beads. These findings agree with (Campolo et al., 2018; Parafati et al., 2019; Marrone et al., 2021;), who found that varying doses of BVE extract significantly impacted the pH of preserved beef treated with the extract. In terms of colour characteristics, control samples

showed a considerable decrease in red colour (*a** parameter) during storage, most likely because of microbial degradation and the resulting rise in pH, often associated with a shift in colour toward green. Meanwhile, the *a** values of treated BVE extract samples, whether encapsulated or not, illustrated comparative stability and suggested that the extract has provided a preventive action against myoglobin oxidation, as previously characterized by Parafati *et al*. (2019). While there were non-significant changes ($p>0.05$) in *a** values between samples containing encapsulated and non-encapsulated BVE, samples having encapsulated BVE showed a more consistent trend, except at four days. That according to (Campolo *et al*., 2018; Hemmatkhah et al., 2020). most packaged and raw minced beef samples demonstrated a significant decrease ($p<0.05$) in the *a** parameter during the first day and throughout storage.

Additionally, the researchers also concluded that both *a** and *b** characteristics, owing to metmyoglobin synthesis, cause a decrease in the *b** value. The Encaps-SDW and SDW samples demonstrate a more severe reduction in the *b** values over storage, likely due to aerobic microorganisms consuming oxygen, resulting in lower oxymyoglobin contributing significantly to the yellow colour formation (Gülçin, 2011; Hemmatkhah et al., 2020). In this instance, the BVE extract encapsulated resulted in reduced *b** parameter changes over the storage period, leading to colour preservation. The addition of BVE extract had a significant impact on hardness at the storage period (8 days), with the highest levels in samples treated or/not treated with BVE extract encapsulated, most certainly associated with the extract's carbohydrate content (Özvural et al., 2016; Parafati et al., 2019). Similarly, because once compared with untreated samples, springiness qualities in BVE and encapsulated BVE extracted samples increased significantly; the providing additional soluble proteins and carbohydrates in the extract may have improved the texture of meat products, resulting in structural resistance higher elasticity after the first component of beef product. Lastly, the cohesiveness characteristic demonstrated the most significant values of meat samples containing encapsulated extract, probably due to alginate's gelling characteristic.

4. Conclusions

One of the main aspects in enhancing quality, prolonging shelf-life, maintaining consumer protection, product safety, and minimizing waste was the microbial control in minced beef. This study follows a trend of identifying the various chemical compounds in red beetroot and evaluating their preventative role in extending food products' shelf life, including meat and its byproducts. Microbiological studies indicate that the addition of BVE and Encaps-BVE to burger preparation improved significantly ($P<0.05$) compared to control samples to total mesophilic bacteria, *Pseudomonas* spp. count, and *Enterobacteriaceae*. Moreover, results revealed that during the storage period, the addition of BVE kept beef minced almost constant in pH (average: 5.2-5.5), while control samples increased significantly ($p<0.05$) in pH values, possibly due to bacterial activity and degradation of protein caused by the production of amines, ammonia, and other essential substances.

Furthermore, after 9 days of the storage period, the sample containing Encaps-BVE demonstrated a steadier trend in terms of colour and texture characteristics than the other treatments. Confident that BVE is an effective technique of microorganisms' growth while storage, further research will be conducted to study the impact of beetroot extract, encapsulated or/not, on the cooked and grilled product's technological features as its sensory characteristics and acceptability. The research results have confirmed the feasibility of using BVE extract, whether encapsulated or/not in alginate beads. In contrast, a natural additive to meat product compositions maintains overall quality characteristics comparable to those of other extracts while also containing a significant inhibitor of antimicrobial agents,

indicating that it could be used as a multifunctional value-added component.

5. References

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PROCESSING METHODS AND STORAGE PERIOD AFFECT THE QUALITY OF PALM OIL

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ABSTRACT

The effects of processing methods and storage periods on oil palm quality were studied in this research. The palm fruits were subjected to four different processing methods; boiling (B), soaking followed by boiling (SWB), steaming (ST) and extraction with petroleum ether (SP). Quality indices, functional; physical properties and selected vitamins were determined. The results showed that free fatty acid values ranged from 5.14 in SP to 6.54 mg/KOH/g in SWB: peroxide value from 2.67 in SP to 10.07meq/kg in SWB, saponification value from 194.48 in ST to 196.82 mg/KOH/g in SWB; iodine value from 49.07 in SP-52.24g/100g in SWB. Free fatty acid, peroxide and iodine values increased as storage time increased except for saponification value which decreased. The moisture content value was from 0.17-0.35 %, specific gravity (0.89-0.92 g/cm^3); smoke point (231.67-240.00℃), flash point (294.00-303.00℃) and fire point (297.33-309.33℃). The soaked and boiled sample (SWB) had the highest values in moisture, smoke and flash point. While steaming (ST) induced the highest values in specific gravity and fire point. The sample extracted with petroleum ether had the lowest values for all the physical properties determined and the highest values for vitamins A (718.97IU/100g) and E (43.95IU/100g. The boiled sample (B) had the lowest values for vitamin A (699.47IU/100g) and vitamin E (38.87IU/100g). Both vitamins decreased as storage time increased. Moisture content and specific gravity increased while smoke, flash and fire points decreased as storage time increased. Emulsion capacity ranged from 62.19-100 % and emulsion stability ranged from 42.70-100 %. Steaming method (ST) produced oil samples with the highest values for both emulsion capacity and stability while extraction with petroleum ether had the lowest values. Both emulsion stability and emulsion capacity increased as storage time increased. Extraction with petroleum ether was the best in terms of good quality, followed by steaming and boiling methods.

1. Introduction

The Oil palm (*Elaeis guineesis*) is a plant that produces very high quantities of oil. Its oil yield is about 3.5 tonnes of oil/ha/year **(**Ngando *et al.,* 2011**).** The Oil palm is believed to originate from West Africa. Both the oil from the fruit and the kernel are edible and commercially important. Palm oil is obtained from the fresh mesocarp while palm kernel oil is obtained from the kernel **(**Poku, 2002). World consumption of palm oil was more than 16.7 million tonnes in 1997/1998 and over 40 million tonnes in 2007/2008. This figure was projected to increase to 70 million tonnes or more by 2020 (Grapevine, 2008). The chemical compositions and physical properties of palm oil and palm kernel oils vary. Both may be used in food and non-food industries **(**Gan Peck Yean, 2012). The oil has the same proportion of unsaturated to

saturated fatty acids, making it unique for many commercial purposes without need for adjusting the processes (Sugano and Tsuji, 1997).

Fresh red palm oil is very healthy for human use since it contains a good proportion of saturated to unsaturated fatty acids and is rich in antioxidants like beta-carotene, tocotrienols, tocopherols, plant squalene, and phytosterol (Oguntibeju *et al.,* 2009). It is rich in 16 carbon saturated fatty acids, palmitic acid, monounsaturated oleic acid and linoleic acid (omega-6 fatty acid) (10%) (Analava and Sutapa, 2009). Palm oil has a unique natural combination of phytonutrients that cannot be found in other vegetable oils (Ahsan *et al.,* 2015). Its minor components which constitutes about 1% includes carotenes, vitamin E, sterols, pigments, and fatty acids (Embrandiri *et al.,* 2013).

The oil is light yellow or red in colour because of its high content of carotenoids some of which are precursors of vitamin A (Ugwu *et al.,* 2002). It is one of the most popularly consumed oil in the world (Bazlul *et al.,* 2010). By- products of palm oil processing include palm kernel, palm kernel cake, palm frond, palm trunk and palm bunch. These by-products have numerous uses. Palm oil is mainly used for human consumption, animal feed and other industrial applications. Nigerians use palm oil as a major cooking oil. It is therefore a good source of energy in the diet (Babatunde, 1987). The free fatty acid (FFA) content, moisture, saponification value and peroxide value are indices of palm oil quality (Madubuike *et al.,* 2015). Fresh palm oil contains high amounts of vitamins A and E. It is extensively used across the world today (up to 33%); followed by soya oil with 31% (I.C.E.X, 2014). Palm oil especially the stearin and olein fractions may be directly blended with other oils to improve oxidative stability (De Marco *et al.,* 2007). It may be inter-esterified with other oils in order to meet the *trans*-free fat requirements of the food industry (Gee, 2007).

Palm oil is the richest dietary source of beta-carotene and contains a total of ~500 – 800 mg of carotenoids/kg oil, which is ~15 times higher than the carotenoid content of carrot (Sundram *et al.,* 2003). The quality of palm oil determines its application. It is also related to the processing method. In Nigeria, the traditional (mostly manual), semi-mechanized and mechanized processors are major methods used in the oil processing industry (Web 1). To a large extent, small holders/traditional processors dominate the sector accounting about for 80 % (Izah and Ohimain, 2013).

This study will investigate the quality of palm oil processed using four different methods. The research will provide information on the best processing method for producing good quality palm oil which can be adopted by small scale palm oil processors.

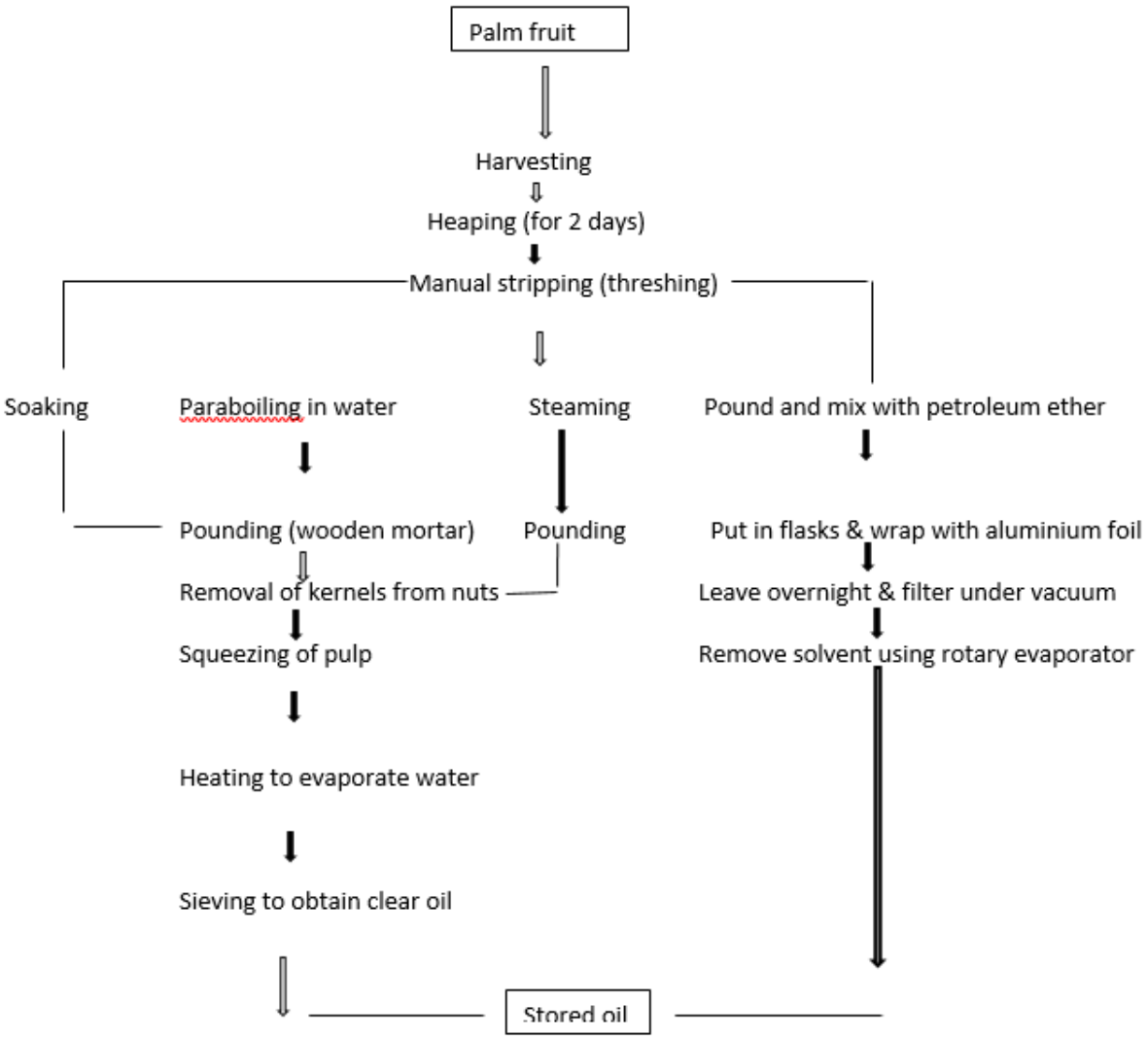
2. Materials and methods

2.1 Sample collection and preparation

Palm fruit was harvested by cutting with sharp knives when fully ripened from Egede in Udi Local Government Area of Enugu State. The palm fruits were subjected to four different processing methods; boiling (B), soaking before boiling (SWB), steaming (ST) and cold extraction with petroleum ether (SP). Five kg each of the harvested fruits were used for each of the different processing methods. Scheme 1 describes the processing of palm fruits.

In all the methods, five (5) kg of the fresh fruit were harvested and heaped on the ground for two days after which they were threshed to free the fruit from the bunch. In the boiling (B) and soaking with boiling (SWB) methods, the palm fruits were parboiled in water in the ratio of 1:2 in a cooking pot for 30 minutes and then pounded in a wooden mortar to separate pulp and nuts. The nuts (palm kernels) were removed and the pulp manually squeezed to obtain a red viscous fluid (oil, fiber, water, impurities), which was heated for 30 minutes for traces of water to evaporate and was finally sieved using metal basket to obtain a clear red palm oil. The processed palm oil was stored in an air tight plastic container at room temperature (30 °C) until needed for analysis. In steaming method (ST), the same quantity of the fresh fruit bunches was harvested and treated as in the boiling method. The palm fruits were steamed using a steaming pot which was constructed in

such a way that during steaming, the palm fruits do not touch the water aided by separating the fruit from the water with a wire sieve. The fruits were steamed for 30 minutes and then ground in a locally constructed machine until pulp and nuts were obtained. The nuts were removed and the pulp was treated as in the other processing methods. The processed palm oil was stored in an air tight plastic container at room temperature (30 ℃) until needed for analysis.



Scheme 1. Processing of palm fruits

In extraction with petroleum ether (SP), the samples were collected for extraction of the oil according to the method by Alina *et al.* (2012). Petroleum ether (b.pt 40-60ºC) was used as the extraction solvent. The ratio of the quantity of oil samples to petroleum ether used was 1:3. Forty (40) g of the ground sample was homogenized with 120 ml of petroleum ether and left overnight. The flasks used were wrapped with aluminum foil to avoid the samples from being exposed to the light. It was subsequently filtered under vacuum through a filter paper (Whatman No.1) in Buchner a funnel to remove the ground fiber from the solvent. The filtrate was then transferred into round bottom flask and attached to a rotary evaporator (Model R-300). The petroleum ether solvent was removed after extraction at 40 ºC. The extracted palm oil was stored in an air tight plastic container at room temperature (30 ℃) until needed for analysis. Freshly extracted palm oil

and samples stored for 30, 60 and 90 days were used for analysis.

In all the methods, five (5) kg of the fresh fruit were harvested and heaped on the ground for two days after which they were threshed to free the fruit from the bunch. In the boiling (B) and soaking with boiling (SWB) methods, the palm fruits were parboiled in water in the ratio of 1:2 in a cooking pot for 30 minutes and then pounded in a wooden mortar to separate pulp and nuts. The nuts (palm kernels) were removed and the pulp manually squeezed to obtain a red viscous fluid (oil, fiber, water, impurities), which was heated for 30 minutes for traces of water to evaporate and was finally sieved using metal basket to obtain a clear red palm oil. The processed palm oil was stored in an air tight plastic container at room temperature (30 ℃) until needed for analysis. In steaming method (ST), the same quantity of the fresh fruit bunches was harvested and treated as in the boiling method. The palm fruits were steamed using a steaming pot which was constructed in such a way that during steaming, the palm fruits do not touch the water aided by separating the fruit from the water with a wire sieve. The fruits were steamed for 30 minutes and then ground in a locally constructed machine until pulp and nuts were obtained. The nuts were removed and the pulp was treated as in the other processing methods. The processed palm oil was stored in an air tight plastic container at room temperature (30 ℃) until needed for analysis.

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2.2.Determination of oil yield

The % yield of oil was calculated using the formula given as follows:

$$Y = \frac{Me}{Mm} \times \frac{100}{1} \qquad (1)$$

Where: Y = oil yield, Me = mass of oil extracted, Mm = mass of loose fresh fruit.

2.3.Determination of Vitamins

The colorimetric and Futher-Meyer methods of the Association of Vitamin Chemists described by Kirk and Sawyer (1991) was used to determine Vitamins A and E.

2.4.Determination of quality indices

The free fatty acid, peroxide, iodine and saponification values were determined using the AOAC method (2005).

2.5.Determination of physical properties

Smoke and flash points were determined according to the method described by Pike (2003). The moisture content and specific gravity of the oil were determined by the AOAC method (2005).

2.6.Determination of functional properties

The emulsion capacity and stability of the oil was determined by the method described by Yasumatsu *et al.* (1972).

2.7.Data Analysis

Analysis of variance was used to determine significant differences ($p<0.05$) among treatment means. Mean separation was done using the SPSS version 20.0. Separation of means was carried out by Duncan Multiple range test and values were reported as means and standard deviation.

3.Results and discussions

Figure 1 shows the yield of oil from the four processing methods. The oil yield ranged from 14.00 % (SWB) - 19.60 % (SP). Extraction with petroleum ether (SP) had the highest oil yield (19.60 %).

The variations in the oil yield for the four processing methods adopted could be as a result of the ability of the different methods applied to loosen the matrix of the plant tissues to allow fat extraction.

According to Owolarafe *et al.* (2002), during digestion, the cells are completely ruptured and lose their integrity to release the oil.

When digestion is properly done, a homogenous mash without undigested fruits is formed. This enhances oil yield (Aletor *et al.,* 1990).

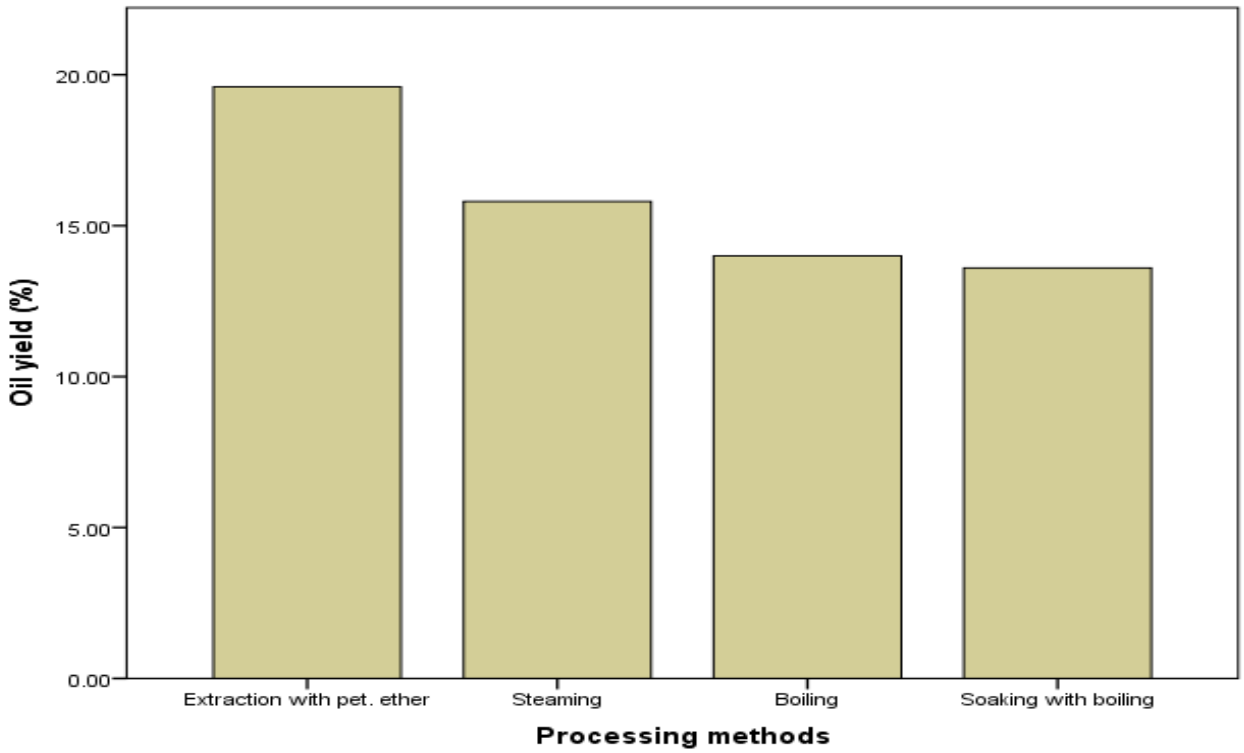

Figure 1. Effects of processing methods on oil yield

3.1. Physical properties of palm oil

The moisture contents of the processed palm oils are shown in the Figure 2. The moisture content for freshly extracted oil samples ranged from 0.17 (SP) – 0.35 % (SWB). Samples ST, B and SWB were statistically similar ($p>0.05$). The higher moisture content recorded in SWB could be as a result of the processing method applied where the fruits were soaked in water over night before parboiling. The moisture contents of the fresh palm oil (0.17 – 0.35 %) was higher than the recommended limit (0.10 %) (Aletor *et al.,* 1990). Ngando *et al.* (2011) reported 0.22; 0.23 - 0.32 and 0.08 moisture content for of palm oil produced in Cameroun using traditional, semi-mechanized and mechanized methods. Zu *et al.* (2012) reported the moisture content of palm oil produced in Ghana as 0.79 – 1.59 %. Udensi and Iroegbu (2007) reported 0.14 – 0.60 % as the moisture content of fresh palm oil sold in main market of Abia State Nigeria which is similar to the result of this study. Agbaire (2012) reported 0.14 – 0.17 % as the moisture content of palm oil sold in Delta state, Nigeria which is also close to the result of this study. After 30 days of storage, the moisture content of palm oils from the four processing methods ranged from 0.39 (SP) – 0.93 % (SWB). There were significant ($p<0.05$) differences in the moisture content values. After 60 days of storage, the moisture contents of palm oil samples from the four processing methods ranged from 0.46 (SP) -1.34 % (SWB). There

were significant ($p<0.05$) differences among the moisture contents of the samples. After 90 days of storage, the moisture contents of palm oil samples from the four processing methods ranged from 0.62 (SP) – 1.56 % (SWB). There were also significant ($p<0.05$) differences among the moisture contents of the samples.

High moisture content promotes rancidity, aids spoilage and short shelf-value while low moisture content promotes stability during storage of palm oil samples. According to Poku (2002) and Orji and Mbata (2006) the moisture content of palm oil depends directly on the final extraction and clarification processes. Moisture contents of the fresh and stored palm oil fell within the limiting range of 0.09 – 3.1 % reported by Denenu and Eze (1983) as acceptable standard which will not cause refining and storage problems. Recommended safe moisture content for fresh oil as reported by SON (2000) and NIS (1992) is 0.29 %. The differences observed in the moisture content of the fresh palm oil for samples B and SWB could be as a result of the processing methods adopted. Moisture content observed in all the samples during storage were within the acceptable standards with the exception of samples processed using boiling and soaking /boiling when they were stored for 60 – 90 days (1.06 %, 1.22 % and 1.34 %, 1.56% respectively as shown in Figure 2). The recommended maximum safe moisture level for palm oil storage is 1 %.

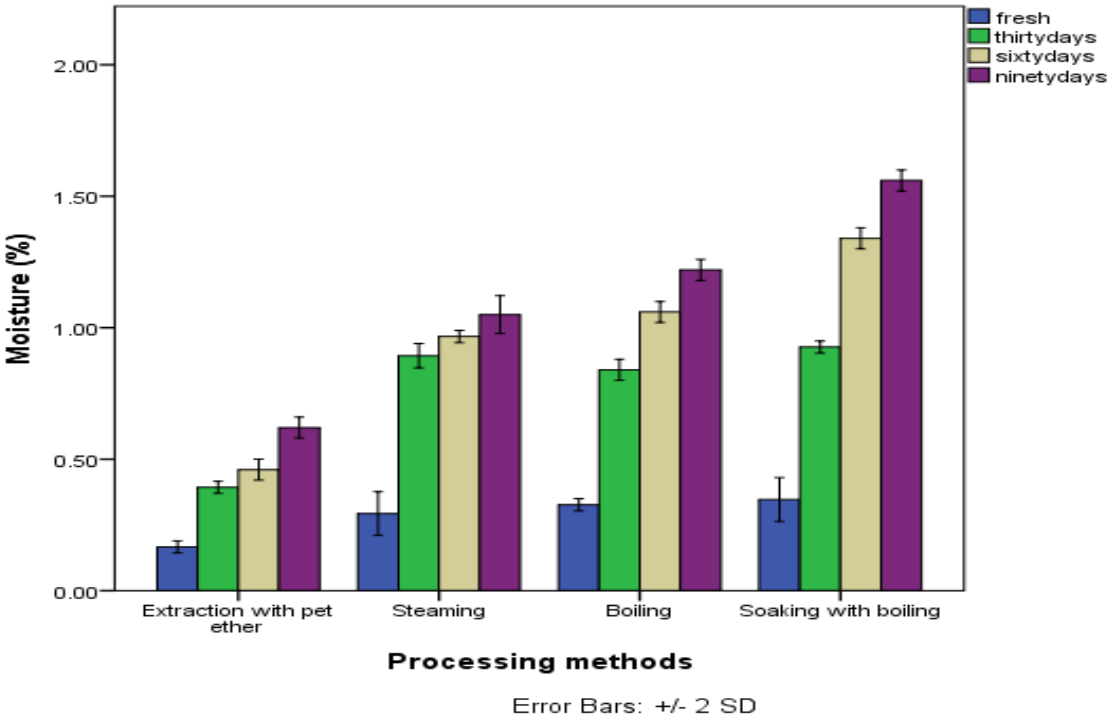

Figure 2. Changes in moisture content of palm oil during the storage period of 90 days

The specific gravity values of the processed palm oils are shown in Table 1. The specific gravity for freshly prepared oil sample for the four processing methods ranged from 0.89 – 0.92 g/cm^3 Sample SP had the least value (0.89 g/cm^3) while sample B had the highest value (0.92 g/cm^3). Samples B and SWB were statistically similar. During storage, the palm oil samples maintained the same specific gravity values after an initial rise within the 30 days of storage. There were no significant ($p>0.05$) differences among samples SP, ST and B within the first 30 days of storage and also for all the processing methods for 60 and 90 days of storage.

Udensi and Iroegbu (2007) reported 0.83 – 0.88 g/cm^3 as the specific gravity of palm oil sold in key markets of Abia State, Nigeria.

Agbaire (2012) reported 0.859 –0.885 g/cm^3 as the specific gravity of palm oil sold in key markets of Delta state. The specific gravity of the palm oil showed that palm oil is less dense than water, therefore will float. The values obtained were very much close to the standard range of 0.898 – 0.907 g/cm^3 approved by Standard Organization of Nigeria (SON) (2000).

Table 1. Changes in specific gravity of the palm oil stored for 90 days (B = Boiling; SWB = Soaking before boiling; ST = Steaming; SP= Cold extraction with petroleum ether. Values with different superscript down the column are significantly different ($p<0.05$))

Sample	Fresh	30 days	60 days	90 days
SP	0.89a	0.92a	0.92a	0.92a
ST	0.91c	0.92a	0.92a	0.92a
B	0.92b	0.92a	0.92a	0.92a
SWB	0.91c	0.92a	0.92a	0.92a

Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$). SP = Solvent extraction; ST = Steaming; B = Boiling; SWB = Soaking followed by boiling.

The smoke point of the processed palm oils are shown in the Figure 3.The smoke point for the fresh and stored palm oil for the four processing methods ranged from 231.67 (SP) – 240.00 ℃ (SWB). There were no significant ($p>0.05$) differences between samples ST and B for freshly prepared palm oil; amongst samples ST, B and SWB after 30 days of storage; between samples SP and B and also between samples ST and SWB after 60 days of storage and between samples SP, B and SWB after 90 days of storage. Smoke point is the temperature at which a fat or oil produces a constant wisp of smoke when heated. It is a useful index of the suitability for frying. The Canadian specifications indicate that frying oil should have a smoke point above 200 ℃ (Appelqvist and Ohlson, 1972). Figure 3 indicates that the processed palm oils fulfilled this requirement and can be used as a frying oil.

The flash point of the processed palm oils are shown in the Figure 4 .The flash point for the fresh and stored palm oil for the four processing methods ranged from 294.00 (SP) – 303.00 ℃ (SWB). There were no significant ($p>0.05$) differences among samples ST, B and SWB for freshly prepared oil sample; samples B and SWB after 60 days of storage and samples ST and B after 30 and 90 days of storage. The Flash point is the temperature at which the decomposition products produced from frying oils can be ignited. This temperature ranges from 275 ℃– 330 ℃ for different oils and fats according to Canadian specifications (Appelqvist and Ohlson, 1972). The fire point of the processed palm oils are shown in the figure 5.The fire point for the fresh and stored palm oil form the four processing methods ranged from 297.33 (for SP) – 308.67 ℃ (for SWB). There were no significant ($p>0.05$) differences between the fire points of samples ST and SWB for freshly prepared samples.

After 30 and 60 days of storage, there were no significant ($p>0.05$) differences between samples SP and SWB as well as ST and B. There were no significant ($p>0.05$) differences between samples ST and B after 90 days of storage. The Fire point is the temperature at which oil combustion is sustained.

The high value obtained for these physical properties indicate that palm oil is suitable for frying. These physical parameters decreased with increase in storage time. This could result from active *lipase* present in the palm oil which causes the hydrolysis of triacylglycerols to fatty acid and glycerol thereby increasing the free

fatty acid content which impairs oil quality and subsequently affects the frying quality of the oil.

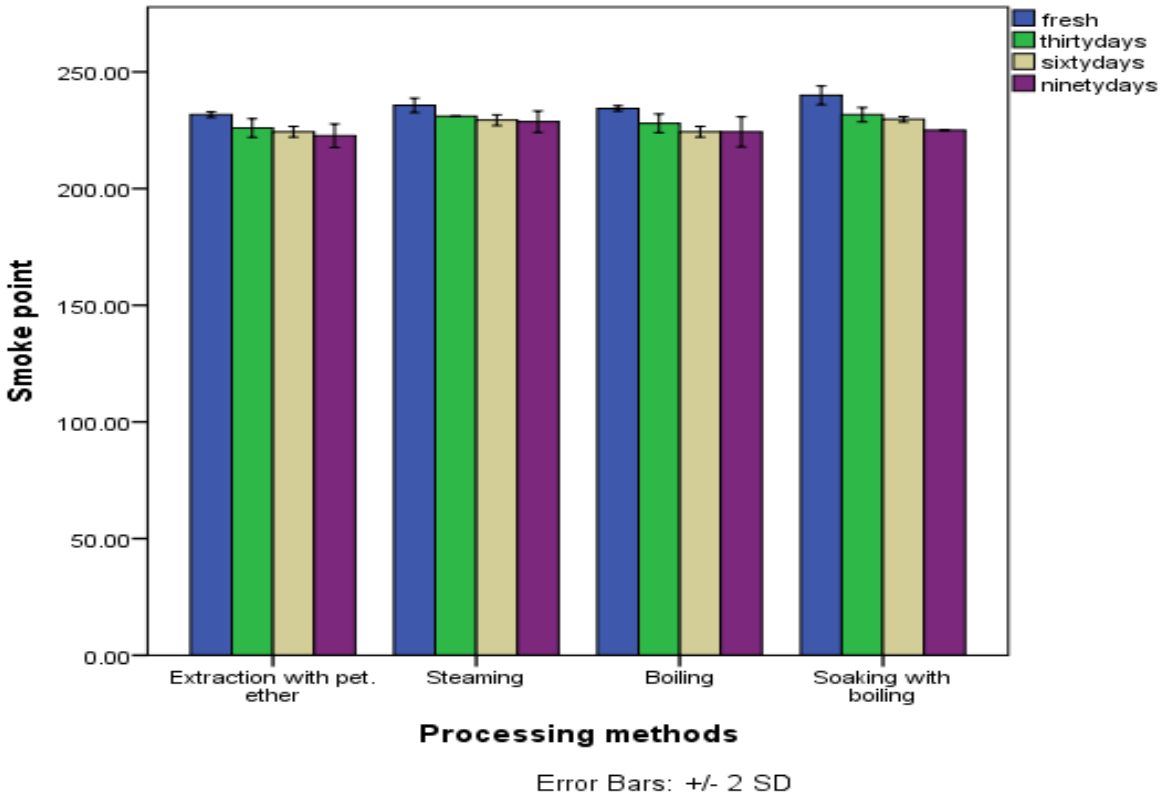

Figure 3. Changes in smoke point of palm oil during the storage period of 90 days

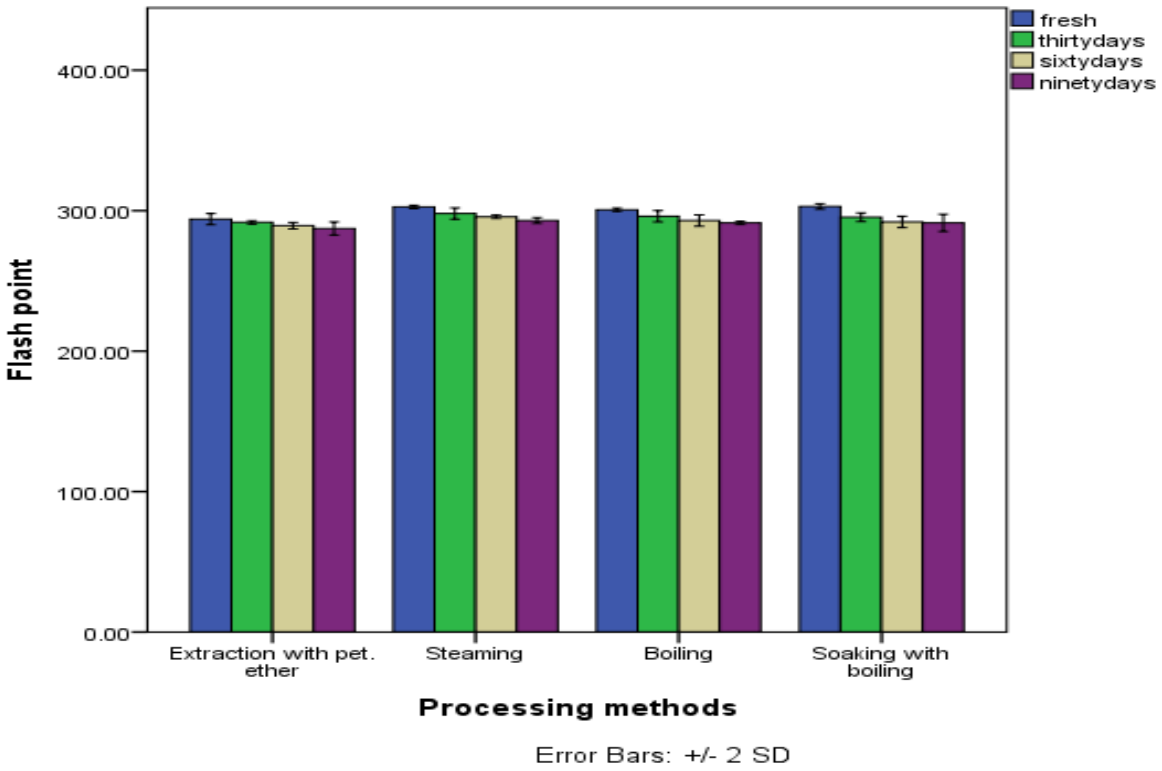

Figure 4. Changes in flash point of palm oil during the storage period of 90 days

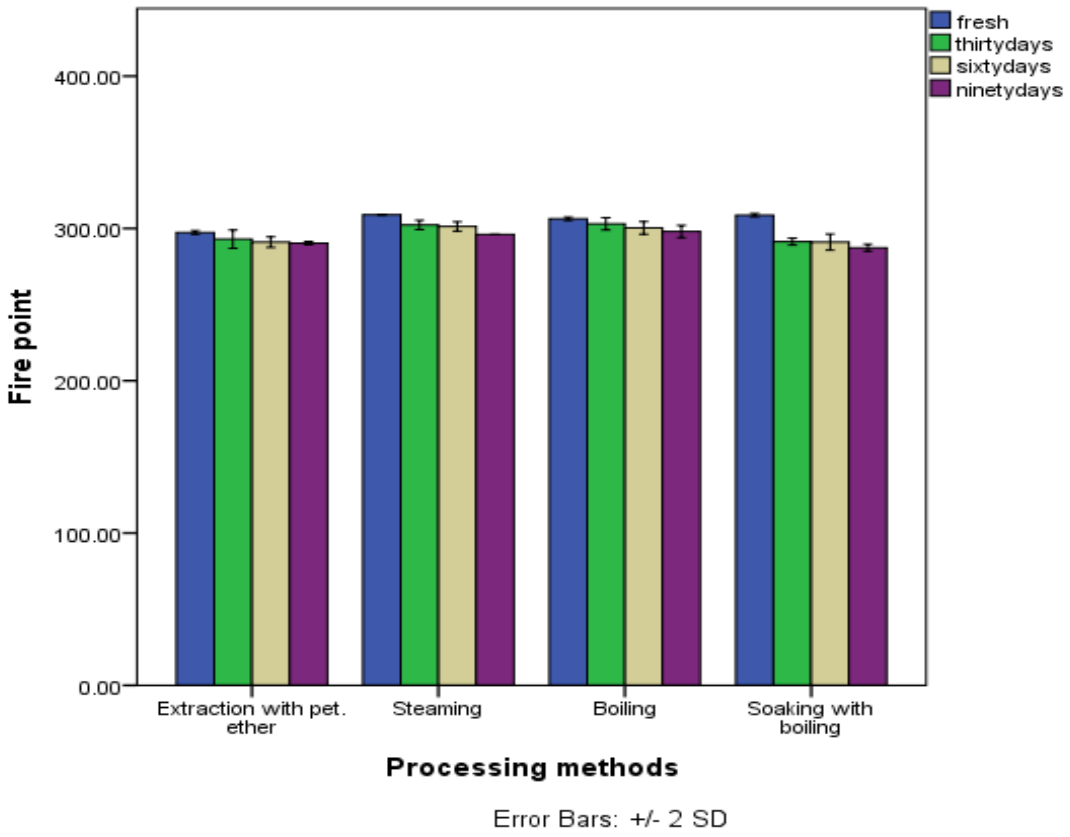

Figure 5. Changes in fire point of palm oil during the storage period of 90 days

3.2. Functional properties of the processed palm oil

The emulsion capacity of oil samples from the various processing methods are shown in Figure 6. The emulsion capacity for freshly prepared oil samples ranged from 62.19 (for SP) – 100.00 % (for ST). After 30 days of storage, the emulsion capacity of the oil samples ranged from 68.62 (for SP) – 100.00 % (for ST). After 60 days of storage, the emulsion capacity of the oil samples from the four processing methods ranged from 74.63 (for SP) – 100.00 % (for ST). After 90 days of storage, the emulsion capacity of the oil samples ranged from 79.00 (for SP) – 100.00 % (for ST). There were significant ($p<0.05$) differences in the emulsion capacities of palm oil from the four processing methods. Emulsion capacity (EC) indicates a molecule's ability to act as an agent that facilitates solubilization or dispersion of two immiscible liquids (Sánchez-Zapata *et al.,* 2009). These high levels of emulsion capacity suggest that palm oil would be highly desirable for a wide variety of both naturally occurring as well as manufactured materials in the industries such as food industries (Shahin *et al.,* 2011). The values of the emulsion stability for the various processing methods are shown in figure 7. The emulsion stability for freshly prepared palm oil samples ranged from 42.70 (for SP)–100.00 % (for ST). After 30 days of storage, the emulsion stability of palm oil from the four processing methods ranged from 44.93 (for SP –100.00 % (for ST). After 60 days of storage, the emulsion stability of palm oil from the four processing methods ranged from 48.77 (for SP)–100.00 % (for ST). By 90 days of storage, the emulsion stability of the oils ranged from 58.47 (for SP) – 100.00 % (for ST). There were significant ($p<0.05$) differences in the emulsion stability of palm oil samples from the four processing methods. Emulsion stability (ES) is the ability to retain the integrity of an emulsion (Sánchez-Zapata *et al.,* 2009). The high emulsion stability also suggest that palm oil could be used for a wide variety of manufacturing processes

especially in the food industries (Shahin *et al.,* 2011).

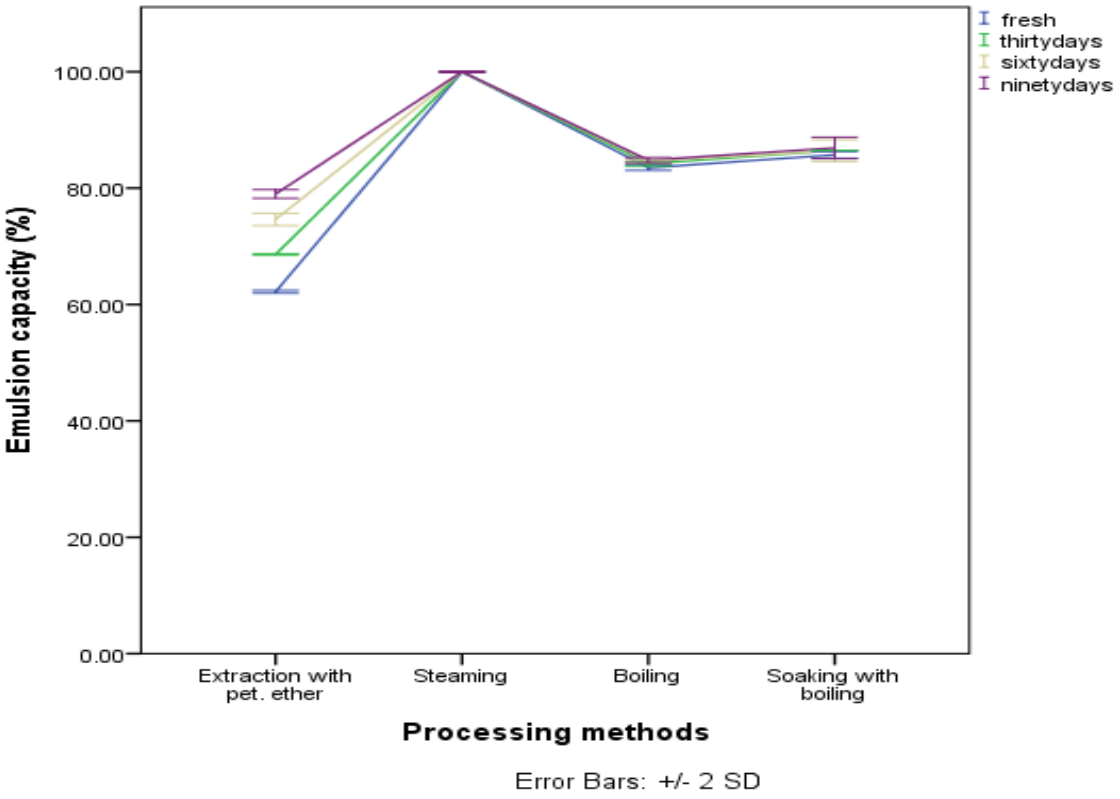

Figure 6. Changes in oil emulsion capacity during short – term storage

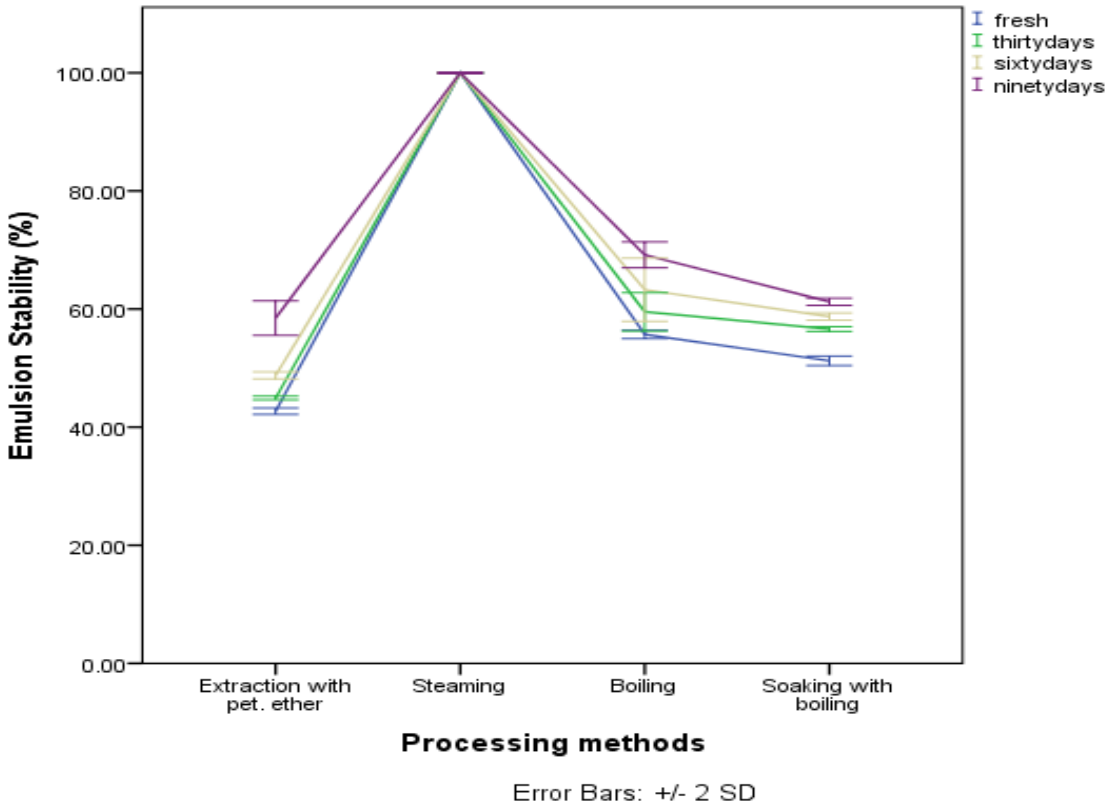

Figure 7. Changes in emulsion stability of palm oil during the storage period of 90 days

3.3. Chemical properties of the processed palm oil

The free fatty acid content of the processed palm oils is shown in Table 2. Free fatty acids of the freshly prepared oils from the four processing methods ranged from 5.14 (for SP) – 6.54 mg/KOH/g (for SWB). There were significant ($p<0.05$) differences in the free fatty acid contents at the onset (immediately after extraction). After 30 days of storage, the free fatty acids of oils from the four processing methods ranged from 8.74 (SP) – 11.41 mg/KOH/g (for SWB). There were significant ($p<0.05$) differences among the free fatty acid content of the oils within the 30 days of storage. After 60 days of storage, the free fatty acid contents of the oils ranged from 9.26 (for SP)– 13.34 mg/KOH/g (for SWB). There were significant ($p<0.05$) differences among the free fatty acids after 60 days of storage. After 90 days of storage, the free fatty acids of the oils ranged from 11.46 (for SP) – 15.80 mg/KOH/g (for SWB). There were no significant ($p>0.05$) differences between samples ST and B.

Free fatty acid content is a very important quality index of oil, and should not exceed 5 %, (expressed as palmitic acid) (Commission du Codex Alimentarius/FAO/OMS, 2005). Fatty acids are components of triacylglycerol molecules. The presence of free fatty acid residues in palm oil indicates poor oil quality (Agbaire, 2012). They result from lipase activity in the mesocarp of the oil palm fruit and which are responsible for the hydrolysis of triacylglycerols (Ngando *et al.,* 2006). The high FFA level reported in this study could be linked with the postharvest handling practices employed during oil palm processing, such as period of fermentation (Ohimain *et al.,* 2012). Ohimain and Izah (2013) have reported a fermentation period of 2–3 days after bunch welcome in a semi mechanized palm oil mill. Hartley (1988) reported that bruising of ripe oil palm fruits could raise the FFA because it contains auto-lipolytic enzymes which split the fruit oil to fatty acid and glycerol. Okechalu *et al*. (2011) reported that the exposure of palm fruit to sunlight prior to fermentation also enhances the formation of free fatty acids. Tagoe *et al.* (2012) reported that microbial invasion of the palm fruit and the FFA of the processed palm oil increases with the period of storage. Basically, FFA content of vegetable oil is an indication of the level of deterioration through the action of lipases in the fruit following cell disruption (Hiol *et al.,* 2000; Houria *et al.,* 2002; Ngando *et al.,* 2011; Ohimain *et al.,* 2012). The high FFA reported in this study could be credited to the level of exposure to sunlight, level of ripeness prior to harvesting and period of fermentation.

Table 2. Changes in the free fatty acid contents of the palm oil stored for 90 days (B = Boiling; SWB = Soaking before boiling; ST = Steaming; SP= Cold extraction with petroleum ether. Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$))

Sample	Fresh	30 days	60 days	90 days
SP	5.14^{a}±0.16	8.74^{a}±0.50	9.26^{a}±0.00	11.46^{a}±0.42
ST	5.61^{b}±0.0	9.82^{b}±0.28	11.13^{b}±0.16	13.43^{b}±0.06
B	6.26^{c}±1.16	10.66^{c}±0.28	12.34^{c}±0.00	15.80^{b}±0.81
SWB	6.54^{d}±0.16	11.41^{d}±0.16	13.34^{d}±0.52	15.80^{c}±0.16

Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$). SP = Solvent extraction; ST = Steaming; B = Boiling; SWB = Soaking followed by boiling.

The peroxide value of the processed palm oils is shown in the Table 3. The peroxide values of the freshly prepared oils for the four processing methods ranged from 2.67 (for SP) – 10.07 meq/kg (for SWB). There were no significant ($p>0.05$) differences between

samples ST and B for the peroxide value. The peroxide values for both samples SP, ST and B were below the standard 10 $mEqkg^{-1}$ for fresh oils. Pearson (1976) noted that fresh oil usually should have peroxide values well below 10 $mEqkg^{-1}$.

The increase of peroxide value in sample SWB could be as a result processing method adopted. Peroxide value was shown by Ihekoronye and Ngoddy (1985) to be a function of processing method, storage environment and packaging. After the first 30 days of storage, the peroxide values of the four processing methods ranged from 9.80 – 18.20 meq/kg. Sample SP had the least value (9.80 meq/kg) while sample SWB had the highest value (18.20 meq/kg).

There were significant ($p<0.05$) differences among the peroxide values after 30 days of storage. After 60 days of storage, the peroxide values of the palm oil samples from the four processing methods ranged from 11.40 (for SP)– 21.00 meq/kg (for SWB. After 90 days of storage, the peroxide values of samples from the four processing methods ranged from 14.13 (for SP) – 23.13 meq/kg (for SWB). There were significant ($p<0.05$) differences among the peroxides values after 90 days of storage.

The peroxide value is an index of rancidity in oils often referred to as lipid peroxidation or oxidative degradation. It is used to measure the stability of fats by measuring the peroxides and hydro peroxides formed during the initial stages of oxidation and thus estimate the extent of spoilage of the oil. Peroxidation generates carcinogenic free radicals (Rossel, 1999). It has been shown by Ngando *et al*. (2011) that this value increases with storage, which suggests that sample SWB method of processing produces an oil with a shorter shelf-life.

Table 3. Changes in the peroxide value of the palm oil stored for 90 days (B = Boiling; SWB = Soaking before boiling; ST = Steaming; SP= Cold extraction with petroleum ether. Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$))

Sample	Fresh	30 days	60 days	90 days
SP	2.67^{a}±0.12	9.80^{a}±0.20	11.40^{a}±0.20	14.13^{a}±0.12
ST	9.53^{b}±0.12	13.20^{b}±0.20	15.60^{b}±0.00	17.93^{b}±0.12
B	9.73^{b}±0.12	15.20^{c}±0.00	17.80^{c}±0.20	21.20^{c}±0.00
SWB	10.07^{c}±0.23	18.20^{d}±0.20	21.00^{d}±0.20	23.13^{d}±0.12

Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$). Sp = Solvent extraction; ST = Steaming; B = Boiling; SWB = Soaking followed by boiling

The saponification value of the processed palm oils is shown in Table 4. The saponification values for freshly prepared palm oil samples ranged from 194.48 (for ST) – 196.82 mg/KOH/g (for SWB). There were no significant ($p>0.05$) differences among samples SP, ST and B also SP, B and SWB.

After 30 days of storage, the saponification values of the oil samples ranged from 187.00 (for ST) – 190.74 mg/KOH/g (for SP). There were no significant ($p>0.05$) differences among samples SP, B and SWB after 30 days of storage. After 60 days of storage, the saponification values of the oil samples ranged from 181.86 (for sample B) – 184.66 mg/KOH/g (for SWB).

There were no significant ($p>0.05$) differences between samples SP and SWB and also between ST and B. After 90 days of storage, the saponification values of the oils ranged from 177.19 (for sample B) – 181.86 mg/KOH/g (for sample SWB). There were no significant ($p>0.05$) differences between samples SP and ST.

Saponification value is indirectly related to the molecular weights of triglycerides. It is

inversely proportional to the average molecular weight of fatty acids (Muhammad *et al.,* 2011).

The saponification value gives information about the solubility in water and soap formation (Akinola *et al.,* 2010; Ohimain *et al.,* 2013).

TZS and Codex standard requirements specify that the saponification value of unblended palm oil should be within the range 194-202 mg KOH/g oil.

The saponification values showed that the palm oils are within the range of specified standard requirements. Saponification value increases as molecular weight and chain length decreases.

Table 4. Changes in the saponification value of the palm oil stored for 90 days (B = Boiling; SWB = Soaking before boiling; ST = Steaming; SP= Cold extraction with petroleum ether. Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$))

Sample	Fresh	30 days	60 days	90 days
SP	195.88^{ab}±0.81	190.74^{b}±0.01	184.19^{b}±0.81	179.99^{b}±0.81
ST	194.48^{a}±0.81	187.00^{a}±0.81	182.33^{a}±0.00	179.05^{b}±0.81
B	195.42^{ab}±0.81	189.34^{b}±1.40	181.86^{a}±0.81	177.19^{a}±0.81
SWB	196.82^{b}±0.81	189.34^{b}±0.00	184.66^{b}±0.81	181.86^{c}±0.81

Values shows the mean of triplicate analysis and ± standard deviation. Values with different superscript down the column are significantly different ($p<0.05$). SP = Solvent extraction; ST = Steaming; B = Boiling; SWB = Soaking followed by boiling.

The iodine value of the processed palm oils is shown in the Figure 8. The iodine values for freshly extracted oil samples ranged from 49.07 (for SP) – 52.24 g/100g fat (for SWB). There were no significant ($p>0.05$) differences between samples B and SWB. The iodine values obtained for freshly prepared oil samples were within the standard range of 45 – 53g/100g fat as recommended by SON (2000) and NIS (1992). After the first 30 days of storage, the iodine values of samples ranged from 52.24 (for SP) – 59.22g/100g fat (for SWB). There were no significant ($p>0.05$) differences between samples ST and B.

After 60 days of storage, the iodine values of the oil samples ranged from 54.78 (for sample ST) – 61.95g/100g fat (for sample SWB). There were no significant ($p>0.05$) differences between samples SP and B. After 90 days of storage, the iodine values of oil samples ranged from 55.41 (for sample ST) – 64.93g/100g (for sample SWB). There were significant ($p<0.05$) differences among the values.

Iodine value is the quantity of iodine absorbed by one gram of the oil to saturate the sigma bond. It is a sign of the level of unsaturation and susceptibility of oil to oxidation and rancidity (Agbaire, 2012). Iodine value determines the stability and shelf life of oil.

High iodine value makes the oil to be unstable thereby affecting other downstream application beside food **(**Ohimain *et al.,* 2013).

The iodine value may be used to identify adulteration of palm oil with any other vegetable or animal fat (Ekwenye, 2006). It is a chemical parameter that characterized oil based on the level of unsaturation. Any substance, which can boost the degree of unsaturation in an oil sample, will raise the iodine value of the oil.

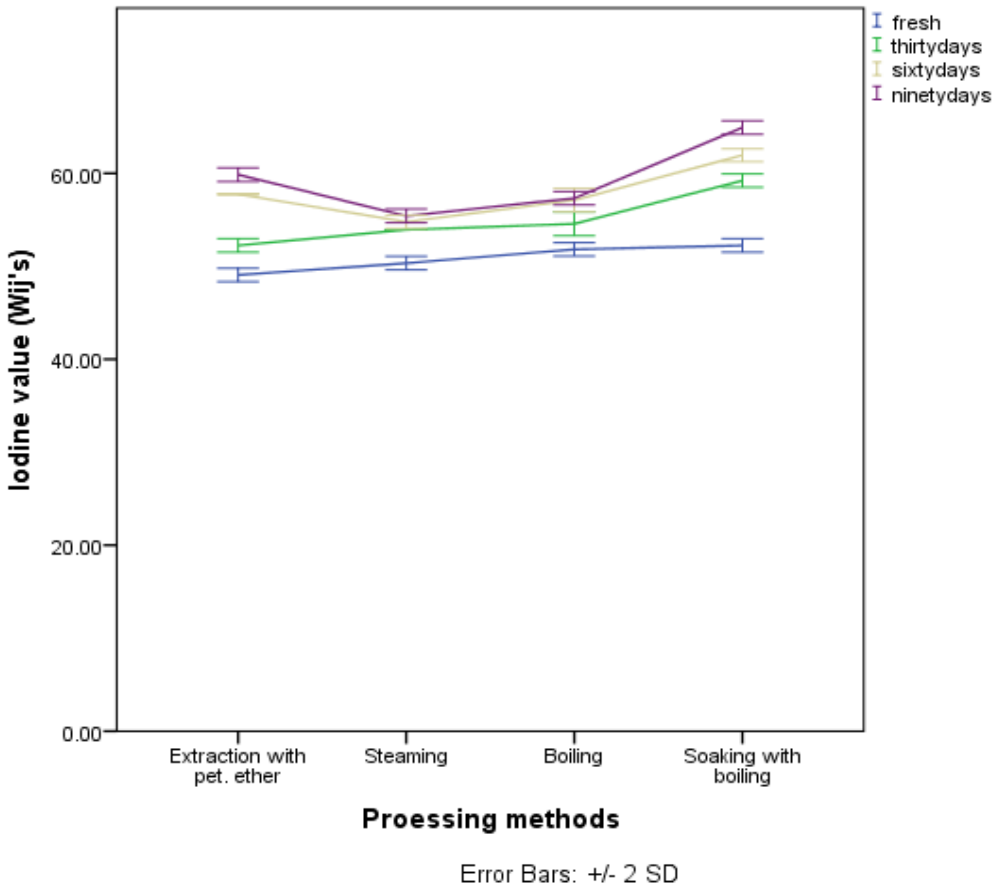

Figure 8. Changes in iodine value of palm oil during the storage period of 90 days

3.4. Vitamin content of the processed palm oil

Vitamin A contents of palm oil from various processing methods are shown in figure 9. The vitamin A contents for freshly prepared oil samples ranged from 699.47 (for SWB) – 718.97 IU/100g (for SP). There were no significant ($p>0.05$) differences between ST and B and as well as B and SWB. After the first 30 days of storage, the vitamin A content of the oils ranged from 659.08 (for SWB) – 699.33 IU/100g (for SP). After 60 days of storage, it ranged from 622.05 (for sample B) – 636.41 IU/100g (for sample SP). By 90 days of storage, it ranged from 581.04 (SWB) – 596.15 IU/100g (for SP). There were significant ($p<0.05$) differences among the vitamin A contents of the samples after 30, 60, and 90 days of storage.

The variation in the vitamin A contents of the fresh palm oil was as a result of the processing methods employed. There were decreases in the vitamin A contents of the palm oil during storage. At room temperatures above 30^{o}C, phytonutrients break up (Alyssa *et al.,* 2009). Frias *et al.* (2009) confirmed that the storage period has significant influence on vitamin A content. According to Duarte *et al.* (2011) the stability of vitamin A depends on production and storage conditions, e.g. absence of oxygen in the packaged product; storage in the absence of light and at a temperature not exceeding 30 °C. However, the values obtained for both fresh and stored palm oils were within the range of 500 – 2000 mg/kg set by (SON, 2000).

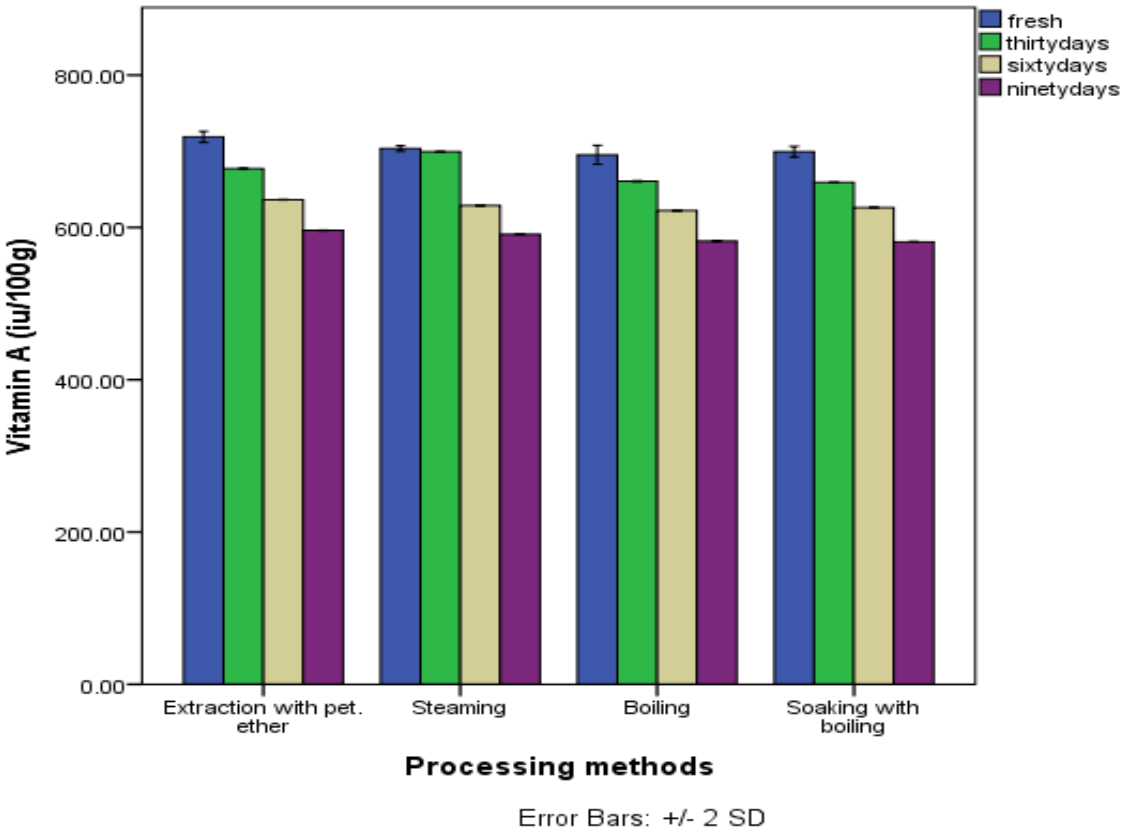

Figure 9. Changes in vitamin A of palm oil during the storage period of 90 days

The vitamin E contents of palm oil from the various processing methods are shown in figure 10. The vitamin E contents for freshly prepared samples ranged from 38.87 – 43.95 IU/100g. Sample SP had the highest value (43.95 IU/100g) while sample B had the least value (38.87 IU/100g). There were no significant ($p>0.05$) differences between samples ST and SWB. After 30 days of storage, the vitamin E contents of oils from the four processing methods ranged from 37.65 – 41.65 IU/100g. Sample SP had the highest value (41.65 IU/100g) while sample SWB had the least values (37.65 IU/100g). There were no ($p>0.05$) significant differences between samples ST and SWB. By the 60th day of storage, the vitamin E contents of the oils ranged from 35.11 – 38.99 IU/100g. Sample SP still had the highest value (38.99 IU/100g) while sample SWB had the least values (35.11 IU/100g). There were no ($p>0.05$) significant differences between ST and B. By the 90th day of storage, the vitamin E contents of palm oil samples from the four processing methods ranged from 34.10 – 35.77 IU/100g. Sample SP had the highest value (35.77 IU/100g) while sample SWB had the least value (34.10 IU/100g). There were significant ($p<0.05$) differences among the vitamin E contents of the oils after 90 days of storage.

The difference in the vitamin E contents of the fresh palm oil is also as a result of the processing methods employed. There were decreases in the vitamin E contents of the palm oil during storage. These vitamins are naturally occurring bioactive compounds which are susceptible to heat and light (Choo and Bonnie, 2000). Dominguez *et al.* (2014) observed that cooking loss of vitamins depends on mass transfer during thermal treatment, lead to cooking loses of vitamins. Different cooking methods affect losses in different ways.

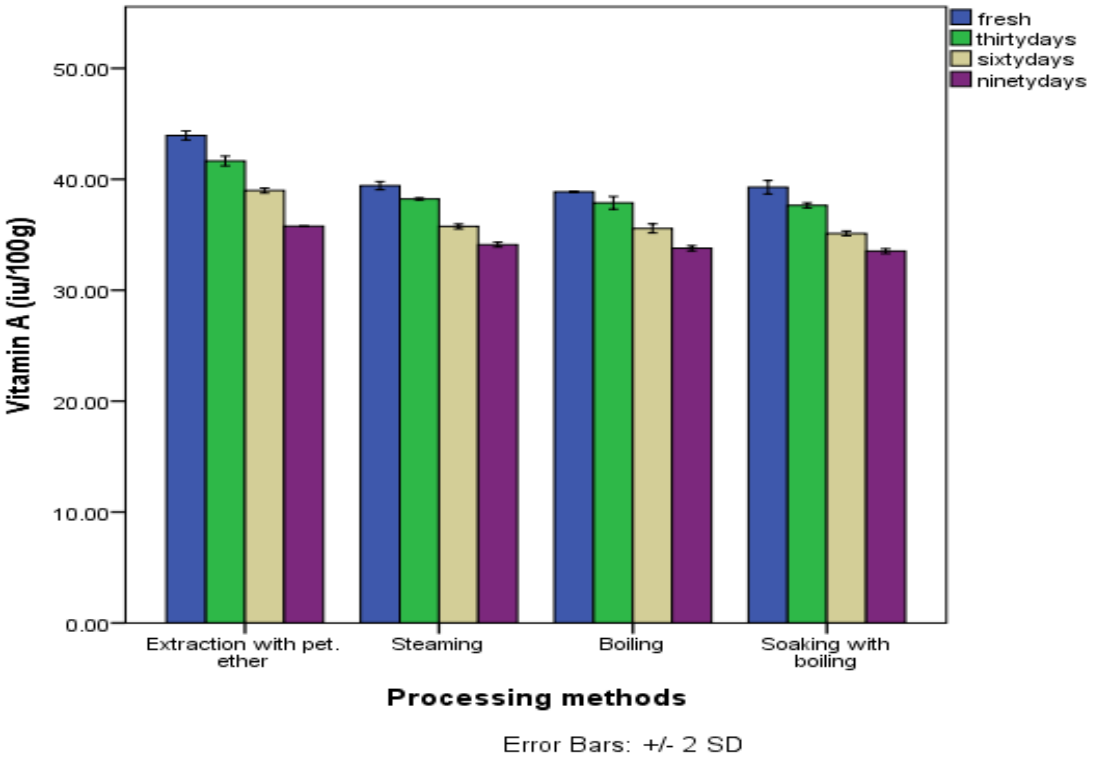

Figure 10. Changes in vitamin E of palm oil during the storage period of 90 days

4. Conclusions

This study has shown that the methods of palm oil processing and the storage time significantly affected the quality of oil produced. Findings from this research show that the values obtained vary with different processing techniques adopted. Extraction with petroleum ether was the best in terms of good quality, followed by steaming method and finally boiling and boiling with soaking. The quality parameters of the palm oil changed progressively with storage time. As storage time increased, free fatty acid, peroxide value and iodine value increased while saponification value decreased. The vitamins A and E contents of the palm oil changed progressively with storage time. As storage time increased, the vitamins decreased also. The physical and functional properties of the processed palm oil were also affected with storage time. Based on the discoveries from this research work, it is obvious that the samples obtained through extraction with petroleum ether and steaming were the best in terms of quality. Therefore, it is recommended to consumers for consumption. It is recommended that the storage study be extended to 6 to 12 months for further researches and the use of food grade solvents for extraction be researched on. It is also recommended that other improved methods of processing palm oil should be imbibed in order to improve the quality as well as the storage stability (shelf - life) of palm oil. Furthermore, different packaging and storage methods should be investigated, to ascertain the effects on the quality of palm oil.

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DIMENSIONAL AND AERODYNAMIC PROPERTIES OF GLOSA HYBRID WHEAT

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ABSTRACT

In post-harvest operations the physical characteristics and aerodynamic properties of grains and seeds are important. The objective of this paper was to evaluate the dimensional and aerodynamic properties of GLOSA hybrid wheat. Geometrical mean dimensions and aerodynamic properties were calculated, based on the measurement of 1000 grains. The moister content of wheat was 12.5%, with a 40 – 43 g per 1000 grains and hectoliter mass (HLM) of 76 – 79 kg/hl. The mean value for length was 6.10 mm, the width was 3.02 mm and the mean thickness was 2.58 mm. The results show a 99.70% frequency of dimensions between the three-standard deviation of the mean. The theoretical terminal velocity of 11.5 m/s is closely related to the experimental value of 11.9 m/s. The obtained data can be used for machine settings in conveying, sorting,or cleaning processes of grains.

1.Introduction

Since ancient times, ever since the very first food revolution, cereals have had special importance for human food. Among the grains, wheat is the most cultivated cereal in Europe, being a staple raw material in the food industry.

Achieving high quality in wheat crops begins by selecting the best varieties, depending on the area of cultivation and climatic conditions. Since the harvesting stage and the post-harvest processes, the aim is to ensure a high quality of the raw material (Balc 2016). After harvest, grains are subjected to cleaning, sorting, transport, drying, and storage processes (Stefanescu 2003). The functioning principles of the machinery and installations in the post-harvest phase are based on the physical and aerodynamic properties of the grains. Machinery and installations involved in the conditioning processes of the grainsbefore milling have special importance for obtaining high-qualityproducts. Knowing all the physical properties of cereal grains is important also in process analysisstarting from sowing (Csizmasia 2008) and harvesting (Gheres 2020, Pruncu 2020). Furthermore, the design of machines in post-harvest processes for cleaning, sorting, and transport requires knowledge of the geometric and pneumatic characteristics of the grains. For this purpose, the engineers can use image analysis of geometric parameters (Firatligil-Durmus 2010).

The characteristics of wheat grain, such as geometric dimensions, shape, mass, density depend primarily on the variety chosen (Beral 2020, Markowski 2013), the plant development conditions, cultivation area, and climatic conditions (Mandea 2018) as well as the moisture content.

The dimensions of the grains define their shape, in the case of wheat is ellipsoid. The shape of the grains can be expressed

mathematically through the factors of sphericity (Kaliniewicz 2015). The analysis of the shape of the grains is also important in terms of pneumatic properties.

Studies of the pneumatic properties of cereals and seeds (Chavoshgoli 2014, Khoshtaghaza 2006, Kumar 2020, Matouk 2005, Nalbandi 2010, Poleak 2016, Shahbazi 2014, Shahbazi 2015a, Shahbazi 2015b) are required in airflow-based sorting, cleaning,and conveying processes (Ghafori 2011).

The terminal speed of grain grains depends on the drag coefficient. Several theoretical methods (Bagheri 2016, Haider 1989, Tran-Cong 2004) and experimental methods (Chavoshgoli 2014, Poleak 2016) for determining the drag coefficient for grains, have been developed in the literature.

For wheat varieties which are grown in Romania, the physical properties are given by Stefanescu 2003: geometric dimensions are within the range (4.0 – 8.6 mm) for length, for width (1.4 – 3.8 mm), thickness between (1.4 – 3.8 mm), absolute mass of 1000 grains between 20 – 42 g, true density of 1.2 – 1.5 kg/dm^3 and bulk density of 0.67 – 0.83 kg/dm^{3}and a terminal speed of 8.9 – 11.5 m/s.

2.Materials and methods

The analyzed materialwas the GLOSA wheat, developed by the National Agricultural Research and Development Institute from Fundulea, Romania. It resulted from the complex hybrid combination of Oflabrad "S", Dor"S", and Bucur varieties. The average height of the plant is 85-95 cm, being similar or slightly superior toFlamura 85 and Fundulea 4varieties. The ear is white, awned,with a cylindrical shape, and has medium density. The grains are medium-sized, elongated in shape, and reddish.The 1000-grain weight was 42 – 43 g and has a hectoliter mass (HLM) of 76-79 kg/hl. The Glosais an early variety, drought-resistant autumn wheat. Recommended for the southern part ofRomania due to climatic conditions, is the second most cultivated in the country. The wheat production of Glosa reached 10,400 kg/hainInsula Mare a Brailei area.

2.1.Dimensional properties

The study on dimensional properties was based on the measurement of 1000 grains (N=1000) with a digital caliper having 0.01 mm accuracy. The length (L), width (W), and thickness (Th) of the first 100 measured grainsare shown in table 1. Based on the measured dimensions the maximum (L_{max}, W_{max}, Th_{max}) and minimum values (L_{min}, W_{min}, Th_{min}) of sizes were determined.The number ofindividual classes was(m_i=20, i=1...20).To obtain the class interval (λ) the formulabelow was used (where*L* subscript relates to length size of the grain):

$$\lambda_L = \frac{L_{max} - L_{min}}{m_i}, \text{[mm]} \quad (1)$$

The lower limits (L_{lowi}, W_{lowi}, Th_{lowi}) and upper limits (L_{upi}, W_{upi}, Th_{upi}) of class intervals were determined based on the expression bellowed:

$$m_{L1} = [L_{low\,1},\ L_{up\,1});\ L_{low\,1} = L_{min};\ L_{up\,1} = L_{min} + \lambda_L$$

$$m_{L2} = [L_{low\,2},\ L_{up\,2});\ L_{low\,2} = L_{up\,1};\ L_{up\,2} = L_{\min 2} + \lambda_L$$

... (2)

$$m_{Li} = [L_{low\,i},\ L_{up\,i});\ L_{low\,i} = L_{up\,(i-1)};\ L_{up\,i} = L_{\min i} + \lambda_L$$

...

$$m_{L20} = [L_{low\,20},\ L_{up\,20}];\ L_{low\,20} = L_{up\,19};\ L_{up\,20} = L_{max}$$

Table 1. GLOSA wheat dimensionsfor the first 100 grains

Nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
L [mm]	6.37	5.93	5.4	6.46	6.08	5.99	5.83	5.35	5.84	6.23	6.5	6.71	6.17	6.01	6.45	6.27	6.08	6.09	5.75	6.43	6.03	6.38	5.87	6.14	6.36
W [mm]	3.36	3.23	3.41	3.44	3.1	3.19	2.82	3.02	2.96	3.1	2.81	3.35	3.16	2.98	3.26	3.29	2.96	2.87	2.69	3.48	3.01	3.25	2.9	3.62	3.39
Th [mm]	2.59	2.95	2.73	2.38	2.79	2.89	2.77	2.81	2.74	2.53	1.99	3.19	2.89	2.8	3.15	3.11	2.76	2.66	2.53	2.64	2.8	3.16	2.5	3.16	2.88
L [mm]	6.21	6.36	6.37	6.77	5.86	6.37	6.36	6.6	6.44	6.46	6.22	6.14	6.46	6.25	6.21	6.51	6.2	6.75	6.06	5.9	5.92	5.73	6.2	5.76	6.27
W [mm]	3.04	2.92	3.04	3.05	3.00	3.26	2.92	3.54	2.99	3.39	3.4	3.19	3.29	2.83	3.45	2.92	3.26	3.06	2.98	3.03	2.54	2.86	3.12	2.91	3.13
Th [mm]	2.95	2.18	2.76	3.00	2.59	2.96	2.64	2.47	2.57	2.62	2.66	2.43	3.08	2.6	2.73	2.72	2.88	2.83	2.69	2.33	1.93	2.4	2.24	2.47	2.56
L [mm]	5.99	5.89	6.54	5.95	6.19	6.18	6.22	6.28	5.77	6.2	6.21	6.05	5.89	5.58	6.11	6.26	6.1	6.00	5.82	6.59	6.21	6.09	6.63	5.97	6.11
W [mm]	2.94	3.06	2.97	3.08	2.87	3.02	3.07	3.00	3.02	2.93	2.74	2.96	3.12	3.15	2.75	3.02	2.94	2.67	2.79	3.18	3.53	3.1	3.09	2.91	2.79
Th [mm]	2.17	2.89	2.61	2.93	2.81	2.7	2.49	2.65	2.71	2.46	2.62	2.78	2.73	2.28	2.56	2.59	2.69	2.12	2.52	2.68	2.83	2.32	2.71	2.51	2.68
L [mm]	5.97	6.52	6.54	6.26	6.18	5.47	5.93	5.89	6.18	6.12	5.89	6.13	5.65	5.74	5.69	6.14	5.98	6.13	6.16	6.51	6.34	5.45	6.08	5.8	6.43
W [mm]	2.99	2.74	3.25	2.77	3.33	2.91	3.13	3.26	3.08	3.14	3.05	2.99	2.96	3.55	3.53	3.24	2.82	3.2	3.13	3.12	2.6	3.06	3.04	2.93	3.39
Th [mm]	2.53	2.54	2.81	2.59	2.98	2.37	2.49	2.58	2.98	2.42	2.81	2.57	2.82	2.96	2.92	2.78	2.74	2.58	3.00	2.77	2.48	2.25	2.59	2.45	2.8

The frequency of occurrence of length, width, and thickness(f_{Li}, f_{Wi}, f_{Thi}),defined by the occurrence of values in individualclasses for each size(n_{Li}, n_{Wi}, n_{Thi}) was calculated with:

$$f_{Li} = \frac{n_{Li}}{N} \cdot 100, [\%] \quad (3)$$

The mean value of individual classes for each size(M_{Li}, M_{Wi}, M_{Thi}) was determined by:

$$M_{Li} = \frac{L_{Low\,i} - L_{up\,i}}{2}, [\text{mm}] \quad (4)$$

The mean value of each size (M_L, M_W, M_{Th}) can be calculated with expression (5):

$$M_L = \frac{\sum_{i=1}^{m} n_{Li} \cdot M_{Li}}{N}, [\text{mm}] \quad (5)$$

The deviation from the mean value inindividual classes (D_{Li}, D_{Wi}, D_{Thi})was calculated with equation (6) to obtain the standard deviation (σ_L, σ_W, σ_{Th}) with expression (7):

$$D_{Li} = M_{Li} - M_L, [\text{mm}] \quad (6)$$

$$\sigma_L = \sqrt{\frac{\sum_{i=1}^{m} (D_{Li})^2 \cdot n_{Li}}{N}}, \quad (7)$$

Statistically, 99.7% of the measured dimensions should fall within the limits of three standard deviations of the mean (M-3σ, M+3σ).

2.2.Aerodynamic properties

The study of aerodynamic properties is important inthe airflow sorting and cleaning processes of seeds.Considering an analogy between a glider and a wheat grain, the aerodynamic forces are represented in figure 1.

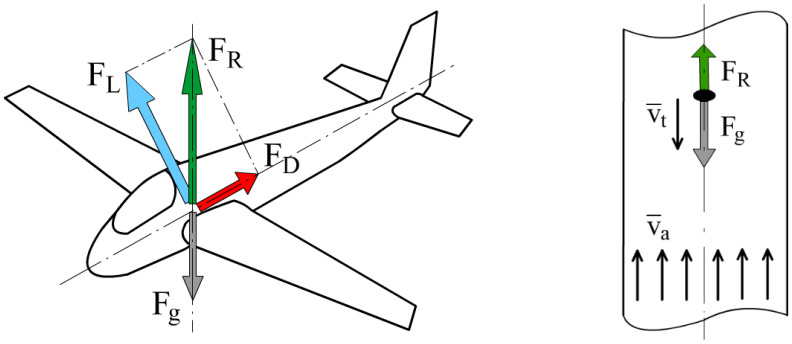

Figure 1. The analogy of aerodynamic forces acting on a glider and wheat grain.

Where: F_g is the force of gravity, F_L is the force of lift, F_D is the drag force, and F_R is the resultant of aerodynamic forces. The two forces acting on a free-falling grain are the force of gravity and resultant of aerodynamic forces. At the start of the falling phase, $F_R < F_g$ and the grain will accelerate. When $F_R = F_g$ the grain has no acceleration, will reach the maximum and constant falling speed, known as the terminal velocity, v_t. In the case of an airflow, with a

velocity of v_a, acting on the grain from below, three situations can be considered: $v_t > v_a$ the grain will fall, $v_t = v_a$ the grain will float, and $v_t < v_a$the grain will be lifted.

The mathematical expression used for terminal velocity, also adopted in the literature (Mujumdar 2015) is given below:

$$v_t = \sqrt[2]{\frac{2 \cdot m_g \cdot g \cdot (\rho_g - \rho_a)}{C_d \cdot A_g \cdot \rho_g \cdot \rho_a}}, [m/s] \quad (8)$$

Parameter $m_g=42 \cdot 10^{-6}$ kg,is the weight of wheat grain, g=9,81 m/s^{2}is the gravitational acceleration. Thedensity of grainρ_g inkg/m^{3}was calculated with equation (9), ρ_a=1.2047 kg/m^3 is the density of air at 20 °C.

$$\rho_g = \frac{m_g}{V_g}, [kg/m^3] \quad (9)$$

Parameter $A_g$is the surface area of the grain section normal to the airflow, and $V_g$is the theoretical volume of the grainobtained by expressions(10)and (11). Where d_g is the equivalent diameter of the grain and was determined with equation (12) based on mean values of length ($M_L=6.1 \cdot 10^{-3}$ m), width ($M_W=3.01 \cdot 10^{-3}$ m), and thickness ($M_{Th}=2.58 \cdot 10^{-3}$ m).

$$A_g = \frac{\pi \cdot d_g^2}{4}, [m^2] \quad (10)$$

$$V_g = \frac{4}{3} \cdot \pi \cdot \left(\frac{d_g}{2}\right)^3, [m^3] \quad (11)$$

$$d_g = \sqrt[3]{M_L \cdot \frac{(M_W - M_{Th})^2}{4}}, [m] \quad (12)$$

The value for the drag coefficient C_d can be adopted from literature or can be determined theoretically (Bagheri 2016).For this study,a drag coefficient of C_d=0.5 was adopted, a commonlyused value in the literature (Mujumdar 2015).

For the experimental study of floating speedthe behavior of the grains in an observation column with monitored airflow was used.

It was observed that the grains begin to float at an airspeed of 9.5 m/s (Figure 2.a). At the airspeed of 11.9 m/s, a balance occurred between the descending and climbing grains in the observation column (Figure 2.b). For an airspeed higher than 13 m/s in the observation column, all the grains were lifted and transported pneumatically.

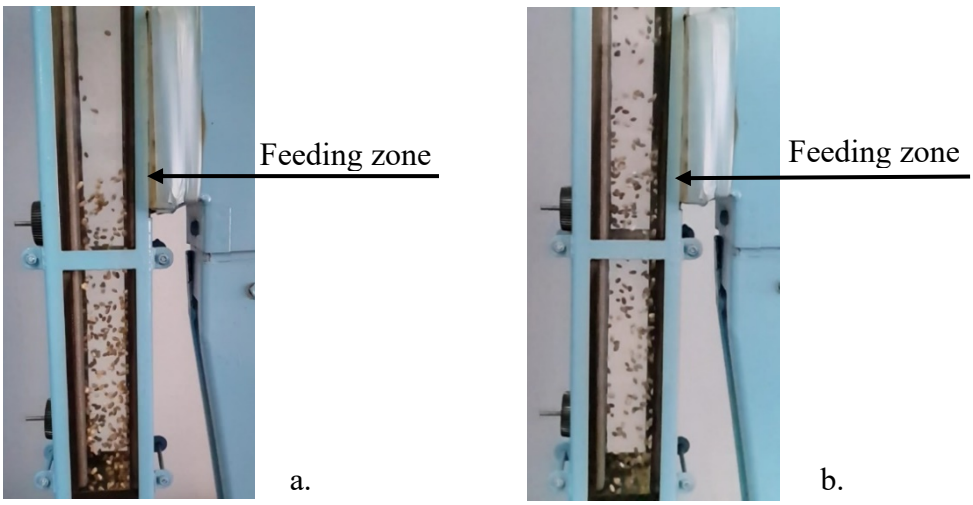

Figure 2. Observation column during experimental measurements:
a – floating velocity at 9.5 m/s, b – floating velocity at 11.9 m/s.

3.Results and discussions

The maximum, minimum, and mean values of dimensions, standard deviations, calculated, and measured terminal velocity for the GLOSA wheat are shown in Table 2.

Table 2. Mean values and standard deviations of grain dimensions and terminal velocity

Dimension	Max. Value, mm	Min. Value, mm	Mean, mm	Standard Deviation	Calculated terminal velocity, m/s	Measured terminal velocity, m/s
Length	6.99	5.03	6.10	0.27067	11.5	11.9
Width	3.63	2.46	3.02	0.19075		
Thickness	3.21	1.88	2.58	0.22828		

The maximum and minimum geometric dimensions established for the analyzed GLOSA wheat fall within the intervals given in the literature according to Stefanescu 2003. The average dimension found for length was 6.1 mm, the width was 3.02 mm and for thickness, a value of 2.58 mm was determined.The standard deviations show a higher dispersion of values for length size, while in the case of width the dimensions are closer to the mean value.

The calculated terminal velocity (for C_d=0.5) was 11.5 m/s and the measured floating velocity was 11.9 m/s with a difference of3.4%.

The one-standard deviation of the mean (M-σ, M+σ),two-standard deviation of the mean (M-2σ, M+2σ), three-standard deviation of the mean (M-3σ, M+3σ)and the frequency occurrence of dimensions are presented in table 3.

Table 3. Standard deviations of the mean and frequency occurrence of dimensions

Dimension	One-standard deviation of the mean	Frequency, %	Two-standard deviation of the mean	Frequency, %	Three-standard deviation of the mean	Frequency, %
Length	(5.83, 6.37)	83.8	(5.56, 6.64)	97.8	(5.29, 6.92)	99.7
Width	(2.83, 3.21)	67.3	(2.64, 3.41)	95.3	(2.45, 3.60)	99.8
Thickness	(2.40, 2.80)	67.6	(2.12, 3.03)	95.8	(1.89, 3.26)	99.9

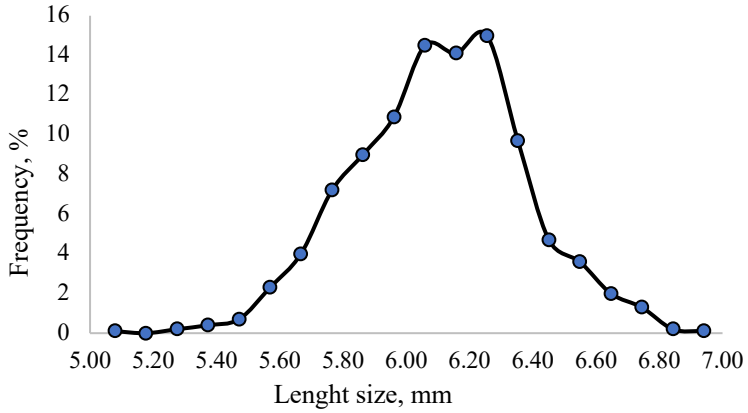

Figure 3. Frequency occurrence of length valuesin classes against the size range.

In the case of width and thickness, the standard deviations of the mean show a good correlation with the 68-95-99.7 rules.For length, the one and two-standard deviation of the mean shows higher values for frequency occurrence than expected and in the case of the three-standard deviation of the mean, the 99.7%rule is respected.

The frequency occurrence of lengthvaluesin classes plotted against the size rangeis shown in figure 3.

The highest occurrence of 145 and frequency of 14.5% of length values are in class 11 with interval limits of (6.01, 6.11), as shown in table 4.

The frequency occurrence of width values in classes plotted against the size rangeis shown in figure 3.

Table 4. Frequency and occurrence of length values

Class	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Interval, [mm]	5.03	5.13	5.23	5.32	5.42	5.52	5.62	5.72	5.81	5.91	6.01	6.11	6.21	6.30	6.40	6.50	6.60	6.70	6.79	6.89
Occurrence	1	0	2	4	7	23	40	72	90	109	145	141	150	97	47	36	20	13	2	1
Frequency, [%]	0.1	0	0.2	0.4	0.7	2.3	4.0	7.2	9.0	10.9	14.5	14.1	15.0	9.7	4.7	3.6	2.0	1.3	2.0	1.0

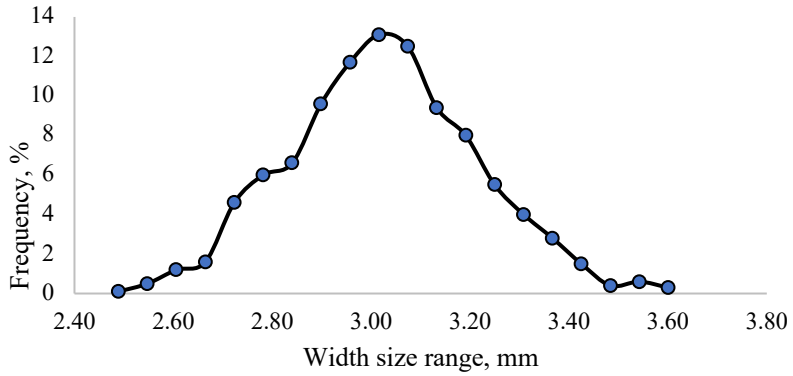

Figure 4. Frequency occurrence of width values in classes against the size range.

The highest occurrence of 131 and frequency of 13.1% of width values are in class 10 with limits of (2.99, 3.05).

The frequency occurrence of thickness values in classes plotted against the size rangeis shown in figure 4.

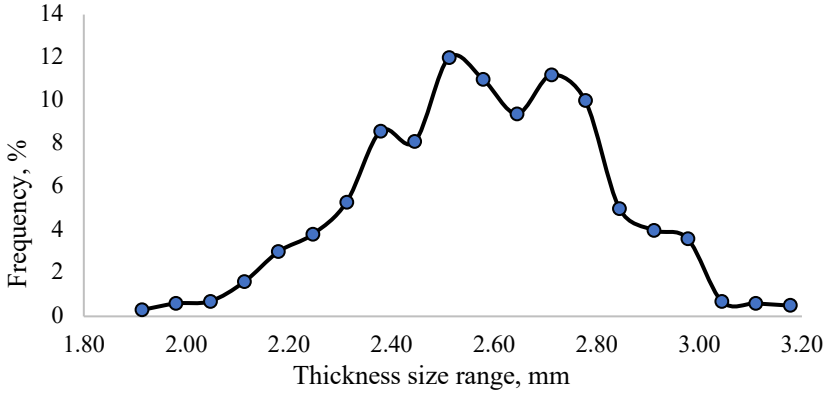

Figure 5. Frequency occurrence of thickness values in classes against the size range.

The highest occurrence of 120 and frequency of 12.0% of thickness values are in class 10 with interval limits of (2.48, 2.55).

4.Conclusions

In this paper, the geometrical dimensions, and aerodynamic properties of GLOSA wheat were analyzed. The value found for the mean length was 6.10 mm with a standard deviation of 0.27.The mean width was 3.02 mm with a standard deviation of 0.19.The mean thickness was 2.58 mm with a standard deviation of 0.228. Mean dimension and standard deviation values of grains are important in choosing the size of sieve openings in mechanical cleaning and sorting operations.

The calculated terminal velocity of 11.5 m/s was very closely related to the measured value the floating velocity of 11.9 m/s. The GLOSA wheat's terminal velocity is in the upper limit of the given interval of (8.9, 11.5 m/s) found in the literature. Terminal velocity of grains is critical in pneumatic cleaning, sorting, and conveying processes.

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