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RESTRAIN HYPERCHOLESTEROLEMIA WITH ORANGE AND MANDARIN VOLATILE AND FOLDED OILS

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ABSTRACT

The findings of this study proved that orange peels contain more twice oil yield over mandarin peels (0.24% and 0.12%, respectively). Concentrated orange and mandarin peel essential oils were obtained by fractional distillation. The chemical constituents of original orange and mandarin oils (OO and MO) and their fivefold (5FOO and 5FMO) were fractionated and identified by GC/MS and GC. Limonene was the major monoterpene component in orange and mandarin oils, 89.65% and 65.57%, respectively, followed by myrcene (3.95%) and γ-terpinene (23.07%) in orange and mandarin oils, respectively. Octanal (1.47%) and linalool (1.5%) were the abundant oxygenated components in orange and mandarin oils, respectively. The decrement percentages of limonene were 18.58 and19.25% in five fold orange and mandarin oils, respectively. The major oxygenated component in 5FOO was the alcoholic β- Cis-terpeneol (4.27%). The main esters of mandarin oil, methyl N-methyl anthranilate and geranyl acetate were increased to 0.45% and 1.05%, respectively in 5FMO. In general, total oxygenated compounds percentages increased by 6.9 and7.3 times after folding of orange and mandarin oils, respectively.

The above mentioned oils, pure limonene and synthetic antioxidant (BHT) were orally administrated for hypercholesterolemic rats for four weeks. No significant differences were recorded among groups of rats administrated with all different tested oils in body weight gain (%) or feed intake (g) ($p \leq 0.05$). In general, all rat groups administrated with orange and mandarin oils and their concentrates, showed improvement in HDL levels nearly to normal level compared to the negative control. Thus, the decrement of serum cholesterol level among cholesterol-fed groups did not correlate with the amount of limonene consumed by rats and may be related to other minor components associated with limonene and shared in the antioxidant effect. The efficiency of folded oils on hypocholesterolemic rats did not affect by decreasing limonene by deterpenation process.

1. Introduction

Orange (*Citrus sinensis*) and mandarin (*Citrus reticulata*) fruits are belonging to Rutaceae Family which follows up genus Citrus (Bourgou, *et al*., 2012). Citrus peel volatile oils, as by- products of the citrus processing, have wide uses primarily they are used as aroma flavor in many food, pharmaceutical and cosmetic products (Stuart *et al.,* 2001_a). Recently, other applications make use of limonene, the major compound of the oil extracted from citrus peels, as a green solvent

for lipids extraction (Mamidipally and Liu, 2004 and Virot *et al*., 2008).

Citrus essential oils are a mixture of volatile compounds consist mainly of monoterpene hydrocarbons, which produce high levels of unsaturated and unstable components by oxidation and hydration in the presence of many factors such as light and heat (Ferhat, 2007). The deterpenation process, concentration of citrus essential oils, separates the relatively odorless and flavorless hydrocarbons from the oxygenated components which are more highly odoriferous and flavored. Deterpenation is a relative term and the complete removal of terpene hydrocarbons from citrus oil is not usually achieved or even desired. In practice, any oil which has removed 50% or more of its terpene hydrocarbons could be described as terpeneless despite the fact that the percentage of remaining hydrocarbons is still high (Tzamtzis *et al*., 1990). Citrus oils from which terpenes have been removed are called folded oils as the remaining flavorful oxygenated compounds are more concentrated. The degree of concentration is often calculated from the ratio of the principal constituent in the concentrated oil to that in the prime oil from which it was made (Bettini, 2007). Folded orange oils are used as a food ingredient and also as a flavor enhancer in beverages. They can be incorporated into new and existing formulations to enhance fragrance and color.

Hypercholesterolemia and its associated cardiovascular diseases (CVD) represent one of the greatest worldwide economic, social and medical challenges that we are currently facing (Olshansky *et al*., 2005). Hypercholesterolemia also plays a pivotal role in the development of atherosclerosis, which is a leading cause of death. The relationship between plasma lipid and lipoprotein concentrations and the risk of developing cardiovascular disease (CVD) on the basis of dietary fat type is well documented (Krauss *et al*., 2001). In addition, the high level of cholesterol in blood have been found to be a major risk factor for the development of atherosclerosis (Brunzell *et al*., 2008). It is noteworthy that, Ancient Egyptians have used aromatic oils as early as 4500 BC in cosmetics and ointments. They made a mixture of herbal preparations from aniseed, cedar, onion and garlic in medicine (Baser and Buchbauer, 2010). Also, the use of aromatic oils more than 700 substances including ginger, myrrh, sandalwood and cinnamon as being effective for healing, in China and India, for a long time in ancient era (Pauli and Schilche, 2009).

D-limonene, 1-methyl-4-(1-methylethenyl) cyclohexane, is a mono cyclic monoterpene that is mainly present in citrus essential oils with lemon-like odors. Santiago *et al.* (2010) studied the effect of d-limonene on blood pressure, plasma lipids, circulatory lipid per-oxidation byproducts and antioxidant status in young male Wistar rats fed a high fat diet. They ascertained that d-limonene should be considered as a promising lipid lowering agent and antioxidant activities with blood pressure-lowering properties.

D-limonene is listed in the Code of Federal Regulations as generally recognized as safe (GRAS) as a flavoring agent in common foods such as fruit juices, soft drinks, baked foods, ice cream and desserts. In humans, D-limonene has demonstrated low toxicity after repeated dosing for up to one year. The studies showed that D-limonene inhibits lipid per-oxidation and prevents free radical-induced damage, physical and psychological stress and stress-induced hypertension. D-limonene has also been reported to have important biological activities, such as antioxidant properties, anti-inflammatory activities and chemo-preventive or chemotherapeutic properties against several types of cancer as mentioned by Jing *et al.* (2013).

Consequently, the present study has been carried out to investigate the deterioration in chemical composition, terpenes and oxygenated components, of original orange and mandarin peel oils after concentration, folding, by GC/MS, as well as evaluate the effect of administrated original and concentrated oils, depending on their limonene content, on

growth performance, serum lipid profile as well as serum liver and kidney functions of hyper-cholesterolemic rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials

Mature orange (*Citrus sinensis*) and mandarin (*Citrus reticulata*) fruits, balady variety (the local variety in Egypt) were purchased from from El-Shabrawey Farm, Wadi-Elmolak, Sharkia Governorate, Egypt and Al-Amal Farm, Wadi El Natrun, Beḥeira Governorate, Egypt.

2.1.2. Authentic Compounds

Twenty authentic hydrocarbons (GC-grade), including limonene(C8–C23 n-alkanes), as well as Butylated hydroxy toluene (BHT) were obtained from Merck–Schuchardt, Munich, Germany.

2.1.3. Experimental animals

Forty eight male Albino rats (Wister strain, in bred, with average weight 140-150 g) were obtained from the Research Institute of Ophthalmology, Giza, Egypt.

2.2. Methods

Extraction of orange and mandarin peel oils

The oils of both orange and mandarin peels were extracted by applying cold pressing method according to the method of Ahmad and Rehman (2006), the washed peels were shredded to small pieces (~ 0.5 x 0.3 cm), cold pressing was done at ambient temperature by a manual piston. The obtained extract was centrifuged at 4000 rpm for 20 min. at 4°C. After centrifugation, the separated oil was then treated with anhydrous sodium sulfate .The oil was kept in opaque glass bottles at -18°C, until used. Oil yield was calculated as follows: Oil yield % (w/w) = (quantity of obtained oil / quantity of used peels) ×100

2.2.1. Concentration of essential oils (Folding)

orange and mandarin essential oils were concentrated by vacuum distillation (10 mm bar, 45-55°C for2.5-3hr.) to obtain fivefold oils according to the method described by Stuart *et al*. (2001_b). The obtained oils were stored at 2ºC, until analysis and utilization.

Identification and determination of essential oils volatile components

Two µl of each essential oil were injected and analyzed (GC) using a Hewlett Packard model 5890 instrument equipped with a flame ionization detector (FID) and a DB-5 fused silica capillary column (60m x0.32 mm. id and film thickness of 0.32 µm). The oven temperature was programmed from 60˚C to 200˚C with rate 3˚C /min. Helium (1 cm3/min) used as a carrier gas. The temperatures of injection port and detector were 220˚C and 250˚C, respectively. The retention indices (Kovats index) of the separated peaks were calculated (using hydrocarbons C 8-C 23) and their area percentages were calculated with a Hewlett Packard 3396 integrator as reported by Adams (1995).

GC-mass spectroscopic analysis was carried out using a Hewlett-Packard 5972 GC-MS system equipped with the same column and condition used in GC analysis .The ionization voltage was70eV, mass range m/z39-400 amu. Components were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology, NIST) and compared with those authentic compound and published data, as well as on comparison of their indices with those of authentic compounds as mentioned by Adams (1995).

2.2.2. Biochemical experiment

The experimental animals were kept in an environmentally controlled room (temperature 25◦ ± 2 C, humidity: > 60%) with regular light-dark cycle. The animals were housed individually in polypropylene aerated cages with screen bottoms and provided diet and water *ad libitum*. The rats were fed on a basal diet consisted of 21.70% casein, 53.30% corn starch, 15% sucrose , 5% corn oil , 4% mineral mixture and 1.00% vitamin mixture (Tebib *et al.*,1997) for 10 day. After adaptation

period, the rats were randomly divided into eight groups (six rats each) as follows: Group 1 (negative control): Animals received a basal diet. Group 2 (positive control): Animals received high fat and cholesterol diet (H.F.C.D) which prepared as basal diet , except that corn starch and corn oil contents were decreased to 39.92 and 2.00%, respectively, and the diet was supplemented with 15% beef tallow,1.0% cholesterol,0.2% choline bitartrate and 0.18% cholic acid , as described by Terpstra *et al.* (2002). Group 3 (OO): Animals received H.F.C.D and orally provided with 440mg/Kg body weight (BW)/ day of orange oil (which contain 400 mg/Kg BW of limonene). Group 4 (5FOO): Animals received H.F.C.D and orally provided with440mg/Kg BW/ day fivefold orange oil (which contain 320 mg/Kg BW of limonene). Group 5 (MO): Animals received H.F.C.D and orally provided with 440mg/Kg BW/ day mandarin oil(which contain 280 mg/Kg BW of limonene). Group 6 (5 FMO): Animals received H.F.C.D and orally provided with440mg/Kg BW/ day fivefold mandarin oil (which contain 320 mg/Kg BW of limonene). Group 7 (LIM): Animals received H.F.C.D and orally provided with 440 mg/Kg BW/ day pure limonene (Ahmad and Beg, 2013). Group 8 (BHT): Animals received H.F.C.D and orally provided with 20 mg/Kg BW/ day synthetic antioxidant (Brewer, 2011).

Animals groups, except negative control group, were fed on a high fat and cholesterol diet (H.F.C.D) for 3 weeks to raise the serum cholesterol level for groups. After 3 weeks, different groups were fed a high fat and cholesterol diets and administrated with different tested original oils, concentrated oils and synthetic antioxidant (BHT) for 4 weeks. The administrations of the original and concentrated oils were done by oral route using a calibrated 1 ml syringe with attached polyethylene canola as described by Vasudeva and Sharma (2012). At the end of experimental period, the rats were fasting over night and sacrificed and then, internal organs were weighed.

The daily dose of original and concentrated oils was specified not to exceed than 1/10 LD_{50} of limonene (LD_{50} = 4400 mg/kg body weight) (Anon, 2009). Original, concentrated and pure limonene was dissolved in 0.5 ml corn oil as a carrier and the doses were applied daily.

At the end of the experiment, feed consumption (intake) ratio was calculated as mentioned by Gorinstein *et al*. (2007) and Ruangchia and Lien (2012). The body weight gain (BWG, %) was calculated as follows: Body weight gain (%) = (Final weight – Initial weight / Initial weight) X 100. After 8 weeks, rats were scarified then liver and kidneys were excised immediately then cleaned and weighed. Internal organs percentages (liver, kidneys, heart, brain, spleen and lunges) were calculated according to Atangwho *et al*. (2012), as follows: Organs weight (%) = (wt. of organ/ final body wt. of rat) × 100

Blood samples, overnight fasted rats for each group, were taken at the beginning of the experiment and each 15 days interval during experiment period. The blood samples were collected from the eye plexuses by fin glass capillary tubes according to the method of Schermer (1967). The collected blood was put into a dry clean centrifuged glass tube without coagulant to prepare serum sample. Blood was left for 15 min at room temperature to coagulate then the tube were centrifuged at 3000 rpm for 10 min and the clean supernatant serum was kept at -20ºC to determine blood constituents.

2.2.3. Biochemical analysis

Plasma lipid profile was determined. Serum total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined according to the method of Tietz, (1976). Total lipids (TL) was determined as described by Zollner and Kirsch (1962). Triglycerides (TG) were determined according to the methods of Fossati *et al*. (1982). Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), as liver function enzymes, were determined colorimetrically as

described by Sherwin, (1984) and Young (1997), respectively, one unit is defined as 1μmol NADH oxidized per min. Regarding, kidney functions, uric acid was determined as mentioned by Friedman and Young, (2001), while Creatinine was determined in blood serum, according to the method of (Tietz, 1995). Glucose level was determined in blood serum of experimental rats using enzymatic method ascribed by Young *et al*. (1972).

2.2.4. Statistical analysis

The collected data of biological evaluation was statistically analyzed using Analysis of Variance (ANOVA) as mentioned by Rao and Blane (1985). An average of three replicates, unless, otherwise stated, was used in calibrations. The standard deviation (SD) was also calculated.

3. Results and Discussion

3.1. Yield and chemical composition of orange and mandarin peel oils and their concentrates

The yield of cold pressed orange and mandarin peel oils was calculated. The results indicated that the orange peels contain about twice the oil yield of mandarin peels, where, they recorded (0.24±0.05) *vs*. (0.12±0.17) % (w/w). These results confirmed those of orange oil obtained by Kamal *et al*. (2011) and Badee *et al*. (2011). Njoroge *et al.* (2005) recorded 0.13% -0.15% as a range for the oil yield obtained from mandarin peels.

The chemical components of cold pressed orange and mandarin peel oils (OO and MO, respectively) and their fivefold oils, (5FOO and 5FMO, respectively), were fractionated and identified by GC and GC/MS, and the identified components are shown in Table 1.

The results illustrated in Table (1) indicated that orange oil chromatogram included 28 volatile components belong to seven chemical groups. The predominant chemical group was monoterpenes (96.21%). Five monoterpene compounds were identified in orange oil, the main component is limonene (89.65%), followed by myrcene(3.95%). In addition, 6 sesquiterpene hydrocarbons were identified as trace components (representing 0.22% of oil components) in orange oil. Moreover, three terpene alcohols compounds were identified (as trace compounds), representing 0.80% of oil components. Furthermore, seven aldehydes compounds representing 1.92% of orange oil components were identified (as minor compounds), octanal (1.47%) was the major of them. Orange oil profile components are extended to include one trace oxide component namely, trans-limonene oxide(0.01%), and one trace ester component, namely caryophyllene acetate (0.08%).Finally, orange oil components included five trace oxygenated sesquiterpenes representing 0.67% of total oil components.

As for the chemical composition of mandarin oil, the data in Table (1) indicated that, it was consisted of 17 volatile chemical component belong to six chemical groups. Monoterpenes was the major chemical group (95.12%), it included 8 volatile chemical components. Limonene and γ –terpinene were the predominant components, 65.57% and23.07%, respectively, while, β-pinene and myrcene represented minor components in monoterpenes group. However, Sesquiterpenes was a minor chemical group in mandarin oil(1.4%) consisted from3 volatile chemical components. Moreover, one compound belongs to alcohols group and another belongs to aldehydes, namely Linalool(1.5%) and Neral (0.34%), respectively, were identified. Three trace esters (0.5%) were also detected in mandarin oil.

Fractional distillation is a common industrial method for folding citrus essential oil because of its low cost and the odor profiles of the produced oil. Besides, it is accepted technique in the flavor and fragrance industry as mentioned by Flesher (1994).

Table 1. Chemical constituents of orange and mandarin oils and their fivefold concentrates by GC/MS

Components	K.I*	OO	5FOO	MO	5FMO
Monoterpenes					
α-Thujene	929	--	--	0.45	--
α-Pinene	937	1.31	0.09	2.07	--
Sabinene	978	0.76	0.07	0.22	--
β-Pinene	982	--	--	1.98	0.26
Myrcene	997	3.95	--	1.76	0.79
Δ-3-Carene	1011	--	--	--	--
α-Terpinene	1018	0.54	0.52	--	--
ρ-Cymene	1027	--	--	0.30	0.08
Limonene	1036	89.65	72.99	65.57	52.95
γ –Terpinene	1064	--	--	23.07	22.79
Total	--	**96.21**	**73.67**	**95.12**	**76.87**
Sesquiterpenes					
α-Copaene	1367	0.07	--	--	--
β-Cubabene	1390	--	0.12	--	--
β- Caryophyllene	1418	--	0.48	--	0.12
γ- Elemenene	1431	0.01	0.22	0.38	1.08
Humulene	1440	--	0.31	--	--
β-Farnesene	1458	0.01	0.1	0.74	0.42
D-Germarcrene	1486	0.01	--	0.28	0.06
Valencene	1491	0.04	--	--	--
α-Farnecene	1508	--	0.80	--	0.05
Δ-Cadinene	1514	0.08	0.15	--	2.21
Total	--	**0.22**	**2.18**	**1.40**	**3.94**
Alcohols					
Linalool	1096	0.22	0.43	1.50	4.90
Cis-p-mentha 2,1- dienol	1121	0.31	--	--	0.24
β- Cis-terpeneol	1142	--	4.27	--	1.53
ρ-mentha 1- dien -8- ol	1149	--	--	--	2.49
Terpen-4-ol	1171	--	0.17	--	0.41
α- Terpineol	1190	--	--	--	0.41
Trans-Carveol	1218	0.27	3.01	--	2.96
Geraniol	1254	--	0.18	--	--
Perilla alcohol	1294	--	0.82	--	--
Total		**0.80**	**8.88**	**1.50**	**12.77**
Phenols					
Carvacrol	1300	--	1.66	--	--
Total	--	--	**1.66**	--	--
Aldehydes				--	--
Octanal	1001	1.47	1.62	--	--
Nonanal	1108	--	0.13	--	0.34
Citronellal	1159	0.03	3.60	--	0.60
Decanal (N)	1204	0.26	0.76	--	0.74
Neral	1247	0.03	--	0.34	--
Geranial	1270	0.08	2.52	--	0.17
Undecanal	1306	0.01	1.56	--	0.48

Dodecanal	1408	0.04	0.26	--	--
Total		**1.92**	**10.45**	**0.34**	**2.33**
Oxides					
Cis-limonene oxide	1136	--	--	--	0.07
Trans-limonene oxide	1139	0.01	1.18	--	--
Caryophyllene oxide	1581	--	0.21	--	0.24
Total	--	**0.01**	**1.39**	**--**	**0.31**
Esters					
Citronellyl acetate	1294	--	--	0.09	--
Geranyl acetate	1383	--	0.73	0.11	1.05
Methyl N-methyl anthranilate	1402	--	--	0.30	0.45
Caryophyllene acetate	1697	0.08	--	--	--
Total	--	**0.08**	**0.73**	**0.5**	**1.50**
Oxygenated Sesquiterpenes					
Z-Nerolidol	1533	0.06	--	0.11	1.08
E-Nerolidol	1564	0.12	0.77	--	--
β-Bisabolol	1675	0.17	--	--	--
α-Bisabolol	1682	0.08	--	--	--
β-Sinensal	1701	0.24	--	--	--
Total	--	**0.67**	**0.77**	**0.11**	**1.08**
Total Non- oxygenated compounds (%)		**96.43**	**75.85**	**96.82**	**80.71**
Total oxygenated compounds (%)		**3.48**	**23.98**	**2.45**	**17.94**
Total identified components (%)		**99.91**	**99.83**	**99.27**	**98.65**

* Kovats retention indices calculated for DB-5 capillary column in GC in reference to C8-C23 n-alkane
OO: Orange oil, MO: Mandarin oil 5FOO: Five-fold orange oil 5FMO: Five-fold mandarin oil.

Results in Table (1) indicated that the percentage of the major components limonene decreased from 89.65% in the original orange oil to 72.99% in five-fold oil (5FOO), 18.58%, decreasing percentage. Otherwise, vacuum distillation process led to decrease limonene, from 65.57 to 52.95% in five-fold oil (5FMO), 19.24% decreasing percentage. Whereas, the second major monoterpene components γ-terpenene, not found in orange oil, slightly affected by distillation (1.21%, decreasing percentage). The high percentage of limonene in folded orange oil was observed by Vora, *et al.* (1983), who concentrated orange peels oils of Valencia and Midseason varieties by vacuum distillation to 10 fold and recorded that the percentage of limonene was decreased from 95.17 to 79.91% in ten-fold orange oil. Although, myrcene was completely disappeared from 5FOO after concentration, three components namely, α-Pinene and α-Terpinene were variably decreased (-93.13%, -90.79% and-3.70%, respectively). As for the rest of monoterpenes in 5FMO. Three components variably decreased, β-Pinene, Myrcene and p-Cymene (-87.76, -55.11 and -73.33%, respectively). In general, total Monoterpenes in 5fold orange and mandarin oil were reduced by 23.43 and 19.19%, respectively, from their original values after concentration.

Regarding the apparent considerable increases in sesquiterpenes of 5 folded orange and mandarin oils (9.91 times and 2.81times, respectively), it could be noticed that three volatile sesquiterpene compounds present in original orange oil were completely disappeared in concentrated oil. While, γ-Elemenene, β-Farnesene and Δ-Cadinen were variably increased after folding process. The increments in sesquiterpenes in the investigated folded oils might be due to the highly boiling range of sesquiterpenes as less volatile components. Hereby, Vora *et al.* (1983)

determined the quantitative composition of ten-fold orange oil comparing with the original oil and stated that some sesquiterpene components increased after deterpenation process. As for the increments in sesquiterpenes of 5FMO, it might be due to their high boiling range. Concerning the predicted increases in the oxygenated components due to folding process of orange and mandarin oils, total alcoholic components showed the highest increments in5fold orange and mandarin oil(11.1 times and 8.51 times in 5FOO and 5FMO, respectively). It is well known that folding process of orange oil led to disappear one of the three alcoholic volatile components in the original oil, simultaneously, four alcohol components directly appeared in 5FOO after folding. As for, concentration process of mandarin oil, it led to appear 6 volatile alcohols components which did not present before in the original oils beside the singular original alcohol component, namely; linalool, the characteristic of mandarin oil flavor (Choi and Sawamura, 2002).

With regard to the expected increments in total aldehyde contents of orange and mandarin oils due to folding process, the aldehyde contents increased by 5.44 times and 6.85times in 5FOO and 5FMO, respectively. Furthermore, Phenols components were not found in original orange and mandarin oils, but a unique phenol component was found only in 5 FOO, namely, Carvacrol. As for volatile ester component, trace component was found in original orange oil (Caryophyllene acetate) which disappeared after folding and another ester component (Geranyl acetate) appeared.

It is noteworthy that Methyl-N-methyl anthranilate is the main ester contributes to the overall aroma of mandarin oil as reported by Mazza (1987).

In general, the obtained data (Table 1) indicated that total oxygenated compounds increased 6.89 times and7.32 times in 5FOO and 5FMO, respectively.

3.2. The biological effects of orange and mandarin oil and their concentrates on hyper-cholesterolimic rats

The changes in growth performance, internal organs weight % as well as serum chemistry parameters of hype- cholesterolimic rats were determined after daily oral administrated of orange and mandarin and their concentrated oils for 4 weeks. The LD_{50} of limonene was taken into consideration. So, all doses didn't exceed (1/10) from LD_{50} of limonene constituted 4400 mg/kg BW (440 mg/kg BW) as recommended by Anon (2009). Regarding growth parameters, results in Table 2 illustrated that there are significant ($p \leq 0.05$) differences between different groups in the final body weight and body weight gain (BWG) (%). The significant differences in BWG between positive and negative control groups(G1& G2) and groups administrated original orange and mandarin oils (G3&G5) indicated the impact of limonene in reducing obesity. This conclusion ascribed to the decreasing effect in BWG of G7 administrated limonene. The superior depression in BWG showed in group 8 administrated BHT focus the light on the antioxidant activity of BHT and the natural antioxidant, limonene.

Table 2. Effect of oral administration with orange and mandarin oils and their concentrates on growth performance, internal organs weight % of hypercholesterolemic rats

Parameters	Groups of rats								LSD **
	G1	G2	G3	G4	G5	G6	G7	G8	
Growth performance									
Initial body weight (g)	241.75 ^{a} ± 10.53	239.50^{a} ^{b} ± 13.07	237.50^{a} ^{b} ± 14.43	248.25 ^{a} ± 13.5	222.00 ^{c} ± 18.60	222.00 ^{c} ± 13.63	256.00 ^{a} ± 12.75	231.70 ^{b} ± 11.78	20.42
Final body weight (g)	262.75 ^{a} ± 14.99	261.75 ^{a} ± 21.48	249.50 ^{ab} ± 19.94	264.00 ^{a} ± 14.58	229.70 ^{c} ± 23.82	238.00 ^{b} ± 21.35	271.00 ^{a} ± 18.99	238.00 ^{b} ± 10.86	29.10
BWG* (%)	8.68^{a} ± 1.80	9.29^{a} ± 3.21	5.05^{bc} ± 1.46	6.34^{ab} ± 2.59	3.46^{c} ± 2.41	7.20^{a} ± 6.27	5.85^{b} ± 2.39	2.69^{d} ± 3.24	4.20
Food intake (g/rat/day)	14.99 ^{a} ± 3.88	15.13 ^{a} ± 3.24	15.48 ^{a} ± 4.21	15.11 ^{a} ± 4.36	15.57 ^{a} ± 4.10	15.26 ^{a} ± 4.34	15.88 ^{a} ± 3.52	15.90^{a} ± 3.84	0.00
Internal organs weight (%)									
Liver	2.89 ^{c} ± 0.19	4.06 ^{abc} ± 0.15	4.72 ^{a} ± 0.42	4.21 ^{ab} ± 0.23	4.67 ^{a} ± 0.52	4.45 ^{a} ± 0.53	3.95^{b} ± 0.51	3.81 ^{bc} ± 0.32	0.73
Kidneys	0.59 ^{a} ± 0.18	0.56 ^{a} ± 0.09	0.59 ^{a} ± 0.05	0.53 ^{a} ± 0.05	0.62 ^{a} ± 0.02	0.61 ^{a} ± 0.07	0.58 ^{a} ± 0.05	0.49 ^{a} ± 0.09	0.00
Heart	0.33 ^{a} ± 0.07	0.32 ^{a} ± 0.06	0.28 ^{a} ± 0.04	0.28 ^{a} ± 0.02	0.26 ^{a} ± 0.04	0.25 ^{a} ± 0.03	0.26 ^{a} ± 0.04	0.27 ^{a} ± 0.06	0.00
Brain	0.61 ^{a} ± 0.14	0.49 ^{a} ± 0.10	0.57 ^{a} ± 0.05	0.56 ^{a} ± 0.01	0.58 ^{a} ± 0.03	0.59 ^{a} ± 0.02	0.56 ^{a} ± 0.06	0.52 ^{a} ± 0.08	0.00
Spleen	0.24 ^{a} ± 0.13	0.30 ^{a} ± 0.10	0.33 ^{a} ± 0.09	0.23 ^{a} ± 0.04	0.29 ^{a} ± 0.04	0.29 ^{a} ± 0.07	0.26 ^{a} ± 0.08	0.33 ^{a} ± 0.05	0.00
Lungs	0.65^{a} ± 0.07	0.62 ^{a} ± 0.08	0.63 ^{a} ± 0.06	0.57 ^{a} ± 0.03	0.63 ^{a} ± 0.06	0.59 ^{a} ± 0.05	0.51 ^{a} ± 0.07	0.55 ^{a} ± 0.03	0.00

Means (n= 6 rats± SD) with the same letter between rows are not significantly different at P≥ 0.05.
G1 : Basal diet (negative control). **G2 :** High fat diet +1% cholesterol (HFCD) (positive control).
G3 : HFCD + 440 mg OO contain 400 mg/Kg BW of limonene daily. **G4 :** HFCD + 440 mg 5FOO contain 320 g/Kg/ BW of limonene daily. **G5 :** HFCD + 440 mg MO contain 280 mg/Kg BW of limonene daily. **G6** : HFCD + 440 mg 5FMO contain 230 g/Kg/ BW of limonene daily.**G7 :** HFCD + 440 mg/Kg/ BW pure limonene daily. **G8 :** HFCD +20 mg BHT /Kg BW/daily.
***Body weight gain %** = (Final weight – Initial weight / Initial weight) X 100. **Least significant differences.

Our findings are not in agreement with Ruangchai and Lien, (2012)who found that limonene didn't affect the body weight gain of rats. Jing *et al.* (2013) reported that d-limonene did not prevent body weight gain of mice group fed on high fat diet supplemented with 0.6 g d-limonene /Kg/BW, in compared with groups fed on high fat diet as a positive control. Concerning the internal organs weight (%) of rat groups, the highest liver weight % was observed in the groups of rats G3 & G5, where, they recorded 4.72 and 4.67%, respectively. On

the other hand, the lowest (2.89%) was recorded by the negative control group (G1) followed by (G8). The rats fed on high fat diet have increased in liver weight by fat deposition on their bodies (Milagro *et al*., 2006). Slightly non significant differences were noticed in liver weight % among rat groups administrated original, concentrated orange or mandarin oils (G3, G4 and G5 or G6).

No significant differences ($p \leq 0.05$) were observed among rat groups (G2) and (G7) fed on the same diet plus pure limonene (440 mg/Kg BW). Thus, pure limonene component didn't affect fat accumulation in adipose tissues or increased fat cells numbers and size. No significant ($p \leq 0.05$) differences in kidneys, heart, brain, lungs and spleen weight % among rats fed ($P \leq 0.05$) on a basal diet and those groups fed on high fat diet and cholesterol and orally administrated with the investigated oils. These results are in agreement with those of Osfor *et al.* (2013) who found that the increment in heart and spleen weight of hyper-cholesterolemic rats or that supplemented by *Citrus aurantium* albedo powder compared with basal diet rats, was not significant.

On the other hand, serum lipid profile of rats was determined and the results are shown in Table (3). Results indicated that the serum total lipids (TL) level of rats, fed with different experimental diets, was significantly increased ($p \leq 0.05$) in positive control group (G2) fed with HFCD as compared with negative control (G1 given a basal diet). It could be noticed that (G6) had a lower total lipids level (332.67 mg/dL) than that received HFCD, (G2) which gave the highest TL level (502.24 mg/dL). The results concerning the lowering effect of TL by limonene are supported with the findings of Ruangchai and Lien (2012) and Ahmad and Beg (2013). So, it could be noticed that, serum TL level decreased after oral administration of experimental obese rats groups fed with H.F.C.D for G3, G4, G5 and G6. Moreover, the highest depression effect of TL could be noticed in G3, followed with G6, which could be attributed to the effect of limonene beside other monoterpenes found in orange and folded mandarin oils.

The superior serum total cholesterol (TC) level (143.31 mg/dL) was observed in group fed with H.F.C.D and (0.2 ppm) BHT (G8) followed by positive control G2 (132.96 ml/dL) with no significant ($p \leq 0.05$) differences between them. The average TC levels after four weeks in the cholesterol-fed groups (G3, G4, G5, G6 & G7) showed significantly ($p \leq 0.05$) decrement compared with positive control G2. The results indicated that the decrement of TC level among cholesterol-fed groups did not connect with amount of limonene consumed by rat groups only but may be related with the other minor components associated with limonene and subsequently shared with limonene as antioxidant potency (synergistic effect) as mentioned by Russo, *et al*. (2013) and Sharifi-Rad, *et al.* (2017). Similar data indicated that each of orange and mandarin oils and their concentrates had considerable reduction effects against the increase of serum total cholesterol. The reduction in TC and TG could be explained by the inhibition of two key enzymes in the regulation of cholesterol synthesis: HMG-CoA reductase and Acyl-Co A: Cholesterol acyl transferase (ACAT) (Kim *et al*., 2003). Indeed, limonene inhibited hepatic cholesterol biosynthesis and esterification (Clegg *et al*., 1980). The blockage of cholesterol synthesis by an inhibitor of HMG-CoA reductase (Elegbede *et al.,* 1984). Consequently, limonene reduces synthesis of HMG-CoA reductase at a post-transcriptional level (Clegg *et al*., 1980). Our findings also are in harmony with those reported by Lee, *et al*., (2018), who found antioxidative and cholesterol-lowering activities of lemon essential oil (67.57% limonene) in hypercholesterolemia-induced rabbits, evidenced by its inhibition effects on LDL oxidation and lipid peroxidation and preventive effects against erythrocyte deformation and aortic intimal proliferation. Several mechanisms for limonene protection in biological systems, which include the

deactivation of electronically activated species such as singlet oxygen and the deactivation of reactive chemical species, such as peroxyl or alkoxyl radicals that can be generated within cells, might be precluded the initiation of harmful oxidative reactions (Cao *et al*., 1993).

In general, all tested groups administrated with orange and mandarin oils and their concentrates, showed improve in HDL levels nearly and better than negative control.

Table 3. Effect of oral administration with orange and mandarin oils and their concentrates on lipid profile and kidney functions of hyper-cholesterolemic rats.

Parameters	Groups of rats								LSD*
	G1	G2	G3	G4	G5	G6	G7	G8	
Total lipids (mg/dL)	292.31 ^{cd} ± 26.57	502.24 ^{ab} ± 91.73	209.45 ^{d} ± 30.96	364.71 ^{cd} ± 42.12	403.14 ^{abc} ± 56.66	261.25 ^{cd} ± 122.62	332.67 ^{cd} ± 95.47	513.77 ^{a} ± 157.24	146.80
Total cholesterol (mg/dL)	80.21^{d} ± 5.43	132.96 ^{a} ± 35.21	94.56^{c} ± 7.83	114.85 ^{bc}± 28.22	116.38^{b} ± 19.17	108.28 ^{b} ± 19.17	109.79^{b} ± 16.02	143.31 ^{a} ± 40.11	40.54
Triglycerides (mg/dL)	89.28 ^{ab} ± 5.94	83.51 ^{ab} ± 19.11	49.14 ^{c} ± 6.95	63.61 ^{b} ± 18.58	63.29 ^{b} ± 9.55	55.00 ^{c} ± 11.40	67.85 ^{b} ± 19.67	101.58 ^{a} ± 22.50	26.12
HDL (mg/dL)	39.65^{b} ± 12.35	27.30 ^{c} ± 4.05	45.06 ^{ab} ± 6.95	40.73 b ± 6.03	50.48 ^{a} ± 2.08	37.91 ^{bc} ± 2.62	42.03 ^{abc} ± 19.67	49.61 ^{a} ± 6.82	11.21
LDL (mg/dL)	22.70^{c} ± 8.18	88.95 ^{a} ± 33.59	39.66 ^{b} ± 4.20	61.39 ^{ab} ± 35.17	53.24^{abc} ± 12.42	59.35^{ab} ± 22.33	54.19^{abc} ± 14.72	73.37 ^{a} ± 29.61	29.12
Urea (mg/dL)	46.05 ^{a} ± 4.44	35.95 ^{ab}±7.24	31.53 ^{b}± 5.30	31.27 b ± 5.49	26.90^{c} ± 1.46	28.33 ^{bc} ± 5.59	38.06 ^{a} ± 9.89	41.42 ^{a} ± 2.62	8.24
Creatinine (mg/dL)	0.41^{a} ± 0.08	0.40 ^{a} ± 0.03	0.35 ^{a} ± 0.02	0.47 ^{a} ± 0.11	0.40 ^{a} ± 0.03	0.48 ^{a} ± 0.03	0.44 ^{a} ± 0.14	0.42 ^{a} ± 0.09	0.00

Means (n= 6 rats± SD) with the same letter between rows are not significantly different at P≥ 0.05.
G1 : Basal diet (negative control). **G2 :** High fat diet +1% cholesterol (HFCD) (positive control).
G3 : HFCD + 440 mg OO contain 400 mg/Kg BW of limonene daily. **G4 :** HFCD + 440 mg 5FOO contain 320 g/Kg/ BW of limonene daily. **G5 :** HFCD + 440 mg MO contain 280 mg/Kg BW of limonene daily. **G6** : HFCD + 440 mg 5FMO contain 230 g/Kg/ BW of limonene daily.
G7 : HFCD + 440 mg/Kg/ BW pure limonene daily. **G8 :** HFCD +20 mg BHT /Kg BW/daily.
* Least significant differences.

With regard to findings of LDL level, no significant (p≤0.05) differences were observed among groups fed with HFCD and administrated with orange and mandarin oils (G3and G5), five-fold orange and mandarin oils(G4 and G6), and limonene (G7), of varying of limonene content, in these oils the lowest LDL level was noticed in the group original orange oil (G3). The level of LDL in serum of G5, rats administrated mandarin oil

(280 mg limonene content) compared with the group of rats G7 administrated pure limonene (440 mg limonene content) was not significant. Also, no significant difference between LDL level of rats group G4, administrated five-fold orange oil, 320 mg limonene content, and rats of group G6 administrated five-fold mandarin oil (0.23 g/kg limonene), indicated that the depression in LDL levels among the tested groups was not directly related to the given amount of limonene.

Triglycerides (TG) level of hyper-cholesterolemic rats groups administrated with sweet orange oil G3, and five-fold mandarin oil G6 remained lower than that of rats orally received five-fold orange oil G4 and mandarin oil G5. Also, these groups were significantly lower in triglycerides content than pure limonene group G7 administrated with (440 mg limonene content). These results indicated that there was no liner relation among serum triglyceride level in hyper-cholesterolemic rats groups and amount of limonene orally consumed. Jing *et al.* (2013) showed that D-limonene (5%) as supplementation in mice diet reduced TG by 36.1% and lowered LDL by 20.4% and increased HDL by 18.3% compared to the obese mice. Also, it reduces lipid deposition in the hepatic tissue compared to high fat diet fed mice. Thus, it could be noticed that d-limonene prevent lipid accumulation in obese mice liver and reduce the risk association with metabolic function of lipids in HFD obese mice (Jing *et al.* 2013). The supplementation of the diet with d-limonene at 0.2% significantly reduced serum cholesterol and triglycerides concentration of rats and increase the antioxidant activity of the Serum Superoxide Dismutase, the catalase and the Glutathione Peroxidase as endogenous liver enzymes compared with basal diet group.

The data showed that there were significant decreases in serum urea values between the negative control (G1) and the groups of rats fed on orange oil (G3), fivefold orange oil (G4), mandarin oil (G5), fivefold mandarin oil (G6). These results, besides the non significant changes in creatinine levels (Table, 3) proved that orange and mandarin oils and their concentrates didn't have a detrimental effect on the kidney functions, but the opposite may be true. Similar findings also indicated that d-limonene component was not solely responsible for the slight improvement of kidney functions. The higher of serum urea level in negative control rats group than the level of urea in positive control groups also was observed by Kanthlal *et al*. (2012), who found that the rats fed on high fat and cholesterol diet was lower in serum urea level than those fed on basal diets. The capacity of the hepatocytes in obese rats to synthesize urea from precursors is decreased and the uptake of amino acid by liver and the hepatic activity of the enzymes of urea cycle are also decreased (Barber *et al*., 1985). Hence, there will be a decrease in the urea level in obese rats.

3.3. Effects of orange and mandarin oil and their concentrates on liver function enzymes of hyper-cholesterolimic rats

From the results in Fig. (1), the lowest level of AST enzyme was in the negative control group of rats (G1, 12.00 U/L), while the highest level was with the positive group fed with high fat diet+1% cholesterol (G2, 39.33U/L). It is important to observe that addition of 5fold orange oil (G4) or limonene(G7) strongly prevent the increase of AST level in both rat groups with non significant differences among them and the negative control group (G1). It was observed that all tested essential oils and BHT in groups of rats G3, G5, G6 and G8 considerably decreased AST levels but to slightly higher level than G1. No significance ($p \leq 0.05$) differences were observed among groups fed with orange, mandarin oils, their concentrates and pure limonene in spite of varying of amount of limonene administrated. Thus, the variations of the amount of limonene consumed by rats in different groups don't affect the amount released of AST.

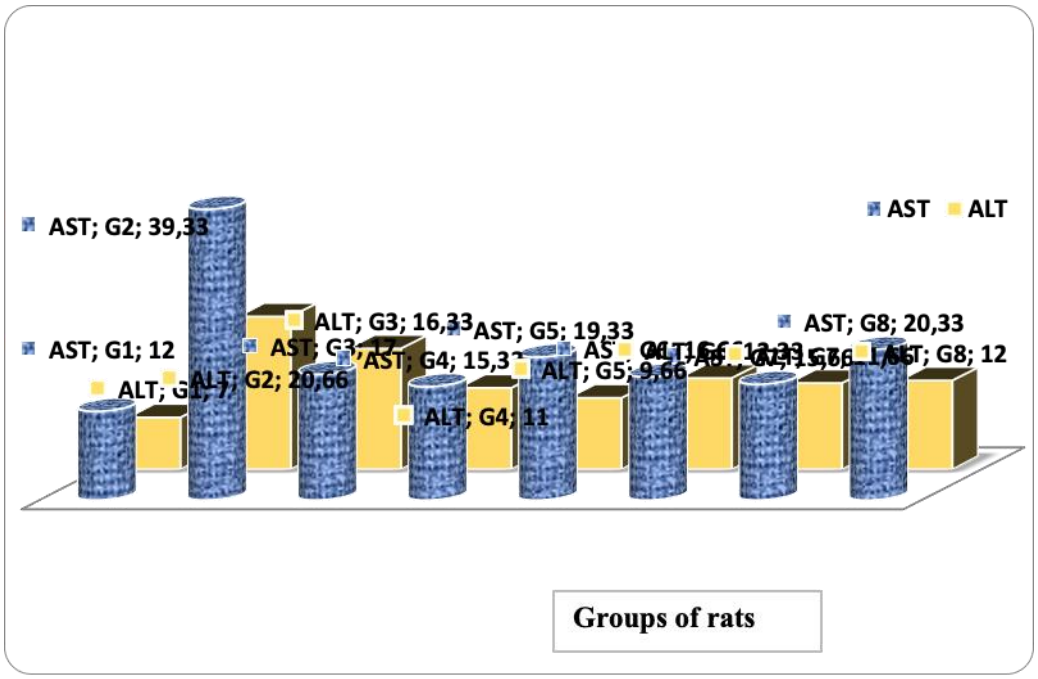

Fig. 1. Effect of oral administration with orange and mandarin oils and their concentrates on liver function enzymes of hyper-cholesterolemic rats.

On the other hand, the five-fold orange and mandarin oils groups (G4 and G6) was more effective in reducing AST activity in serum than their original oils (G3 and G5). A high significant protective effect was noticed of mandarin oil (G5) against release of ALT compared with the positive control G2. All other additions significantly reduced ALT levels, but to lower extents (G3, G4, G6, G7 and G8). Some components of certain species of citrus fruit such as Bergamot orange, as limonene and α-pinene, have hepatoprotective effects as published by Ozbek, *et al.* (2005). In this connection, Karaca, *et al.* (2005) examined hepato-protective activity of Bergamot essential compared with the positive control G2. All other additions significantly reduced ALT levels, but to lower extents (G3, G4, G6, G7 and G8).

It was mentioned that some components of certain species of citrus fruit such as Bergamot orange, as limonene and α-pinene, have hepatoprotective effects as published by Ozbek, *et al.* (2005). In this connection, Karaca, *et al.* (2005) examined hepato-protective activity of Bergamot essential oil, limonene (40%) and linalool (8%), on carbon tetrachloride-induced hepatotoxicity in rats and reported that Bergamot oil reduced the serum ALT level but did not affect the AST level.

3.3. Effects of orange and mandarin oil and their concentrates on serum glucose of hyper-cholesterolimic rats

The data presented in Fig. 2 showed that no significant ($p \leq 0.05$) differences were noticed between the negative control group (G1), pure limonene group (G7) and BHT group (G8)

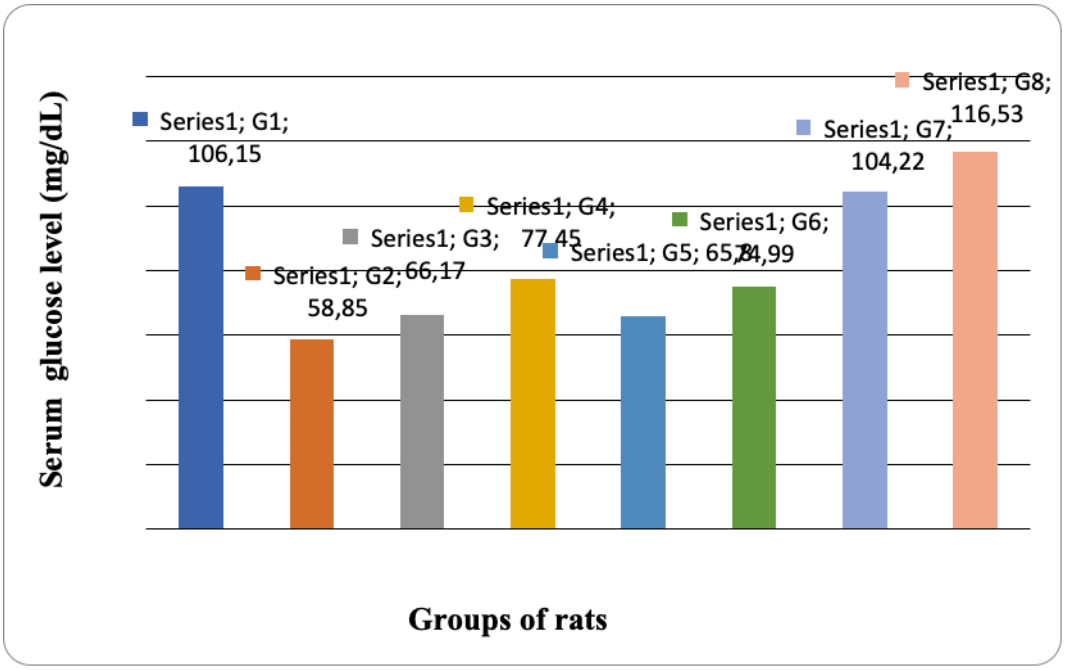

Fig. 2. Effect of oral administration with orange and mandarin oils and their concentrates on serum glucose level of hyper-cholesterolemic rats.

Meanwhile, significant decrements in serum glucose level were detected in the five remaining groups fed on orange oil, mandarin oil or their concentrates (G2, G3, G4, G5 and G6), comparing with the above mentioned three groups (G1, G7 and G8). It's noteworthy to observe that rats fed with orange oil, mandarin oil and their concentrates did not change serum glucose level whether over the normal range 50-136 mg/dL (Gad, 2007).

In general, it's clear that rats fed with orange oil, mandarin oil, and their concentrates tended to show significant decreases in serum glucose levels. More *et al.*, (2014) demonstrated that the combination of limonene and linalool can function very effectively against serum glucose level in diabetic rats by act in synergistic manner.

4. Conclusions

The deterpenation process reduced the percentages of limonene in five fold orange and mandarin oils. Oral administration with orange and mandarin oils and their concentrates, for hypercholesterolemic rats for four weeks exhibited improvement in HDL levels nearly to normal level compared to the negative control, however, the decrement of serum cholesterol level among cholesterol-fed groups did not correlate with the amount of limonene consumed by rats and may be related to other minor components associated with limonene and shared in the antioxidant effect. The efficiency of folded oils on hypocholesterolemic rats did not affect by decreasing limonene by deterpenation process.

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MULTI-OBJECTIVE OPTIMIZATION TO DETERMINE THE COLD DRYING MODE OF GAC (*MOMORDICA COCHINCHINENSIS SPRENG*)

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ABSTRACT

The establishment of the cold drying mode of Gac was based on the solution to multi-objective optimization problem by the restricted area method. Experiments were carried out to set up the mathematical model of objective functions describing the influence of technological factors (temperature of moisture condensation, temperature of cold drying chamber, velocity air (or drying agents) and time of cold drying) in the cold drying process. The restricted area method with R*(Z) optimal combination criterion was applied to solve the multi-objective optimization problem, determining the optimal technological mode of cold drying process (correspondingly 9.83^0C; 44.18^0C; 3.46m/s and 12.36h) in order that the objective functions reached the minimum value in terms of the finished product, including the energy consumption of 2.17kWh/kg, the residual water content of 7.45%, the anti-rehydration capacity of 8.69%, and the loss of total β-carotene and lycopene in Gac of 5.04%.

1. Introduction

Gac is a fruit rich in carotenoid, lycopene which belong to group vitamin A. The β-carotene and lycopene in Gac oil are higher twice more than liver mackerel and about 10 times more than carrots (Apinya Bhumsaidon, et al., 2016). Gac is a valuable fruit in the world because it only distributes in Southeast Asia and Southern China. Many studies confirmed the valuable nutriment of Gac (Anh Le, et al., 2018; A. L. Chai, et al., 2018) such as β-carotene, lycopene, vitamin E, Q, carbohydrates, unsaturated fatty acids, amino acids, mineral, and other microelements for human. The water content of Gac is 74.6%. Currently, Gac has not been popularly used like other fruits, and important nutritional sources of Gac have been untapped effectively (Betty K, et al., 2004; Anh V. Le., et al., 2018). The question is to find methods for processing and preservation of Gac to create valuable products (Haugvalstad. G. H., et al., 2005).

If Gac is dried for preservation purpose by conventional drying method, Gac can be completely destroyed its nutrients because this method is usually carried out at high temperatures (above 100^0C) (Heldman D. R, et al., 1992). If preservation of Gac is carried out at low temperature and low pressure (under points O (4.58mmHg; 0.0098^0C) by the freeze drying method, it can be completely kept natural characteristics of material (Khalloufi S, et al., 2004; Figura LO, et al., 2007) and the quality of products are very good. However, this method is not used widely because of high energy consumption (Dzung N.T, 2016c & 2018c; Dzung N.T, et al., 2018a & 2018b). Therefore, the application of cold drying technology for processing and preservation of Gac is potential method.

There have been a number of researches on establishing and solving the mathematical models of heat and mass transfer for the cold drying applied to many different types of

drying materials, which results in the determination of the kinetics of the cold drying process (Fortes, M., et al., 1999; Gebhart B., 1992). One of the most typical model was the one of Luikov A.V. (1972), from which Holman J., (1992), Vaccarezza.L.M. et al. (1994), they achieved the mathematical models of heat and mass transfer for the cold drying of specific drying materials. Mainly in these researches the kinetics of the cold drying process was focused to establish the technological parameters, but the assessment of the qualified products via the cold drying mode reaching the objectives such as minimum energy consumption or residual water content or the anti-rehydration capacity or the loss of total β-carotene and lycopene in Gac of finished products still remained unsolved (Khalloufi S, et al., 2004; Haugvalstad. G. H., et al., 2005; Figura LO, et al., 2007). The combination of those targets with the determination of the cold drying mode is the problem of multi-objective optimization. However, it is complicated to give an answer to the multi-objective optimization problem. As always, test of the multi-objective optimization problem is a set of optimal Pareto test, each application of different methods will give different optimal Pareto tests. There are currently numerous methods applied to this problem, namely linear combination (Dzung N.T, et al., 2016a), fuzzy data classification and Harrington method (Dzung N.T, 2016c), which reveal the subjective concept of the expert group on deciding the importance of each objective function. Therefore, the utopian point method S(Z) or the restricted area method R*(Z) based on Pareto optimization theory is necessarily replaced to obtain the results more objective (Dzung N.T, et al., 2016b).

According to Dzung et al., (2016a & 2016b), it is obvious that cold drying is a complicated technique because of depending on various technological factors. The determination of cold drying mode required the outputs to reach the minimal level (Fig. 1), including the energy consumption per weight (y_1, kWh/kg), the residual water content (y_2, %), the anti-rehydration capacity (y_3, %) and the loss of total β-carotene and lycopene in Gac (y_4, %) of the cold-dried product (finished product). It should be emphasized that those 4 outputs were affected by the 4 technological factors: temperature of moisture condensation (Z_1, ^{0}C), temperature of cold drying chamber (Z_2, ^{0}C), velocity drying agents (Z_3, m/s) and time of cold drying process (Z_4, h).

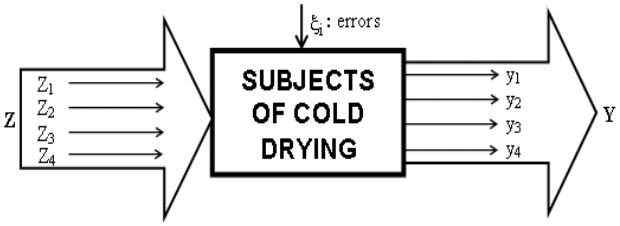

Figure 1. Diagram of cold drying subjects

However, the simultaneous consideration of all these outputs above to reach the minimal level resulted in the standard solution to multi-objective optimization problem (Dzung N.T, et al., 2011). This problem regularly appears in reality and in different fields. The answer to the multi-objective optimization problem was found in the case of the application of the R*(Z) optimal combination criterion (also known as the restricted area method) for the cold drying process of Gac. The multi-objective optimization results were used to establish the technological cold drying mode of Gac which was the closest to the utopian point but the furthest from the restricted area C, (Dzung N.T, et al., 2016a).

2.The fundamentals in multi-objective optimization

2.1. Basic concepts

The technological subjects including m objective functions $f_1(Z)$, $f_2(Z)$, ..., $f_m(Z)$ form the vector of these functions $f(Z) = \{f_j(Z)\} = \{f_1(Z), f_2(Z), ..., f_m(Z)\}$, where j = 1 to m. Every objective function $f_j(Z)$ is affected by n variables Z_1, Z_2, ..., Z_n which form the Z variable vector $Z = \{Z_i\} = (Z_1, Z_2, ..., Z_n)$, where i = 1 to n. These variables vary in the identified domain Ω_Z and the function values

form the domain of the objective function Ωf (in the two-objective optimization problem, the domain can be performed geometrically in the closed curve A–f(ZS)–f(Z)–B–N–M (Fig. 2), (Dzung N.T, et al., 2011).

Every objective function $f_j(Z)$ with Z variable vector $Z = \{Z_i\} = (Z_1, Z_2, ..., Z_n)$, where i = 1 to n, is considered as the one-objective optimization problem. Hence, the m-objective optimization problem can be simply transformed into the problem to find the minimum value for the set of m one-objective optimization problems (Dzung N.T, et al., 2011):

$$f_j\min = f_j(Z_1^{\,j\ opt}, Z_2^{\,j\ opt}, ..., Z_n^{\,j\ opt}) = \text{Min } f_j(Z_1, Z_2, ..., Z_n) \quad (1)$$

$$Z = \{Z_i\} = (Z_1, Z_2, ..., Z_n) \in \Omega_Z \quad (2)$$

$$\text{Where } j = 1 \text{ to } m;\ i = 1 \text{ to } n \quad (3)$$

The utopian plan and the utopian effect [1, 10]: If the variable vector $Z^{UT} = \{Z_i^{UT}\} = (Z_1^{UT}, Z_2^{UT}, ..., Z_n^{UT}) \in \Omega_Z$ is the test for all one-objective optimization problems (1) + (2) + (3), it means that $Z_i^{UT} = Z_i^{jopt}$ with i = 1 to n. Thus, Z_i^{UT} is called the utopian plan or the utopian test of the m-objective optimization problem.

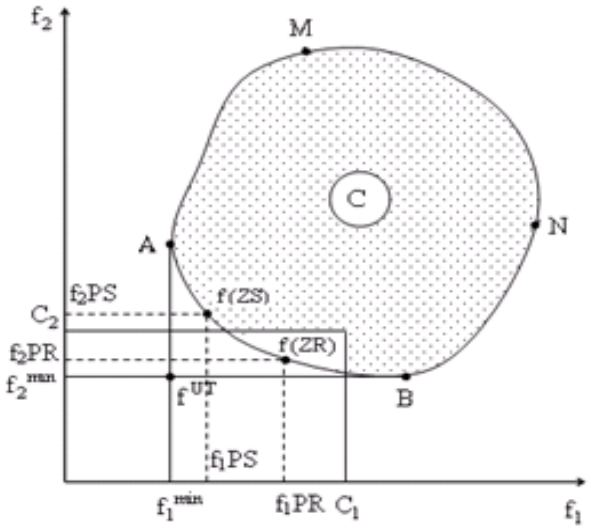

Figure 2. Dimension of objective functions of the two-objective optimization problem

In reality, Z_i^{UT} does not usually exist because it cannot satisfy all of the targets. However, every one-objective optimization problem (1) + (2) + (3) has its own $f_j$min (with j = 1 to m) respectively so $f^{UT} = (f_{1min}, f_{2min}, ..., f_{mmin})$ does exist. Then, $f^{UT} = (f_{1min}, f_{2min}, ..., f_{mmin})$ is called the utopian effect or the utopian point. According to Fig 2, the utopian point f^{UT} of the two-objective optimization problem exists but lies outside the identified domain Ω_f, i.e. the utopian test does not exist.

The dominant plan and the dominated plan [1, 10]: It is assumed that there are two variable vectors $ZQ = \{ZQ_i\}$ and $ZV = \{ZV_i\}$ with i = 1 to n. Then, there exist respectively two function vectors $f(ZQ) = \{f_j(ZQ)\}$ and $f(ZV) = \{f_j(ZV)\}$ with j = 1 to m.

If with all j: $f_j(ZQ) < f_j(ZV)$, ZQ is called the dominant plan (or the dominant test) over ZV, symbolizing: ZQ '>' ZV; and ZV is called the dominated plan (or the dominated test), symbolizing: ZV '<' ZQ,

The optimal Pareto plan (Dzung N.T, et al., 2011): The ZP plan is called the optimal Pareto plan in condition that ZP cannot be dominated by any other plans dependable on the identified domain Ω_Z. Then, f(ZP) would be called an optimal Pareto effect in the set of the optimal Pareto effects $\Omega_f P$. Fig 2 performs the set of the optimal Pareto effects $\Omega_f P$ as the curve A – f(ZS) – f(Z) – B.

Theorem 1 (Theorem Pareto): If the multi-objective optimization problem has the test which is the so-called optimal one according to some definition, this test received has to be the optimal Pareto plan without the dependence on the chosen definition (Dzung N.T, et al., 2011).

Therefore, one test of the multi-objective optimization problem (1) + (2) + (3) found by any method, to be recognized as the optimal by the method chosen, must in advance be certified as the optimal Pareto plan.

2.2.Multi-objective optimization by the utopian point method (UPM)

Considering the m-objective optimization problem (1) + (2) + (3): The optimal values f_{1min}, f_{2min}, ..., f_{mmin} can be determined after

solving each problems, and the fact that the utopian test (the test for the whole system) does not exist still identifies the utopian point $f^{UT} = (f_{1min}, f_{2min}, ..., f_{mmin})$. A S(Z) optimal combination criterion is defined by the following expression (Dzung N.T, et al., 2016b):

$$S(Z) = \sqrt{\left[\sum_{j=1}^{m} s_j^2(Z)\right]} = \sqrt{\left[\sum_{j=1}^{m} \left(f_j(Z) - f_{jmin}\right)^2\right]} \quad (4)$$

It is obvious that S(Z) is the distance from f(Z) to f^{UT}, where $s_j(Z) = |f_j(Z) - f_{jmin}|$. Choosing S(Z) optimal combination criterion as an objective function, the m-objective optimization problem are restated as: Find $ZS = (Z_1S, Z_2S, ..., Z_nS) \in \Omega_Z$ in order that the objective function S(Z) reaches the minimum value:

$$S_{min} = S(ZS) = \text{Min } S(Z) = \min\left\{\sqrt{\left[\sum_{j=1}^{m} \left(f_j(Z) - f_{jmin}\right)^2\right]}\right\} \quad (5)$$

$$Z = (Z_1, Z_2, ..., Z_n) \in \Omega_Z$$

Theorem 2: If ZS of the optimization problem (5) does exist, ZS is the optimal Pareto test of the m-objective optimization problem (1) + (2) + (3), (Dzung N.T, et al., 2016b).

Symbol: $f(ZS) = fPS = (f_1PS, f_2PS, ..., f_mPS)$. With the utopian point method (i.e. the m-objective optimization problem converts into the S optimal combination criterion), the optimal Pareto test ZS will be found to have the optimal Pareto effect f(ZS) = fPS closest to the utopian point $f^{UT} = (f_{1min}, f_{2min}, ..., f_{mmin})$. The case m = 2 (two objectives) is illustrated in Fig 2.

2.3.Optimizing the multi-objective functions by the restricted area method (RAM)

In fact, every objective function $f_j(Z)$ is restricted by the conditions set up by technology (Dzung N.T, et al., 2016a). Such as:

a) Case 1: The obligatory conditions

$$f_j(Z) < C_j, \forall j = 1 \div m, \ \forall Z \in \Omega_Z \quad (6)$$

From (6), the restricted area would be made:

$$C = \{f_j(Z) \geq C_j\}, \text{ with } f_j(Z) \quad (7)$$

The restricted area method suggests the solution to the m-objective optimization problem (1) + (2) + (3) by R*(Z) optimal combination criterion, defined as:

$$R^*(Z) = \sqrt[m]{r_1(Z).\, r_2(Z) ... \, r_m(Z)} = \sqrt[m]{\prod_{j=1}^{m} r_j(Z)} \quad (8)$$

With

$$r_j(Z) = \left(\frac{C_j - f_j(Z)}{C_j - f_{jmin}}\right) \text{ when } f_j(Z) < C_j \quad (9)$$

$$r_j(Z) = 0 \text{ when } f_j(Z) \geq C_j \quad (10)$$

According to (9), if $f_j(Z) \rightarrow f_{j\,min}$ and $\forall f_j(Z) < C_j$, $r_j(Z) \rightarrow r_{jmax} = 1$.

From (9), it can be seen: $0 \leq R^*(ZR) \leq 1$. If $R^*(ZR) = 1$, $ZR = Z^{UT}$ – the utopian test. If $R^*(ZR) = 0$, one of the values of $f_j(Z)$ violates (6), which means that $f_j(Z)$ belongs to the restricted area C (7).

b) Case 2: The obligatory conditions

$$C_{1j} < f_j(Z) < C_{2j}, \quad \forall j = 1 \div m, \ \forall Z \in \Omega_Z \quad (11)$$

From (11), the restricted area would be made:

$$C = \{f_j(Z) \geq C_{2j}; \ f_j(Z) \leq C_{1j}\}, \text{ with } f_j(Z) \in \Omega_f \quad (12)$$

The restricted area method suggests the solution to the m-objective optimization problem (1) + (2) + (3) by R*(Z) optimal combination criterion, defined as (8).

With

$$r_j(Z) = \left(\frac{C_{2j} - f_j(Z)}{C_{2j} - f_{jmin}}\right)\left(\frac{f_{jmin} - C_{1j}}{f_j(Z) - C_{1j}}\right)$$

when $C_{1j} < f_j(Z) < C_{2j}$ (13)

$$r_j(Z) = 0$$

when $f_j(Z) \geq C_{2j}$ or $f_j(Z) \leq C_{1j}$ (14)

According to (13), if $f_j(Z) \rightarrow f_{j\ min}$ and $\forall(C_{1j} < f_j(Z) < C_{2j})$, $r_j(Z) \rightarrow r_{jmax} = 1$.

By choosing R*(Z) as the objective function, the m-objective optimization problem is restated as:

Find $ZR = (Z_1R, Z_2R, ..., Z_nR) \in \Omega_Z$ in order that R*(Z) reaches the maximum value.

$$R^*_{max} = R^*(ZR) = \max\left\{R^*(Z)\right\} = \max\left\{\sqrt[m]{\prod_{j=1}^{m} r_j(Z)}\right\} \quad (15)$$

$\forall Z = (Z_1, ..., Z_n) \in \Omega_Z$

From (13), it can be seen: $0 \leq R^*(ZR) \leq 1$. If $R^*(ZR) = 1$, $ZR = Z^{UT}$ – the utopian test. If $R^*(ZR) = 0$, one of the values of $f_j(Z)$ violates (11), which means that $f_j(Z)$ belongs to the restricted area C (12).

Theorem 3: If the multi-objective optimization problem (15) has its own ZR test, this ZR test is also the optimal Paréto test of the m-objective optimization problem (1) + (2) + (3), (Dzung N.T, et al., 2016a).

Symbol: $f(ZR) = fPR = (f_1PR, f_2PR, ..., f_mPR)$. With the optimal ZR, the optimal Paréto effect $fPR = (f_1PR, f_2PR, ..., f_mPR)$ would be the closest to the utopian point and the furthest from the restricted area C.

3. Materials and methods

3.1. Materials

- The materials used for the cold drying experiments were mature Gac, mainly grown in Tay Nguyen area and Southeastern area of Vietnam (Anh Le, et al., 2018).

- Before the cold drying process, Gac was separated seeds and shells, the water content of Gac was 73.56%.

3.2. Apparatus

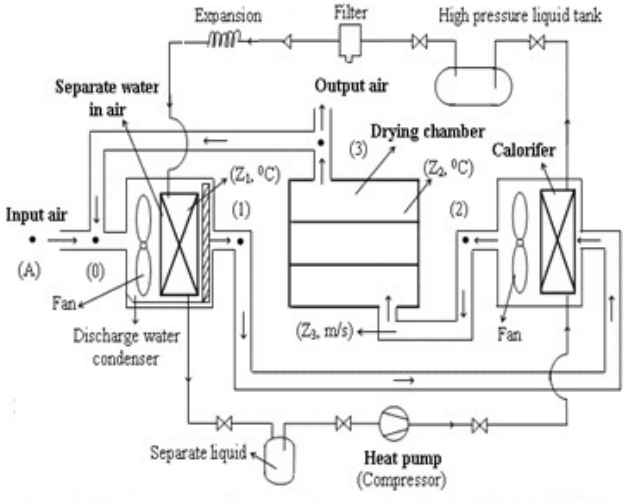

Figure 3. The diagram of cold drying DSL-02 system.

Figure 4. The cold drying DSL-02 system.

- The cold drying system DSL–02 controlled by PLC was used to dry Gac (Fig 3 and Fig 4), (Dzung N.T, et al., 2016a).

- Determining the weight of samples by Satoriusbasic Type BA310S and mass sensor with the range of 0 to 300g and the error of 0.1g.

- Determining the volume of samples by Cylinders with the range of 0 to 500ml and the error of 0.1g.

- Dual digital thermometer (T.P.34-23) and temperature sensor were used to determine the temperature of moisture condensation, the temperature of cold drying chamber during the cold drying process with the range of 0 to 100^0C and the error of 0.5°C.

- Determining time of the cold drying process by timer.

- Determining velocity drying agents by veloccity sensor (DMK-045) with the error of 0.01m/s.

- The equipment of High Performance Liquid Chromatography (HPLC) was used to determine the content of β-carotene and lycopene in Gac.

3.3. Methods

▪ Determining the energy consumption (y_1, kWh/kg product) for 1 kg finished product by Watt meter, (Dzung N.T, et al., 2016a).

$$y_1 = \frac{U.I.\tau.\cos\varphi}{G} \quad (16)$$

Where: G (kg) – weight of finished product; U (V) – number of Voltmeter; I (A) – number of Amperemeter; τ (s) – second; cosφ – power factor.

▪ Determining the residual water content of finished product (y_2, %) by the mass sensor controlled by PLC, (Dzung N.T, et al., 2016a).

$$y_2 = 100 - \frac{G_i}{G_e}(100 - W_i) \quad (17)$$

▪ Determining the anti-rehydration capacity of finished product (y_3, %) indirectly by IR (%), which was the rehydration capacity of the finished product: $y_3 = 100 - IR$, (Dzung N.T, et al., 2016a).

$$IR = \frac{G_1 - G_e}{G_i - G_e}.100\% \quad (18)$$

$$y_3 = 100 - IR = \frac{G_i - G_1}{G_i - G_e}100\% \quad (19)$$

Where: G_i (kg) – weight of initial material used for cold drying; G_e (kg) - weight of finished product; G_1 (kg) – weight of finished product which was soaked into the water at 25^0C until the constant mass (*the saturation of water content*); W_i (%) – initial water content of the material.

The ideal rehyration capacity of product means that the in-water content is equal to the out-water content of product, i.e. $G_1 = G_i$ and $IR_{max} = 1 = 100\%$, $y_{3min} = 0$. In fact, $y_3 > 0$, IR < 100%.

▪ Determining the loss of total β-carotene and lycopene in Gac of finished product (y_4, %) by HPLC method in TCVN 4715 – 90.

$$y_4 = \frac{m_1 - m_2}{m_1}100\% = \frac{\Delta m}{m_1}100\% \quad (20)$$

Where: y_4 – the loss of total β-carotene and lycopene in Gac after cold drying; m_1 and m_2 (mg%) – the total β-carotene and lycopene in Gac before and after cold drying, respectively. The fact that the product achieves the best quality means $y_{4min} = 0$. In fact, $y_4 > 0$.

▪ Orthogonal experimental planning method with degree 2 (Dzung N.T, et al., 2016a).

▪ Establishing and solving 4-objective optimization problem by the restricted area method (Dzung N.T, et al., 2016a).

4. Results and discussion

4.1. Establishing the constituent objective functions of the multi-objective problem

The constituent objective functions of the cold drying process of Gac (including: the energy consumption per weight (y_1, kWh/kg); the residual water content (y_2, %); the anti-rehydration capacity (y_3, %) and the loss of total β-carotene and lycopene in Gac (y_4, %) of the cold-dried product) depended on the technological parameters, including: temperature of moisture condensation (Z_1, ^{0}C), temperature of cold drying chamber (Z_2, ^{0}C), velocity drying agents (Z_3, m/s) and time of cold drying process (Z_4, h). The objective functions y_1, y_2, y_3 and y_4 were described by the experimental planning method with the degree-2 orthogonal experimental matrix. The experimental number was determined as:

$$N = n_k + n_* + n_0 = 2^k + 2k + n_0 = 25 \quad (21)$$

Where: k = 4; $n_k = 2^k = 2^4 = 16$; $n_* = 2k = 2x4 = 8$; $n_0 = 1$, carrying out 25 experiments.

These variables x_1, x_2, x_3, x_4 were coded variables of Z_1, Z_2, Z_3, Z_4 by the following expression:

$$x_j = (Z_j - Z_j^0)/\Delta Z_j \quad (22a)$$

where: $Z_j^0 = (Z_j^{max} + Z_j^{min})/2$;

$\Delta Z_j = (Z_j^{max} - Z_j^{min})/2$;

$Z_j^{min} \le Z_j \le Z_j^{max}$

The value of the star point:

$$\alpha = \sqrt{\sqrt{N.2^{(k-2)}} - 2^{(k-1)}} = 1.414 \quad (22b)$$

And conditions of the orthogonal matrix:

$$\lambda = \frac{1}{N}\left(2^k + 2\alpha^2\right) = 0.8 \quad (22c)$$

Mathematical model of objective functions was defined by the following expression [1, 2, 8, 10]:

$$y = b_0 + \sum_{j=1}^{k} b_j x_j + \sum_{j\neq i;j=1}^{k} b_{ji} x_j x_i + \sum_{j=1}^{k} b_{jj} x_j^2 \quad (23)$$

The experimental conditions established the parameter levels affecting the cold drying process shown in table 1.

Table 1. Parameter level design

Parameters	Z_1 (^{0}C)	Z_2 (^{0}C)	Z_3 (m/s)	Z_4 (h)
- α (-1.414)	7.93	32.93	1.17	9.17
Low -1	10	35	2	10
Central 0	15	40	4	12
High +1	20	45	6	14
+ α (1.414)	22.07	47.07	6.83	14.83
Deviation ΔZ_i	5	5	5	2

4.1.1. Establishing the mathematical model of objective functions y_1, y_2, y_3 and y_4

The experiments were carried out with all of the parameter levels in table 1 to determine the value of the objective functions. The result was summarized in table 2.

Table 2. The degree-2 orthogonal experimental matrix, k = 4, n_0 = 4, n = 28, α = 1.414, λ = 0.8

N		x_0	x_1	x_2	x_3	x_4	x_1x_2	x_1x_3	x_1x_4	x_2x_3	x_2x_4	x_3x_4	x_1^2-λ	x_2^2-λ	x_3^2-λ	x_4^2-λ	y_1	y_2	y_3	y_4
2^k	1	1	-1	-1	-1	-1	1	1	1	1	1	1	0.2	0.2	0.2	0.2	1.21	11.34	12.76	4.32
	2	1	1	-1	-1	-1	-1	-1	-1	1	1	1	0.2	0.2	0.2	0.2	1.31	10.34	11.63	4.65
	3	1	-1	1	-1	-1	-1	1	1	-1	-1	1	0.2	0.2	0.2	0.2	1.46	9.34	10.51	5.6
	4	1	1	1	-1	-1	1	-1	-1	-1	-1	1	0.2	0.2	0.2	0.2	1.32	8.73	9.82	5.24
	5	1	-1	-1	1	-1	1	-1	1	-1	1	-1	0.2	0.2	0.2	0.2	1.31	9.24	10.4	5.54
	6	1	1	-1	1	-1	-1	1	-1	-1	1	-1	0.2	0.2	0.2	0.2	1.53	8.56	9.63	5.14
	7	1	-1	1	1	-1	-1	-1	1	1	-1	-1	0.2	0.2	0.2	0.2	1.40	9.13	10.27	5.48
	8	1	1	1	-1	1	1	-1	1	-1	1	-1	0.2	0.2	0.2	0.2	1.71	9.83	11.06	5.9
	9	1	-1	-1	-1	1	1	1	-1	1	-1	-1	0.2	0.2	0.2	0.2	1.83	7.25	8.16	4.35
	10	1	1	-1	-1	1	-1	-1	1	1	-1	-1	0.2	0.2	0.2	0.2	1.92	8.04	9.05	4.82
	11	1	-1	1	-1	1	-1	1	-1	-1	1	-1	0.2	0.2	0.2	0.2	1.83	8.34	9.38	5.01
	12	1	1	1	-1	1	1	-1	1	-1	1	-1	0.2	0.2	0.2	0.2	2.09	6.34	7.13	4.81
	13	1	-1	-1	1	1	1	-1	-1	-1	-1	1	0.2	0.2	0.2	0.2	1.77	6.46	7.27	4.88
	14	1	1	-1	1	1	-1	1	1	-1	-1	1	0.2	0.2	0.2	0.2	1.87	7.46	8.39	4.48
	15	1	-1	1	1	1	-1	-1	-1	1	1	1	0.2	0.2	0.2	0.2	2.09	7.73	8.7	4.64
	16	1	1	1	1	1	1	1	1	1	1	1	0.2	0.2	0.2	0.2	2.14	5.89	7.43	6.23

2k	17	1	1.414	0	0	0	0	0	0	0	0	0	1.2	-0.8	-0.8	-0.8	1.43	7.46	8.39	4.48
	18	1	-1.414	0	0	0	0	0	0	0	0	0	1.2	-0.8	-0.8	-0.8	1.25	7.05	7.93	4.26
	19	1	0	1.414	0	0	0	0	0	0	0	0	-0.8	1.2	-0.8	-0.8	1.35	8.45	9.51	5.07
	20	1	0	-1.414	0	0	0	0	0	0	0	0	-0.8	1.2	-0.8	-0.8	1.31	8.31	9.35	4.99
	21	1	0	0	1.414	0	0	0	0	0	0	0	-0.8	-0.8	1.2	-0.8	1.84	7.56	8.51	4.54
	22	1	0	0	-1.414	0	0	0	0	0	0	0	-0.8	-0.8	1.2	-0.8	1.41	8.56	9.63	5.14
	23	1	0	0	0	1.414	0	0	0	0	0	0	-0.8	-0.8	-0.8	1.2	2.25	6.34	7.13	6.81
	24	1	0	0	0	-1.414	0	0	0	0	0	0	-0.8	-0.8	-0.8	1.2	1.61	8.02	9.02	4.81
n_0	25	1	0	0	0	0	0	0	0	0	0	0	-0.8	-0.8	-0.8	-0.8	1.53	7.55	8.49	4.53

The regression equations (24), (25), (26) and (27) were obtained after processing the experimental data, calculating the coefficients, testing the significance of the coefficients by the Student test, and testing the regression equations for the fitness of the experimental results by Fisher test [8]:

$$y_1 = 1.462 + 0.062x_1 + 0.067x_2 - 0.098x_3 + 0.43x_4 - 0.19x_1x_3 + 0.214x_1x_2 - 0.187x_2x_3 + 0.227x_2x_4 - 0.028x_3x_4 - 0.059(x_1^2 - 0.8) - 0.062(x_2^2 - 0.8) + 0.083(x_1^2 - 0.8) + 0.237(x_2^2 - 0.8) \quad (24)$$

$$y_2 = 8.133 - 1.325x_3 - 1.104x_1x_3 + 1.2x_1x_4 + 0.91x_2x_3 - 1.325x_2x_4 + 0.048(x_2^2 - 0.8) \quad (25)$$

$$y_3 = 9.15 - 1.49x_3 - 0.27x_1x_2 - 1.242x_1x_3 + 1.35x_1x_4 - 1.024x_2x_3 + 1.491x_2x_4 + 0.689(x_2^2 - 0.8) \quad (26)$$

$$y_4 = 4.978 + 0.106x_1 + 0.3x_2 - 0.396x_3 + 0.66x_4 - 0.7x_1x_3 + 0.81x_1x_4 - 0.83x_2x_3 + 0.634x_2x_4 - 0.14x_3x_4 - 0.29(x_1^2 - 0.8) + 0.44(x_4^2 - 0.8) \quad (27)$$

4.1.2. Establishing the mathematical model of multi-objective functions

The technological parameters (x_1, x_2, x_3 and x_4) of the cold drying process of Gac had the simultaneous impact on these objective functions (y_1, y_2, y_3 and y_4) with the identified domain $\Omega_x = \{-1.414 \le x_1, x_2, x_3, x_4 \le 1.414\}$. Thus, the four-objective optimization problem determining the technological cold drying mode of Gac was restated as: Finding in common the test $x = (x_1^{opt}, x_2^{opt}, x_3^{opt}, x_4^{opt}) \in \Omega_x = \{-1.414 \le x_1, x_2, x_3, x_4 \le 1.414\}$ in order that:

$$\begin{cases} y_1 = f_{1\min}(x_1^{opt}, x_2^{opt}, x_3^{opt}, x_4^{opt}) = \min f_1(x_1, x_2, x_3, x_4) \\ y_2 = f_{2\min}(x_1^{opt}, x_2^{opt}, x_3^{opt}, x_4^{opt}) = \min f_1(x_1, x_2, x_3, x_4) \\ y_3 = f_{3\min}(x_1^{opt}, x_2^{opt}, x_3^{opt}, x_4^{opt}) = \min f_1(x_1, x_2, x_3, x_4) \\ y_4 = f_{4\min}(x_1^{opt}, x_2^{opt}, x_3^{opt}, x_4^{opt}) = \min f_1(x_1, x_2, x_3, x_4) \\ \forall x = (x_1, x_2, x_3, x_4) \in \Omega_x \end{cases} \quad (28)$$

Where: $y_1 < C_1 = 2.5$;
$C_{21} = 5.2 < y_2 < 8.5 = C_{22}$;
$y_3 < C_3 = 10.5$;
$y_4 < C_4 = 4.5$ (29)

The establishment of the cold drying mode of Gac was based on factors including: economic, technicality and quality of the product obtained. Experimental results were obvious that: if the energy consumption for the production of 1 kg product was higher than 2.5kWh, it would increase the product price and difficult commercialization. If the anti-rehydration capacity of the final product was greater than 10.5%, Gac would be denatured, not able to recover the original of its quality. As a result, quality of product was reduced. If the loss of total β-carotene and lycopene in Gac of the final product was greater than 5.5%, natural color and flavor of Gac would be destroyed and nutritional value of product reduced. In addition, if the residual water content of the final product was greater than 8.5%, the microorganisms would be capable to grow, develop and damage products. On the other hand, if residual water content of final

product was less than 5.2%, the final product would be completely denatured. According to [6, 10], if the multi-objective optimization problem was solved by the utopian point method, value of the objective functions (y_1, y_2, y_3 and y_4) would not satisfy conditions (29) so the multi-objective optimization problem have to be solved by the restricted area method.

4.2. Solving the one-objective optimization problems

These one-objective optimization problems were found to achieve: $y_{1min} = miny_1(x_1, x_2, x_3, x_4)$; $y_{2min} = miny_2(x_1, x_2, x_3, x_4)$; $y_{3min} = miny_3(x_1, x_2, x_3, x_4)$; $y_{4min} = miny_4(x_1, x_2, x_3, x_4)$, with the identified domain $\Omega_x = \{-1.414 \leq x_1, x_2, x_3, x_4 \leq 1.414\}$. By the meshing method programmed in Matlab 7.0, the results of optimal parameters of every objective function (24), (25), (26) and (27) limited in the experimental domain were summarized in table 3.

Table 3. Minimum tests of each one-objective optimization problem (Material drying of Gac)

j	y_{jmin}	$x_1^{j\ opt}$	$x_2^{j\ opt}$	$x_3^{j\ opt}$	$x_4^{j\ opt}$
1	1.21	-1.000	-1.000	-1.000	-1.000
2	5.31	-1.414	1.414	1.414	1.414
3	7.03	-0.563	-0.987	0.856	1.023
4	4.13	-0.978	0.753	0.974	-0.907

According to the table 3, the utopian points were indentified: $y^{UT} = (y_{1min}, y_{2min}, y_{3min}, y_{4min}) = (1.21, 5.31, 7.03, 4.21)$. However, the utopian plan did not exist, because of $x^{jopt} = (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}, x_4^{jopt}) \neq x^{kopt} = (x_1^{kopt}, x_2^{kopt}, x_3^{kopt}, x_4^{kopt})$ where j, k = 1 to 4, and $j \neq k$.

4.3. Solving the multi-objective optimization problem by the restricted area method

The purpose of the experiment was to reach the target of cold drying process of Gac which was expressed by 4 regression equations (24), (25), (26) and (27) but the test satisfying all function values (y_{1min}, y_{2min}, y_{3min}, y_{4min}) could not be found. Hence, the idea of the four-objective optimization problem was to find the optimal Pareto test for the optimal Pareto effect $y(xR) = yPR = (y_1PR, y_2PR, y_3PR, y_4PR)$ closest to the utopian point $y^{UT} = (y_{1min}, y_{2min}, y_{3min}, y_{4min}) = (1.21, 5.31, 7.03, 4.21)$.

Establishing the R*-objective combination function $R^*(y_1, y_2, y_3, y_4) = R^*(x_1, x_2, x_3, x_4) = R^*(x)$ as the followings:

$$\begin{cases} R^*(x) = \sqrt[4]{r_1(x).r_2(x).r_3(x).r_4(x)} = \sqrt[4]{\prod_{j=1}^{4} r_j(x)} \\ \Omega_x = \{-1.414 \leq x_1, x_2, x_3, x_4 \leq 1.414\} \end{cases} \quad (30)$$

Where: with conditions (29), thus $r_1(x)$, $r_2(x)$, $r_3(x)$ and $r_4(x)$ can be established as (9) and (13):

$r_1(x) = (2.5 - y_1)/(2.5 - y_{1min})$ when $y_1 < 2.5$;
$r_1(x) = 0$ when $y_1 \geq 2.5$
$r_2(x) = [(8.5-y_2)/(8.5-y_{2min})][(y_{2min}-5.2)/(y_2 - 5.2)]$ when $5.2 < y_2 < 8.5$
$r_2(x) = 0$ when $y_2 \leq 5.2$ or $y_2 \geq 8.5$
$r_3(x) = (10.5 - y_3)/(10.5 - y_{3min})$ when $y_3 < 10.5$
$r_3(x) = 0$ when $y_3 \geq 10.5$
$r_4(x) = (5.5 - y_4)/(5.5 - y_{4min})$ when $y_4 < 5.5$
$r_4(x) = 0$ when $y_4 \geq 5.5$

With: y_{1min}, y_{2min}, y_{3min}, y_{4min} were showed in Table 3

The four-objective optimization problem needed to indentify $xR = (x_1R, x_2R, x_3R, x_4R) \in \Omega_x$ in order that $R^*(x_1R, x_2R, x_3R, x_4R) = Max\{R^*(x_1, x_2, x_3, x_4)\}$. The maximum value of (30) was determined by the meshing method programmed in Matlab 7.0:

$R^*_{max} = Max\{R^*(x_1, x_2, x_3, x_4)\} = 0.671$

With: $x_1R = -1.034$;
$x_2R = 0.835$;
$x_3R = -0.271$;
$x_4R = 0.182$;

Then, transforming into real variables:

$Z_1^{opt} = 9.83\ ^0C$;
$Z_2^{opt} = 44.18\ ^0C$;
$Z_3^{opt} = 3.46$ m/s;
$Z_4^{opt} = 12.36$ h;

Substituting x_1R, x_2R, x_3R, x_4R into these equations (24), (25), (26) and (27), the optimal Pareto effect was obtained as: y_1PR = 2.09; y_2PR = 7.86; y_3PR = 8.997; y_4PR = 5.07;

The rehydration capacity of the cold-dried product was determined as:

IR = 100 - y_3PR = 100 – 8.997 = 91.003%

By the restricted area method, solving the multi-objective optimization problem with R*-Optimal combination criterion which satisfied the maximum R*-Optimal combination criterion (R^*_{min} = 0.671) was determined the optimal Pareto test (or the technological mode of cold drying process of Gac) as: temperature of moisture condensation was Z_1^{opt} = 9.83^0C, temperature of cold drying chamber was Z_2^{opt} = 44.18^0C, the velocity drying agents was Z_3^{opt} = 3.46m/s and the time of cold drying process was Z_4^{opt} = 12.36h. Corresponding with the optimal Pareto test was determined the optimal Pareto effect as: the energy consumption per weight of 1 kg finished product was y_1PR = 2.09kWh/kg; the residual water content of the finished product was y_2PR = 7.86%; the anti-rehydration capacity of the finished product was y_3PR = 8.997% (Correspondingly IR = 91.003%) and the loss of total β-carotene and lycopene in Gac of the finished product was y_4PR = 5.07%.

Compared with the experimental results from the table 3, these results above were suitable and satisfy with the objectives of the problem.

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

Carrying out the cold drying process of Gac at the optimal Pareto test: temperature of moisture condensation of Z_1^{opt} = 9.83^0C, temperature of cold drying chamber of Z_2^{opt} = 34.18^0C, the velocity drying agents of Z_3^{opt} = 3.46m/s, and the time of cold drying process of Z_4^{opt} = 12.36 hours, the experimental results were determined as: The energy consumption per weight of y_1 = 2.16kWh/kg; the residual water content of y_2 = 7.45%; the anti-rehydration capacity of y_3 = 8.69% (or the rehydration capacity of IR = 100 – y_3 = 91.31%) and the loss of total β-carotene and lycopene in Gac of y_4 = 5.04% of the cold-dried product. Consequently, it was very noticeable that the results from the optimization problems of cold drying process of Gac had the approximation to the experimental results.

Figure 5. Gac was dried by the cold drying method

After Gac was dried by the cold drying method at the optimal Pareto test: Z_1^{opt} = 9.83^0C; Z_2^{opt} = 44.18^0C; Z_3^{opt} = 3.46m/s; Z_4^{opt} = 12.36h. The finished product obtained could be seen in Fig 5.

It was certain that the optimal Pareto test and the optimal Pareto effect of the multi-objective optimization problem of cold drying process of Gac could be possibly applied to determine the technological mode of cold drying process of Gac for using in the industry.

When the velocity air or drying agents and the time of cold drying process were fixed: x_3 = -0.271 and x_4 = 0.182, correspondingly Z_3 = 3.46m/s and Z_4 = 12.36h, the relationship between y_1, y_2, IR = 100 - y_3, and y_4 with 2 variables x_1, x_2 was performed geometrically in 3D (Fig 6, 7, 8, 9).

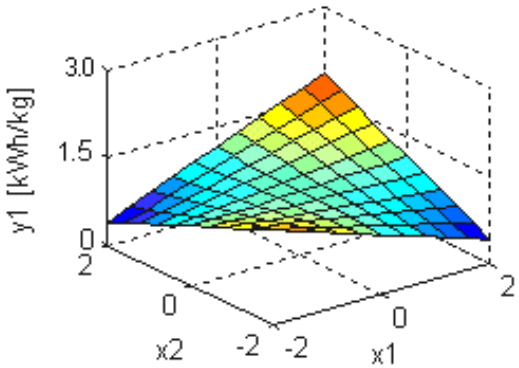

Fig 6. The energy consumption, x3 =-0,271 and x4 = 0.182

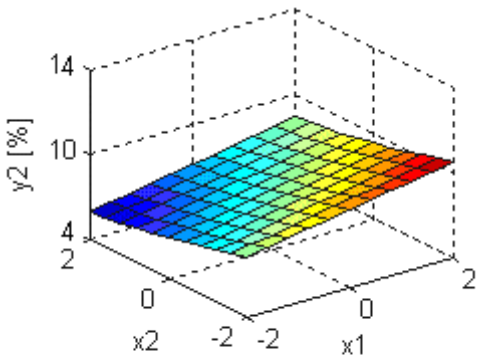

Fig 7. The residual water content of product, x3 = -0.271 and x4 = 0.182

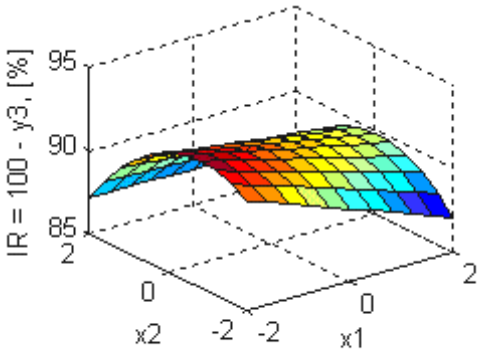

Fig 8. The rehydration capacity of product, x3 = -0.271 and x4 = 0.182

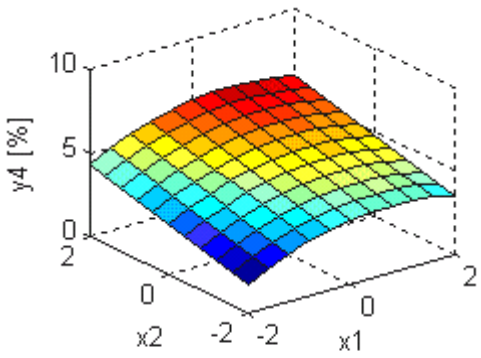

Fig 9. The loss of total β-carotene and lycopene of product, x3 = -0.271 and x4 = 0.182

5. Conclusions

The mathematical models (24), (25), (26) and (27) obtained from the experiments were the experimental statistical models which could well describe the impact of temperature of moisture condensation, temperature of cold drying chamber, velocity air or drying agents and time of cold drying process on the energy consumption per weight, the residual water content, the anti-rehydration capacity, and the loss of total β-carotene and lycopene in Gac of the cold-dried product.

Solving multi-objective optimization problem by the restricted area method determined the optimal technological mode of cold drying process of Gac, with temperature of moisture condensation of 9.83^0C, temperature of cold drying chamber of 44.18^0C, the velocity drying agents of 3.46m/s and the time of cold drying process of 12.36h, resulting in: the energy consumption per weight of the cold-dried product of 2.09kWh/kg; the residual water content of the cold-dried product of 7.86%; the anti-rehydration capacity of the cold-dried product of 8.997% (or rehydration capacity of the cold-dried product of 91.003%) and the loss of total β-carotene and lycopene in Gac of the cold-dried product of 5.07 %.

Therefore, the technological mode of cold drying process of Gac was feasible to be applied to industrial production.

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ICE CREAM WITH ORGANIC KAVILCA (BUCKWHEAT) FIBRE: MICROSTRUCTURE, THERMAL, PHYSICOCHEMICAL AND SENSORY PROPERTIES

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Sensory properties

ABSTRACT

In this study, the effects of functionality of Kavılca fibre on ice cream physicochemical and sensory characteristics were evaluated. The supplemented ice cream samples were found reduced number of intercellular space and diameters because of the existence of filamentous extensions surrounding the protein network. According to DSC, glass transition (T_g'), freezing point (T_f), melting (T_m'), and melting point (T_m) values demonsrated a significant decrease with the rising of fibre concentration. In general assessment, the results have been revealed that Kavılca fibre can improve the quality and sensory properties of ice cream and can be used as a fat substitute.

1. Introduction

Wheat is one of the most widely used cultural plants in human nutrition throughout the world, and besides, it constitutes the basis of basic nutrients in our country and in the other developing countries. There are different varieties of wheat caused by the variations in the number of its chromosomes. In contrast to bread wheat (*Triticum aestivum*), which is used widely today, *Triticum dicoccum* variety is the wheat variety that was consumed widely until the 19th century and is known as "Emmer", "Kavılca", and "Gernik." Kavılca is an ancient wheat variety that has been cultivated for centuries and that is becoming extinct. It is also known as Kavılca, Kabluca, and Yaban Wheat in the Kars region of Turkey (English: Emmer, Latin: *Triticum dicoccum*, Italian: Ferro). This variety, one of the ancestors of wheat, has many properties that are different from those of the modern wheat varieties. Kavılca is also known as “buckwheat” (Hayıt and Gül, 2005; Bilgiçli, 2009). Buckwheat (*Fagopyrum esculentum* Moench.), which belongs to the family Polygonaceae, is an annual plant and it is known that it resembles grains regarding its chemical compound and usage characteristics. It consists of dietary fibre, essential polyunsaturated fatty acids (PUFAs), vitamins (B1, B2, and B6), and antioxidant compounds such as routine and quercetin, and minerals (P, K, Mg, and Fe). Because buckwheat has high nutrient quality, it has a great potential in food industry in terms of functional food production (Dizlek et al., 2009). The incorporation of buckwheat fibre which is rich in fibre content can be included in dairy technology such as ice cream industry as an ingredient to be used in the manufacture of new functional products with high nutritional value and distinct flavour and aroma.

Gluten is excluded from the diet of coeliac patients as it causes serious bowel symptoms

(Hayıt and Gül, 2005; Torbica et al., 2010). Unfortunately, their sole treatment process is seriously sticking to a diet that does not include gluten throughout their whole lives. We define a diet free of wheat, barley, and rye flour as a gluten-free diet (Ciclitira et al., 2005; Urgancı, 2005; Türksoy and Özkaya, 2006). Buckwheat is a food component that meets an ideal gluten-free diet for gluten patients since this variety of wheat does not include gluten (Hayıt and Gül, 2005; Bilgiçli, 2009). Using this wheat, can be a good substitute for the ingredients being used in gluten-free products.

In conclusion, because we thought that Kavılca, an ancient fat variety of wheat, should be kept under protection and its production should be expanded and supported by scientific studies, we produced ice cream with the fibre that we obtained in our study. We aimed to find out how it affected the quality, thermal properties, nutritional and rheological properties of ice cream, and to reveal its profile map of physical and sensorial properties such as increased in volume and melting.

2. Materials and Methods

2.1. Materials

Raw cow milk and cream were purchased from Balacan Farm landing Erzincan province, Turkey and sugar, salep, skim milk powder and emulsifier (mixture containing mono and diglycerides of fatty acids) were obtained from local markets. The organic Kavılca wheat was fetched from a local production at Hacıpiri village of Akyaka district of Kars in Turkey and fibre was produced using a traditional milling machine according to the modified methods of Bauer et al. (2012).

2.2. Physicochemical composition of dietary fibre

Moisture and ash content were determined by the methods described in Martinez et al. (2012) and AACC (1995), respectively. The fibre pH was determined using a pH meter (OHAUS starter 3000; Vyncke, 1981). The contents of consisting of soluble dietary fibre (SDF), total dietary fibre (TDF) and insoluble dietary fibre (IDF) were found using enzymatic method (AACC, 2009).

2.3. Ice cream samples processing

The ice cream samples were produced in the Sofra patisserie, Erzincan, Turkey. First, fat contents of mixes were standardized to 5% by adding cream. The formulation of ice cream mix consisted of skimmed milk powder 4.7%, sugar 18%, a stabiliser salep; 0.6%, emulsifiers 0.2% and Kavılca fibre (KF) added separately at 0.5%, 1%, 1.5% and 2% ratios to experimental groups except control sample. The mixtures were pasteurized at 85^{o}C for 20 min and stored at 4^{o}C for 24 h and the mixes were frozen using an ice cream freezer (Ugur Cooling Machineries Co., Nazilli, Turkey) to the ice cream production. Later each experimental sample was packaged and stored at -22^{o}C. The production and flow chart of the ice cream samples are presented in Figure 1 and 2, respectively

Figure 1. a) organic Kavılca wheat; **b)** flour was produced using a traditional milling machine; **c)** fibre obtained from organic Kavılca; **d)** production of mix; **e)** mix; **f)** production of ice cream.

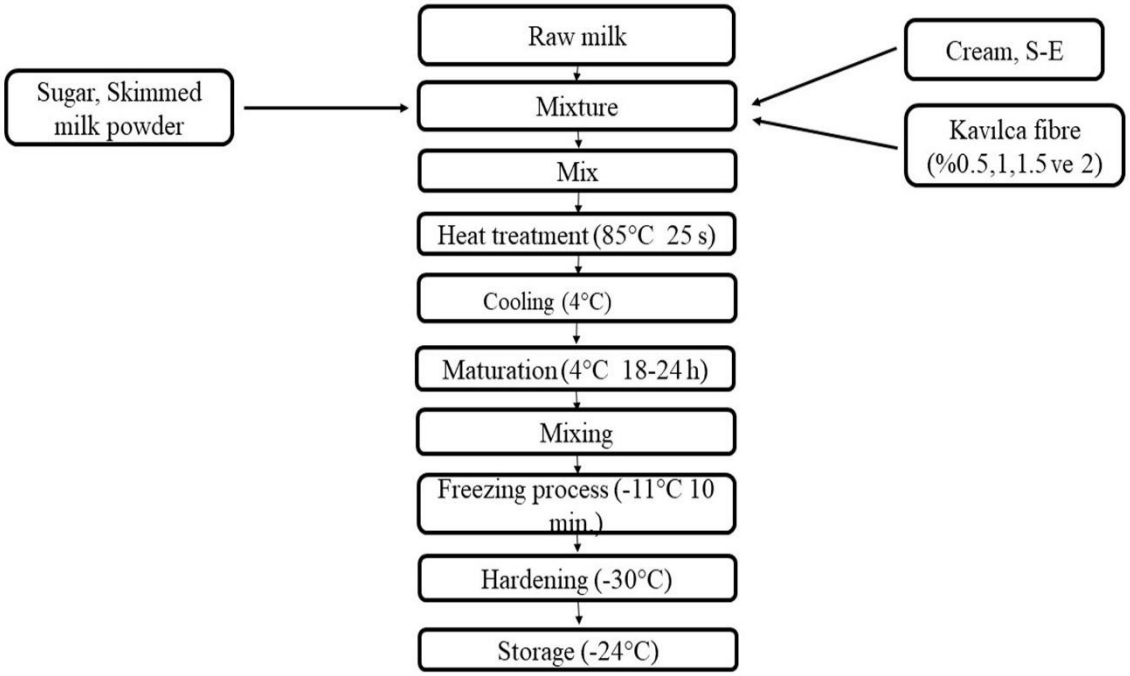

Figure 2. The flow chart of the ice cream samples

2.4. Physicochemical analysis of sample treatments

The moisture, acidity, fat and ash were analyzed according to Kurt et al. (2007) method and pH was measured using a pH metre (Ohaus starter 3000). The apparent viscosity was measured using a digital Brookfield Viscometer, Model DV-I (Brookfield Engineering Lab- Inc, USA; Akın et al., 2007) and overrun were determined in ice cream samples according to the Marshall and Arbuckle (1996) and Güven and Karaca (2002) methods.

2.5. Microstructure analysis

Microstructure analysis in ice cream samples was carried out using Scanning Electron Microscopy (SEM; FEI, Quanta FEG 450) according to the modified methods of Akalın et al. (2012). The samples were weighed aluminium tubes (inner diameter 1.16 mm, length 30 mm) as 0.3 g and the aluminium tube was pushed twice into the ice cream approximately 1–2 cm below the surface. The measurements were recorded with magnification 5000 and 30.000 at 15 kV under low vacuum condition.

2.6. DSC measurements

DSC measurements of thermograms were obtained with using EXTAR DSC 7000 equipped. Aliquots (approximately 15 mg) of each sample were sealed into aluminium pans and then loaded onto the DSC instrument. The samples were prepared following methodology adapted from Blond (1994), and it was applied including this steps: (i) cooling to -80 °C at 10 °C/min, (ii) heating from -80 to -40 °C and annealing at the same temperature for 30 min to promote maximal ice formation, (iii) cooling to -80 °C at 10 °C/min and isothermal holding for 5 min and (iv) heating from -80 to 20 °C at 5 °C/min.

2.7. Sensory evaluation

Sensory properties were carried out in ice cream samples after 1 week of storage according to Lawless and Heymann (2010) and the evaluation was performed by 20 panelists. The 9-point hedonic scale that consisted of points between 1 (dislike extremely) and 9 (like extremely) was used for the specified sensory attributes which were resistance to melting, body and texture, cream aroma, colour, taste and aroma, mouthfeel, gumming structure, iced structure and general acceptability.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 22.0 (IBM Corp., Released 2013). Data were analyzed by analysis of variance (ANOVA) and the differences between means by the ANOVA and Duncan's Multiple Range Test were used to determine significant differences among the results.

3. Results and Discussion

The chemical composition of milk is used to produce Kavılca fibre ice cream was as follows: titratable acidity 0.17% (±0.00) as lactic acid (L.A.), pH 6.37 (±0.03), total solids 11.01% (±0.01), fat 3.09% (±0.00), protein 2.88% (±0.02) and ash 0.66% (±0.02).

Table 1. The gross chemical and physical properties of Kavılca fibre

Analysis	Kavılca fibre
Dry matter (%)	93.72±0.50
pH	6.44±0.01
Ash (%)	2.94±0.00

Total dietary fibre (g/100 g)	50.19±0.79
Insoluble dietary fibre (g/100 g)	46.41±0.55
Soluble dietary fibre (g/100 g)	3.40±0.24

*n.d. -not determine

3.1. Physicochemical qualities of dietary fibres

As can be seen in Table 1, dry matter, pH, ash, total dietary fibre, insoluble dietary fibre and soluble dietary fibre of its Kavılca fibre were found as 93.72±0.50, 6.44±0.01, 2.94±0.00, 50.19±0.79, 46.41±0.55 and 3.78±0.24 g/100 g respectively.

This result accords with the findings of Zhao et al. (2017) who reported that the ice cream samples with flour obtained from yacon (*Smallanthus sonchifolius*) total dietary fibre, insoluble dietary fibre and soluble dietary fibre contents to be 52.02, 39.99 and 4.84 g/100 g respectively.

Our results are supported by the report with previous findings (Prosky et al., 1988; Yılmaz, 2005; Yangılar, 2015a; 2015b).

3.2. Physicochemical of ice cream samples

The average values of chemical analysis results of experimental ice cream samples and Duncan multiple test comparison results were presented in Table 2.

Table 2. The effect of different Kavılca fibre concentrations on some physicochemical properties of ice creams (mean±SD)

Ice cream samples	Total solids (%)	Fat (%)	Titratable acidity (%)	pH	Ash (%)
C1	31.95±0.24^{a}	5.42±0.03^{c}	0.16±0.00^{a}	6.73±0.01^{b}	1.43±0.07^{a}
C2	32.17±0.19^{ab}	5.36±0.09^{bc}	0.18±0.00^{a}	6.69±0.01^{a}	1.49±0.01^{ab}
C3	32.58±0.09^{bc}	5.17±0.04^{abc}	0.20±0.01^{ab}	6.71±0.01^{a}	1.51±0.06^{ab}
C4	32.88±0.08^{c}	5.12±0.24^{ab}	0.21±0.00^{cd}	6.70±0.00^{a}	1.57±003^{ab}
C5	32.95±0.26^{c}	4.91±0.02^{a}	0.23±0.01^{d}	6.68±0.01^{a}	1.62±0.01^{c}

C1, control (without KF); C2, 0.5% (w/w) KF added; C3, 1% (w/w) KF added; C4, 1.5% (w/w) KF added and C5, 2% (w/w) KF added. *Means within the same column with different letters are significantly different ($p<0.05$).

According to the results of variance analysis, the ice cream samples were found to be statistically significant at $p<0.01$ on the examined properties (dry matter, fat, titration acidity, pH, and ash). In their studies in which they produced probiotic ice cream using apple, orange, oat, bamboo, and wheat fibres, Akalın et al. (2018) found that fibre contents affected dry matters of the samples positively and caused them to increase. The average values of the chemical analysis results of the experimental ice cream samples were given in Table 2. According

to the results of the variance analysis, the ice cream samples were found to be statistically significant at $p<0.01$ on the examined properties (dry matter, oil, titration acidity, pH, and ash). When the values of dry matter and ash were compared, the samples were ordered as C1 <C2 < C3 < C4 < C5. Parussolo et al. (2017) reported that the amounts of dry matter and ash of the ice cream samples that they produced using the flour obtained from yacon *(Smallanthus sonchifolius)* increased depending on the increased in concentration.

Regarding the amounts of fat in the ice cream samples, it was identified that the lowest amount of fat was in C5 coded sample (4.91%) and the highest amount of fat was in C1 coded sample (5.42%). Dervişoğlu and Yazıcı (2006) found the fat contents of the ice cream samples that they produced using different concentrations of fibres they obtained from citrus rind to be 6.73% with 0.4% concentration, 6.63% with 0.8% concentration, and 6.40% with 1.2% concentration. The results of the researchers were higher than those of us, but they also found that the ice creams that we produced using fibre caused a decrease in fat content depending on the increased in concentration just like in this study.

As shown in the Table 2, the increased in the fibre concentration statistically increased the titration acidity of the ice cream samples depending on the rate of increased ($p<0.05$). Çakmakçı et al. (2015) reported that the titration acidity values increased in the ice cream samples that they produced using different concentrations of oleaster flour and their studies were consistent with our results. Fluctuations were observed in the pH values of the ice cream samples with fibre addition; however, variations between the samples could not maintain their statistical significance. The highest pH value was found to be 6.73 in the C1 coded sample and the lowest pH value was 6.68 in the C5 coded sample. According to the results of Duncan multiple comparison tests, the samples were ordered C1 < C2 < C3 < C4 < C5 in terms of the amounts of ash.

3.3. Thermal characteristics

The effect of Kavılca fibre concentration on the melting and glass transition temperatures of the ice cream samples were given in Table 3 and the effect of Kavılca fibre on the freezing point temperature and melting point temperature of the ice cream samples were given in Table 4. It was detected that the glass transition temperature and the melting temperature decreased depending on the increased in the concentration when the data that were obtained using DSC device were examined in order to investigate the thermal properties of the ice cream samples that were enriched by using fibre in different concentrations.

Table 3. The effect of Kavılca fibre concentration on the glass transition and melting temperatures of ice creams (mean±SD)

Ice cream samples	T'_g (glass transition temperature)			T'_m (melting temperature)		
	Onset (°C)	Midpoint (°C)	Offset (°C)	Onset (°C)	Midpoint (°C)	Offset (°C)
C1	-51.87±0.11^{b}	-46.90±0.52^{b}	-41.59±2.07^{b}	-36.60±1.68^{d}	-31.46±0.10^{d}	-27.57±0.91^{c}
C2	-57.44±0.00^{e}	-57.21±0.01^{d}	-53.24±0.05^{c}	-35.70±0.00^{cd}	-30.89±0.13^{d}	-27.62±0.02^{c}
C3	-56.32±0.02^{d}	-55.03±0.04^{c}	-53.22±0.31^{c}	-30.54±0.76^{b}	-29.02±0.03^{c}	-28.78±0.01^{c}

C4	-52.18±0.06^{c}	-46.79±0.07^{b}	-41.04±0.40^{b}	-32.21±0.68^{bc}	-27.80±0.28^{b}	-21.34±1.35^{b}
C5	-42.20±0.04^{a}	-41.86±0.26^{a}	-32.89±1.53^{a}	-14.76±1.73^{a}	-11.38±0.26^{a}	-11.04±0.67^{a}

C1, control (without KF); C2, 0.5% (w/w) KF added; C3, 1% (w/w) KF added; C4, 1.5% (w/w) KF added and C5, 2% (w/w) KF added. *Means within the same column with different letters are significantly different ($p<0.05$).

As we can see in Table 3, the highest T_g' values were found in the sample C5 and the samples C1, C4, C3, and C2 followed it respectively. When the results were evaluated from a statistical point of view, the effect of fibre addition on the T_g' and T_m' values of the samples were found to be significant ($p<0.01$) and decreases in glass transition and melting temperatures were detected as the fibre concentration increased. Yüksel (2015a) found that the T_g' and T_m' values of the ice cream samples he produced using different concentrations of blackthorn (*Prunus spinosa*) were parallel to our results. The researcher commented that the decrease in T_g' and T_m' values could be due to the fact that the water content in the ice cream samples had been frozen. Our study results are consistent with the results of the DSC analysis of the ice cream samples that Soukoulis et al. (2009) produced using wheat fibre.

Table 4. The effect of Kavılca fibre concentration on the freezing point temperature and elting point temperature of ice creams (mean±SD)

Ice cream samples	**Initial freezing point T_f (°C)**	**Heat of ice freezing (enthalpy) ΔH (J/g)**	**Initial melting point T_m (°C)**	**Heat of ice melting (enthalpy) ΔH (J/g)**
C1	-12.42±0.65^{b}	128±16.97^{bc}	-2.10±0.43^{a}	109±15.55^{a}
C2	-10.53±0.04^{a}	124±1.41^{ab}	-3.02±0.03^{b}	117±2.82^{a}
C3	-14.50±0.00^{d}	145.50±0.70^{c}	-2.40±0.14^{ab}	94.20±1.13^{a}
C4	-13.43±0.51^{cd}	138.5±2.12^{bc}	-2.59±0.06^{ab}	101.50±2.12^{a}
C5	-10.60±0.77^{a}	104.5±0.70^{a}	-2.36±0.44^{ab}	100.65±4.73^{a}

C1, control (without KF); C2, 0.5% (w/w) KF added; C3, 1% (w/w) KF added; C4, 1.5% (w/w) KF added and C5, 2% (w/w) KF added. *Means within the same column with different letters are significantly different ($p<0.05$).

In Table 4, the highest T_f value was found in the sample C2 and the lowest in the sample C3; and the highest T_m value was found in the C1 and the lowest in the sample C2. The freezing point and the melting point temperatures of the ice cream samples were found statistically significant ($p<0.01$). The results show that the density and composition of fibre might have affected the molecular water of the ice cream.

3.4. Microstructure

Identification of microstructures of foods is important in terms of dimensional regulation of identifiable components that constitute the food, understanding of their interaction with each other, and providing information about their

physical conditions. When we examine a product such as ice cream from a technological point of view, we need to emphasize that the microstructure of a product is important in order to realize a production with the desired textural and sensory properties. The microstructures of the fibre and ice cream samples that were obtained using Scanning Electron Microscope (SEM) are seen in Figure 3. We determined that the microstructure of the fibre was distributed in the gel homogeneously. Sánchez-Alonso et al. (2006) also detected a result of the microstructure of a similar wheat fibre. When we examine the shapes of the ice cream samples, it is obvious that there are significant structural variations between the control sample and the samples containing Kavılca fibre. We also see the result that there are more serum phases and gaps in the control sample in the control groups of the studies conducted by Sandoval-Castilla et al. (2004) and Ramirez-Santiago et al. (2010). However, it was determined that filamentous extensions and serum phases existed in the samples that Kavılca fibre was added into, but the number of gaps and their diameters decreased.

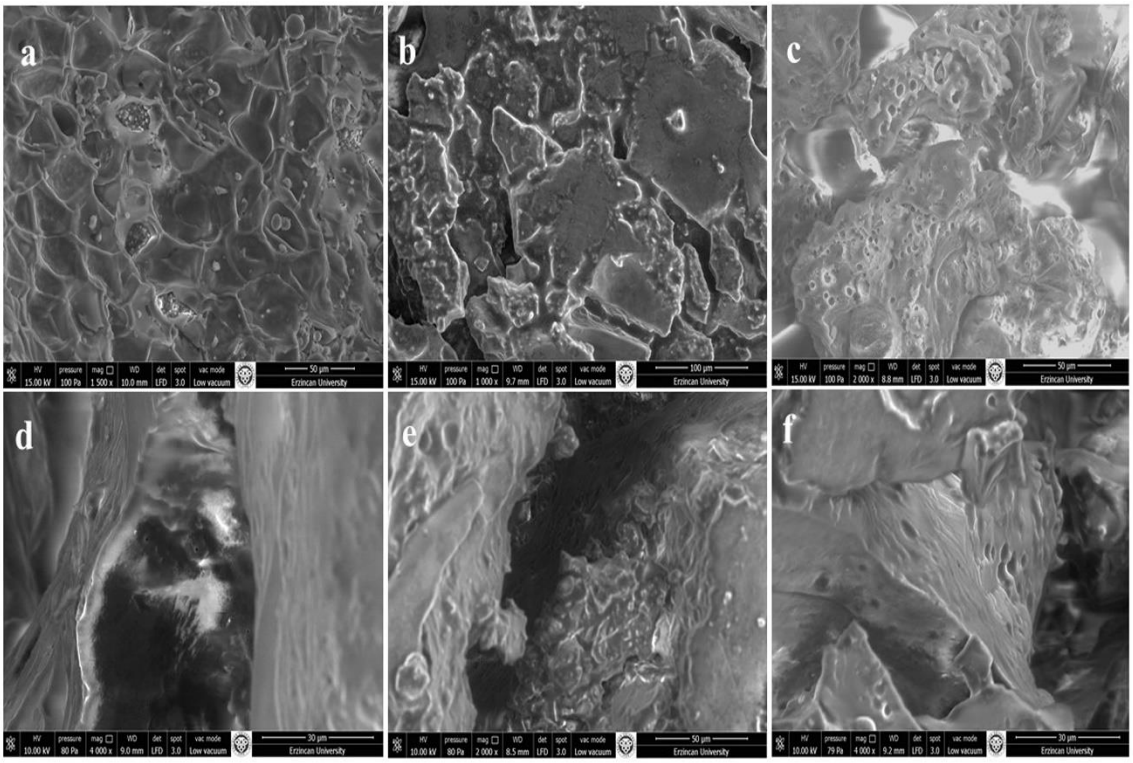

Figure 3. Scanning electron micrographs of ice cream samples and fibre. Labelled arrows: **a.** fibre; **b.** control; **c.** 0.5% (w/w) Kavılca fibre added; **d.** 1% (w/w) Kavılca fibre added, **e.** 1.5% (w/w) Kavılca fibre added, **f.** 2% (w/w) Kavılca fibre added.

In their studies, Soukoulis et al. (2009) reported that the samples with oat and wheat fibres affected viscosity significantly among the ice cream samples that they produced using apple, oat, wheat fibres and inulin, and a tougher structure was formed; and they reported that this was probably because of undissolved materials bound the water. They also reported that the serum phase of the microstructures of the samples containing 4% oat and wheat fibres decreased by means of the fibres that held the water and this caused an increased in viscosity. They detected that the fibres that were comprised of polysaccharides contributed to the effect of apple fibre on viscosity and viscosity increased 3-15 times the control sample. The

researchers reported that the fact that ice creams with apple fibre do not have granular structures, but they contain much pectin had a positive effect on viscosity in consequence of the examination of their microstructures. They already reported in their previous studies that pectin was an anionic hydrocolloid that could interact with positive charges on protein surface (Soukoulis et al. 2007).

Akalın et al. (2018) produced probiotic ice cream using different dietary fibres (apple, orange, oat, bamboo, and wheat) and investigated their microstructures. When they compared the ice cream samples that were produced using serum phase of the control samples and dietary fibre, they found that it was more watery and they reported that only the sample with wheat fibre was effective on the increased in viscosity ($p<0.05$). They reported that apple and orange fibre added ice cream samples were different from the ice creams that were produced using bamboo, oat, and wheat fibre in terms of granular structure. However, they found that a significant increased occurred in viscosity in also the samples produced using apple and orange fibre ($p<0.05$).

3.5. Viscosity analysis

The data on the results of viscosity analysis of the experimental ice cream samples were given in Figure 4. Regarding the values that were obtained at 20 and 50 rpm, it was observed that the sample C5 had the highest viscosity value, and the group C1 (control group) had the lowest.

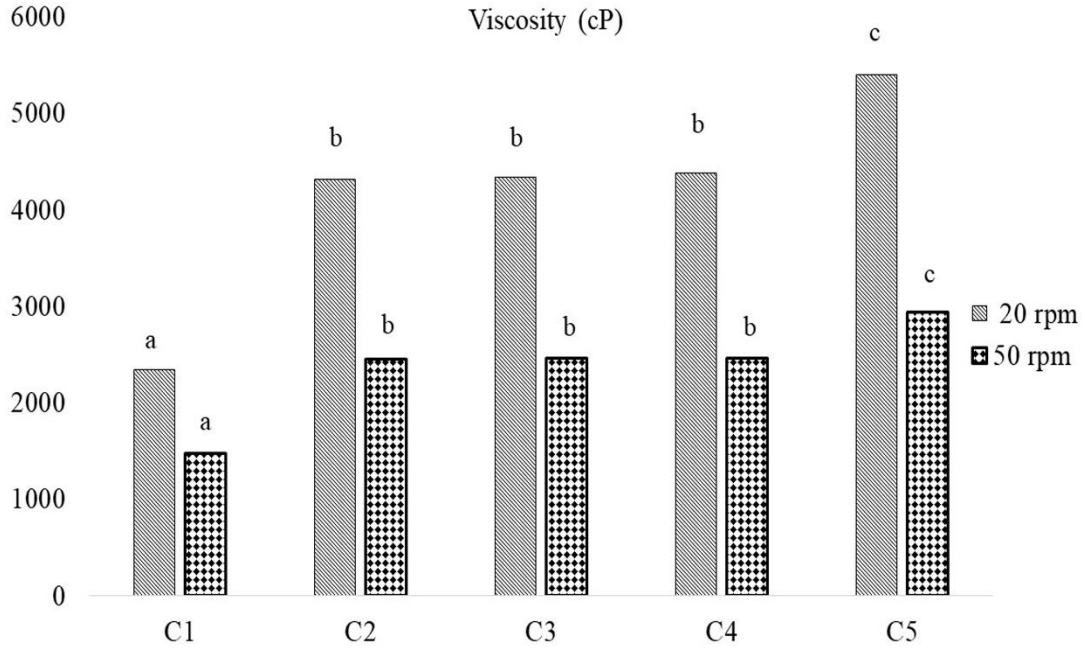

Figure 4. Viscosity values of ice cream mixes. Different letters above the bars indicate significant differences by Duncan multiple range test ($p<0.05$).

In their studies in which they produced probiotic ice cream using apple, orange, oat, bamboo, and wheat fibres, Akalın et al. (2018) reported that the effects of fibre contents on the viscosity of the samples were 0.133, 0.120, 0.082, 0.071, 0.043, and 0.042 for apple, orange, wheat, bamboo, oat, and control samples, respectively. They stated that the fact that wheat

fibre provided the samples a higher viscosity than oat fibre did was because of different fibre compositions and the dissolution rate and the rate of insolubles as Alan et al. (2012) also stated. Rodehutscord et al. (2016) reported that wheat fibre had a higher viscosity when compared with oat fibre.

3.6. Overrun analysis

The average values of the increased in the volume of the samples were presented and arranged in Figure 5 for better comprehension. All the samples were found statistically different from one another in terms of increased in volume.

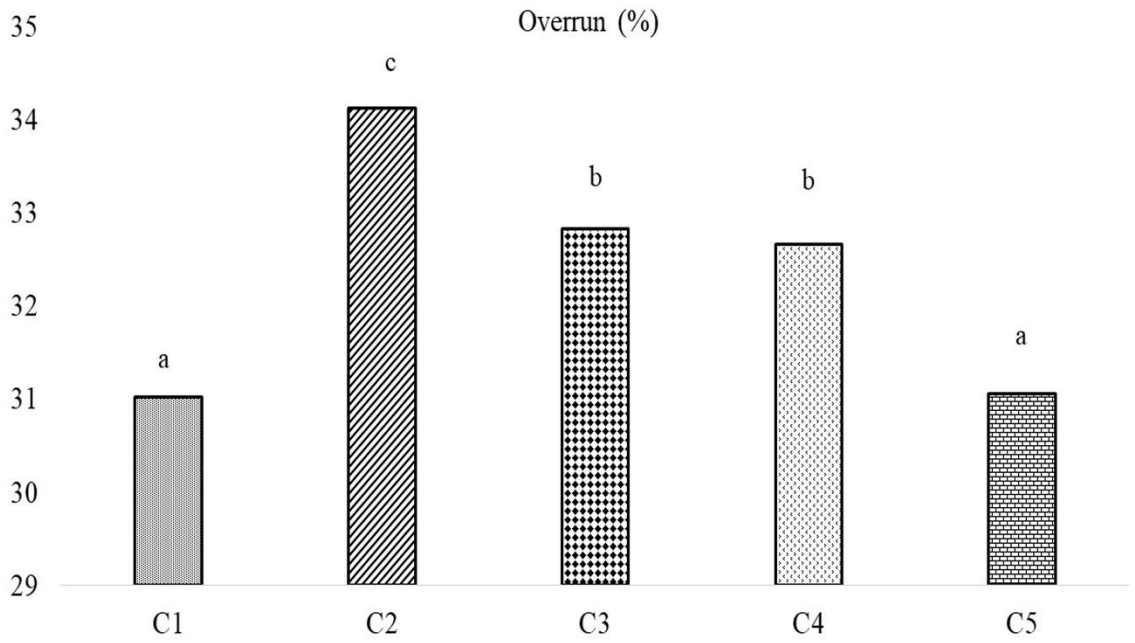

Figure 5. Overrun values of ice cream samples. Different letters above the bars indicate significant differences by Duncan multiple range test ($p<0.05$)

Crizel et al. (2014) reported that the increased in the volume of the samples they produced adding orange fibre in addition to different formulations were lower than those of the control sample and this might be because of the decrease in the fat content of the samples. It was reported that the decrease in volume increased caused the control groups to have a higher melting rate and this was caused by the air cells that exist in the structure of ice cream (Marshall et al., 2003). A study that confirms this finding was conducted by Akalın et al. (2018). The researchers detected that the ice creams that they produced using wheat fibre had lower melting rate than that of the control group throughout the storage period. They also found that the sample with wheat fibre exhibited a lower melting rate than those of the samples with bamboo and oat fibre throughout the storage period. They stated that it might be because wheat fibre provided the ice cream samples a tougher structure.

3.7. First dripping and complete melting times

As can be seen in Figure 6, times of first drop and full melting were found the earliest in the C1 coded sample and the latest in the C5 coded sample. There are some factors that are effective on the rates of melting of ice creams. The diffusion of heat into the ice cream samples emerges as a reason. The high rate of increased in volume decreases the melting rates because of the diffusion of heat. Fat molecule clusters that exist in the air gaps in the ice creams and their aggregations are effective on the melting of ice creams (Akbari et al., 2016).

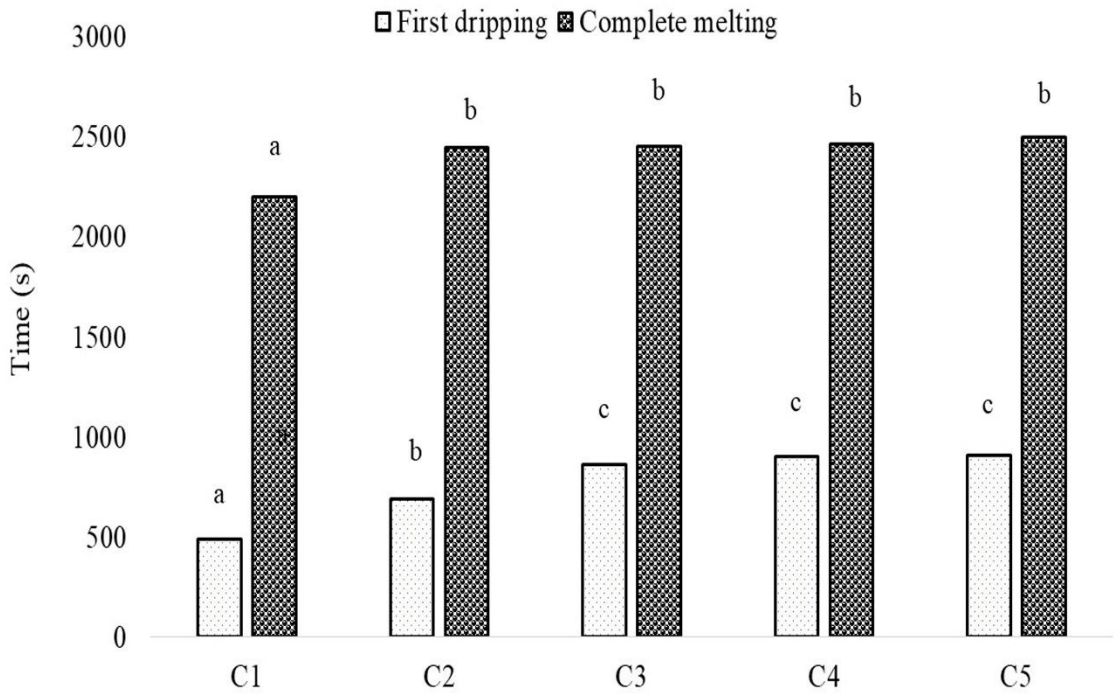

Figure 6. First dripping and complete melting times of ice cream samples. Different letters above the bars indicate significant differences by Duncan multiple range test ($p<0.05$).

The consistency of the mixture, the components that form the mixture, and the structures of the ice crystals also affect the melting rate in the ice cream samples (Javidi et al., 2016). Balthazar et al. (2017) reported that the corn dietary fibre added ice cream sample got the highest score (1.56) among the other ice cream samples in their research in which they studied the melting rates of the ice cream samples that they produced using sheep milk fat, fructo-oligosaccharide, resistant starch, short chain fructo-oligosaccharide, galacto-oligosaccharide, inulin, corn dietary fibre, and polydextrose components. Because dietary fibres have high water binding capacity, they contribute positively to the textural properties of ice creams (Dervişoğlu and Yazıcı, 2006). It is thought that dietary fibre that fruits contain has a role in rising of the first drop and complete melting times depending on the addition of fruits. Also, Muse and Hartel (2004) reported that hardness of ice cream was related to ice phase volume, size of ice crystals, and increased in volume, fat stabilization, and rheological properties of the mixture.

3.8. Sensory attributes

The sensory analysis scores of the ice creams are presented in Table 5 and adding Kavılca fibre into the ice creams significantly affected ($p<0.01$) the sensory scores. The colour value of the control sample got the lowest value, whereas the colour values of the samples C4 and C5 got the highest score. It was detected that the sample C5 had the highest scores in terms of taste and aroma (6.90), resistance to melting (7.20), icy structure (6.70), and colour (7.50). The fact that Karaca et al. (2009) detected that the ice cream samples they produced using carbohydrate-based fat substitutes got lower scores than the control sample in terms of flavour scores shows that the result of their study is not parallel to the results of our study. Because, we can see in Table 5 that Kavılca fibre does not affect the taste and aroma of the samples negatively, and even it improves them.

Table 5. The effect of different Kavılca fiber concentrations on sensory properties of ice creams

Sensory parameters	Samples				
	C1	**C2**	**C3**	**C4**	**C5**
Color	6.9±1.49^{a}	7.35±1.57^{a}	7.30±1.72^{a}	7.50±1.70^{a}	7.50±1.64^{a}
Body and texture	6.60±1.41^{a}	7±1.39^{ab}	6.95±1.44^{ab}	7.45±1.5^{b}	6.75±1.49^{ab}
Resistant to melting	6.75±2.07^{a}	6.55±1.79^{a}	6.85±2.20^{a}	7.15±1.57^{a}	7.20±1.38^{a}
Taste and Aroma	5.6±2.34^{a}	5.40±1.95^{a}	6.30±2.30^{ab}	6.75±2.07^{b}	6.90±1.94^{b}
Creaminess	5.60±2.25^{ab}	5.15±2.27^{a}	5.80±2.45^{ab}	6.40±1.58^{b}	6.10±1.97^{ab}
Mouth feeling	6.30±2.28^{ab}	6.10±2.43^{a}	6.95±2.18^{ab}	7.15±1.54^{b}	6.65±1.84^{ab}
Gumming structure	6.55±2.13^{a}	6.10±2.04^{a}	6.40±2.10^{a}	6.95±1.90^{a}	6.45±2.13^{a}
Iced structure	5.75±2.57^{ab}	5.15±2.54^{a}	5.80±2.18^{b}	6.50±1.90^{ab}	6.70±2.24^{b}
General acceptability	5.55±1.90^{a}	6.05±2.01^{ab}	6.60±1.93^{bc}	7.25±1.53^{c}	6.75±1.89^{bc}

C1, control (without KF); C2, 0.5% (w/w) KF added; C3, 1% (w/w) KF added; C4, 1.5% (w/w) KF added and C5, 2% (w/w) KF added. *Means within the same row with different letters are significantly different ($p<0.05$).

Güven et al. (2003) detected that the structure and texture scores of the Kahramanmaraş ice cream samples they produced using different stabilizers were between 3.50-4.58 and it was seen that the structure and texture scores that the researchers found were lower than the results of our study. It was determined that the samples were ranked from the most liked to the least liked as C4, C5, C3, C2, and C1, respectively. Crizel et al. (2014) reported that the ice cream samples they produced using orange fibre were affected by the fibre negatively in terms of flavour and aroma. However, when we look at their overall acceptability scores, the samples that were produced by using 1.5% orange fibre got higher

scores. Also, when we look at the ice creams produced by adding different ingredients, it was reported that overall acceptability scores of the ice creams that were produced using 10% soy milk was higher when compared with the ones produced using 20% and 30% concentrations and they were liked more (David, 2016); Yüksel et al. (2015b) reported that the ice cream that was produced with 1% concentration got the highest score (7.69) among the ice cream samples produced by using turpentine coffee; Dervişoğlu and Yazıcı (2001) reported that the panelists liked the samples containing 0.75% cola extract, 15% Na_2CO_3 and 0.1% cola aroma the most among the ice cream samples that they produced using cola extract and flavour; and Goraya and Bajwa (2015) reported that the ice cream samples with 10% concentration were liked more among the ice cream samples they produced using a special grape type of India called Indian gooseberry (or amla). These findings show that consumers are open to diversity in ice cream production. Sensory evaluation of the effects of preparing functional products on diversity is very important.

4. Conclusions

In this study, it was determined that Kavılca fibre addition has affected some of the physicochemical properties (dry matter, ash, acidity, pH, and fat) of the ice cream samples. When especially the fat ratio was evaluated, the fact that the fat ratios of the ice cream samples decreased depending on an increased in the fibre concentration revealed the importance of fibre once again in terms of preventing many chronic diseases. Because dietary fibres are used as fat substitutes in reducing the fat content of ice creams. It was detected that there were significant structural variations between the control sample and the samples containing Kavılca fibre in the SEM images of the ice cream samples. It was detected that a protein network of the control sample consisted of serum phase and gaps in which air cells exist, there were filamentous extensions that surrounded protein networks in the samples in which Kavılca fibre was added, the numbers of serum phase and air gaps decreased and their diameters diminished. It was observed that filamentous structures increased, a denser protein network was formed and the structure was harder parallel to the increased in fibre concentration. Regarding the sensory properties, the effect of fibre addition on colour, structure and texture, resistance to melting, flavour and aroma, cream taste, melting in mouth, gummy structure, icy structure, foreign flavour, overall acceptability and fibre structure was found to be important. In conclusion, it was detected that fibre addition contribute positively to the chemical, physical, and sensory properties of ice creams and thus Kavılca fibre can be used in food products.

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MICROSTRUCTURAL CHARACTERISTICS AND ELEMENTAL DISTRIBUTION OF MAGNETIC FIELD PRETREATED SWEET PEPPER

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ABSTRACT

The impact of magnetic field (non-thermal) pretreatment on the microstructures and elemental distribution of sweet pepper was studied. Static and pulse magnetic fields (SMF and PMF) were used in combination with magnetic field strength (8 – 30 mT) and pretreatment time (5 – 25 min) for the study. Blanching (thermal) pretreatment was used as the control. After the pretreatment, all samples were dried at 50 ºC and were analyzed with Scanning Electron Machine (SEM) for microstructures and elemental distribution. Results revealed that, generally, SMFs exhibited undetached outlooks unlike PMFs that are more of visible segregated microstructures. Specifically, SMF – 1 (8 mT & 5 min), PMF – 1 (8 mT & 5 min), SMF – 2 (19 mT & 15 min), PMF – 2 (19 mT & 15 min), SMF –3 (30 mT & 25 min), PMF – 3 (30 mT & 25 min), blanched and fresh samples showed fine spongy, segregated pebbles, partially wrinkled and undetached, bigger sizes of irregular segregated, somewhat eroded surface, smaller sizes of irregular surface with some visible holes, roughened appearance with different sizes of clumps and large puffs with dots of small particles microstructures respectively. Furthermore, the elemental analysis established that magnetic field pretreatment at PMF – 2, PMF – 3, PMF – 1 and SMF – 2 led to significant improvement/better retention in values of most elements (Na, Ca, Mg and P) considered than blanched and fresh samples at 5% probability level.

1.Introduction

Sweet pepper (SP) - (*Capsicum annum*) is a fruit vegetable which is also known as bell pepper. It contains vitamin C, vitamin A, vitamin B and other nutrients in addition to low calorie. Sweet pepper is effective against cataracts, rheumatism, arthritis, lung cancer, diabetes, fever, cold, sores and bruises. Also, it helps in controlling the cholesterol level of human body and stimulates stomach secretion for the enhancement of food digestion (Odewole and Olaniyan, 2016).

Pretreatment is one of the unit operations in food processing value chain that is done to ensure that foods are microbiologically safe for consumption, as well as improving their sensory, nutritional and functional attributes. Also, it can aid further processing of food and extend their storage life. Food pretreatment/processing methods can be broadly grouped into two, these are conventional and non-conventional methods (Neeto and Chen, 2014). The conventional method can also be referred to as traditional or common method. Some typical examples of conventional methods are: blanching, thermal pasteurization, thermal sterilization, parboiling, salting and manual size reduction. Non-Conventional method is also known as emerging or novel method because it

is still evolving and its use is not as popular or common as the conventional method. High Hydrostatic Pressure (HHP), Pulsed Electric Field (PEF), irradiation, pulsed light (Neeto and Chen, 2014), sous vide, microwave heating, ohmic heating and the use of magnetic field are some the examples of the non-conventional method.

The aforementioned statements revealed that both methods of food pretreatment have thermal and non-thermal examples. The non-thermal category preserves the nutritive values of food and has the tendency of reducing the microbiological threats to food (Lipiec *et al*., 2004); whereas, the thermal category may lead to adverse depletion of some heat sensitive nutrients of food. Barbosa-Canovas *et al*. (2005) reported that emerging non-thermal technologies of food pretreatment aim at producing food of better quality than heat-treated foods. It also has the advantages of food processing cost reduction and food value addition characteristics.

Pretreatments can modify the microstructures of food; this can lead to consequential effects on some other properties (nutritional, sensory, functional, physical and mechanical) of food. Heertje (1993) stated that microstructural studies assist in establishing the relationship that exists in the composition, processing and final properties of many food products. Some recent works and vital information exist on the microstructures of foods (Rejaul et al., 2018; Verboven et al., 2018a; Fazaeli et al., 2012; Verboven et al., 2018b; Oladejo et al., 2017a; Oladejo et al., 2017b; Troncoso and Aguilera, 2009; Askari et al., 2004; Antonio et al., 2008; Gudmundsson and Hafsteinsson, 2001; Castro-Giraldez et al., 2011).

Electromagnetism is the concept that leads to the generation of magnetic field due to the flow of current in a conductor (wire) that is either wound or not wound around a core. Electromagnets are temporary magnets; which means, magnetic force can only be felt when current is flowing through the wire. Magnetic fields are classified according to their relative strength as low or high intensity; according to the variation of intensity over space as homogeneous or non-homogeneous; and over time as static or pulsed (Kovacs et al., 1997). The basic theory governing magnetic treatment of food materials could be adapted from the point of view of Dhawi et al. (2009). It was stated that living cells (food inclusive) have charges (in scattered form) which act as endogenous magnets. The endogenous magnets can be affected by exogenous magnet of an external magnetic field (from permanent magnet or electromagnet). This interaction would cause the naturally unpaired or scattered charges present in the internal part of the food materials to be rearranged in another pattern depending on factors such as: type of magnetic field, intensity of the magnetic field (MF), residence time of the product within the magnetic field and inherent characteristics of the food products. Furthermore, it is to be noted that biological membranes used to display strong orientation in magnetic field and cellular tissues are mostly affected by the application of magnetic field (Ordonez and Berrio, 2011). Ions in the cells of living things are responsible for the transmission of the effects of the MF to various parts of the materials.

Some available literatures on the use of magnetic field for food processing are Jia et al. (2015), Hayder et al. (2015); Lipiec et al., (2004); Ordonez and Berrio (2011); Ibara et al., (2015) and Kyle (2015). In all the few available literatures on the use of magnetic field for food pretreatment, microstructural studies and elemental distribution of pretreated foods were not considered. Hence, this study investigates the impact of two types of magnetic fields-Static Magnetic Field (SMF) and Pulse Magnetic Field (PMF), magnetic field strength and pretreatment time on the microstructure and elemental distribution (sodium – Na, potassium – K, calcium – Ca, magnesium – Mg and phosphorus – P) of sweet pepper. This research exposed other useful areas of application of magnet and established a strong basis for further research works in the use of magnet for food processing.

2. Materials and methods

2.1. Materials

The following materials and equipment were used: a magnetic field pretreatment device, electronic weighing balance (OHAUS, Model 201, China), laboratory oven (Model SM9053, England), desiccator, stainless steel knife and tray, Scanning Electron Machine (JEOL, JSM-7600F, Japan) and fresh samples of sweet pepper.

2.2. Sample Preparation

Fresh samples of sweet pepper were washed, cut with a stainless-steel knife, deseeded, measured (100 g) with the electronic weighing balance and pretreated in the magnetic field device. Two types of magnetic field (SMF and PMF) were used in combination with magnetic field strengths in the range 8 – 30 mT and pretreatment time (5 – 25 min). Blanched samples of sweet pepper were used as the control pretreatment. After the pretreatment operation, all samples were immediately dried at 50 ^{o}C inside the laboratory oven, packaged properly and briefly kept inside the desiccator after drying. The pretreatment experiment took place at the laboratory of the Department of Food Engineering, Faculty of Engineering and Technology, University of Ilorin, Ilorin, Nigeria in December 2018. The average temperature and average relative humidity of the laboratory during the drying of all samples were 32 ^{o}C and 63% respectively. After drying, all samples were taken for microstructural analyses on the Scanning Electron Machine (SEM) with the inclusion of Energy Dispersion X-ray (EDX) for elemental distribution.

3.Results and discussions

3.1. Microstructural Characteristics of Magnetic Field Pretreated Sweet Pepper (SP)

The microstructural characteristics of SP under SMF and PMF with different combinations of magnetic field strength and time of pretreatment; and in comparison, with blanched and fresh (untreated) samples are shown in Figures 1(a – d). The microstructures revealed that the applied SMF and PMF under same and different combinations of field strength and pretreatment time led to clear differences in the microstructures of SP. The blanched and fresh samples exhibited microstructural features that are distinctly different from those of the MF pretreated samples.

Generally, there is clear distinction between the microstructures of SMF and PMF pretreatment combinations. SMFs exhibited undetached outlooks unlike PMFs that are more of visible segregated microstructures. The possible reasons for the noticed differences could be attributed to the distinct characteristics of SMF and PMF. SMF is from fully rectified alternating current (AC) to direct current (DC), it has no frequency (Bird, 2010); therefore, its impact on the pretreated product is continuous. On the other hand, PMF is from partially rectified AC to DC (Bird, 2010), as result, it has a non-continuous (pulsating) impact on the pretreated product. The pulsating effect of PMF might have led to the introduction of repeated doses of stress at specific time intervals on the products which most likely caused the noticed segregated microstructures.

Specifically, SMF-1 (8 mT & 5 min) has fine spongy microstructure, PMF-1 (8 mT & 5 min) has microstructure that looked like segregated pebbles of different sizes, SMF-2 (19 mT & 15 min) has partially wrinkled undetached microstructure, and PMF-2 (19 mT & 15 min) shows bigger sizes of irregular segregated microstructure. Furthermore, SMF-3 (30 mT & 25 min) has somewhat eroded surface microstructure, PMF-3 (30 mT & 25 min) has smaller sizes of irregular microstructure with some visible holes. Finally, blanched sample shows a microstructure roughened with different sizes of clumps; the fresh sample shows large puffs with dots of small particles. The implications of the different microstructures could mean there would be better retention/improvement of available nutrients in

the pretreated sweet pepper or otherwise; fast or slow drying rates; better or poor texture, sensory and functional properties.

The microstructural characteristics obtained in this study are in agreement with previous findings in some cases and not in agreement in others. For instance, Vodal et al., (2012) reported that only blanching pretreatment was unable to affect pore size distribution of freeze dried winter carrot, but more pores were achieved when blanching was combined with fast freeze drying. Otero et al. (2000) discovered that the microstructures of peach and mango fruits were maintained to a great extent after using histological techniques to analyze the modification done to their microstructures. Damage was not done to the microstructure of dried osmo-pretreated apple slices, but increase in porosity was achieved (Askari et al., 2004). Modification in terms of formation of pores within the microstructure (tissue) of dried osmo-pretreated sweet potato was achieved (Antonio *et al*., 2008). Pretreatment with distilled water and ultrasound of 28 kHz for maximum of 60 min did not cause significant effect on the microstructure of sweet potato slices of 3 mm thickness, whereas, the combined effect of the ultrasound pretreatment and osmotic dehydration with sucrose solution of 35 % (w/v) led to highest effect on the microstructure of the product (Oladejo et al., 2018b). The microstructures of fried sweet potato showed that ultrasound pretreatment before frying led to lesser uptake of oil (a positive effect) than sweet potato not pretreated (Oladejo et al., 2018a). Also, the combined effect of Pulse Electric field (PEF) and high pressure (200 – 300 MPa) caused more adverse effect on the microstructure of chicken meat, salmon and roes than PEF alone (Gudmundsson and Hafsteinsson, 2001). The modification effect on extracellular spaces of the microstructure of kiwi fruits pretreated with osmotic solution led to liquid occupying those spaces, whereas, air filled the extracellular spaces of fresh (untreated) kiwi fruit (Castro-Giraldez et al., 2011).

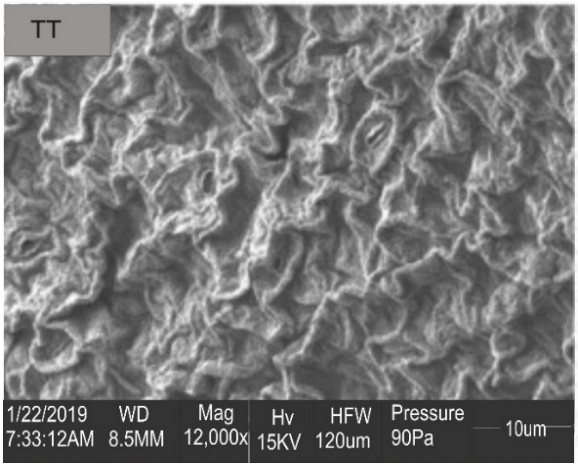

Figure 1a (i): Microstructure of SP at SMF-1

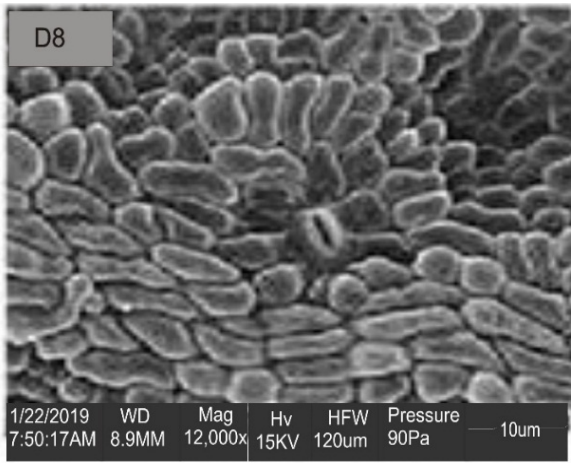

Figure 1a (ii): Microstructure of SP at PMF-

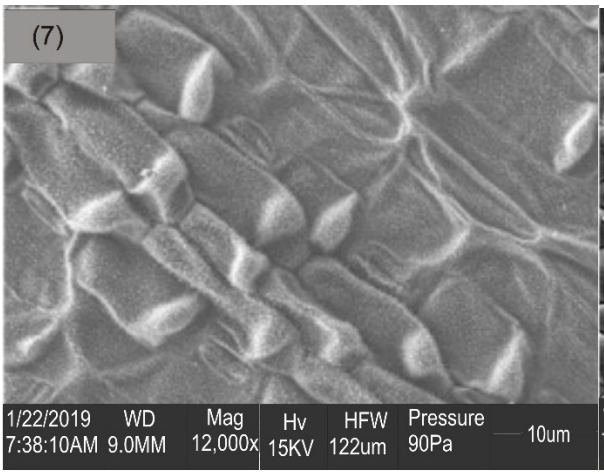

Figure 1b (i): Microstructure of SP at SMF-2

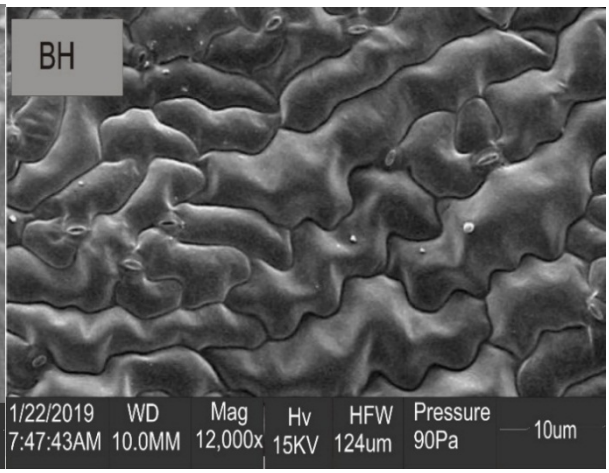

Figure 1b (ii): Microstructure of SP at PMF-2

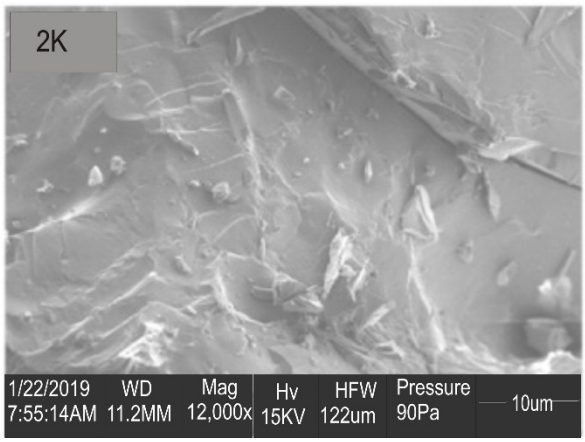

Figure 1c (i): Microstructure of SP at SMF-3

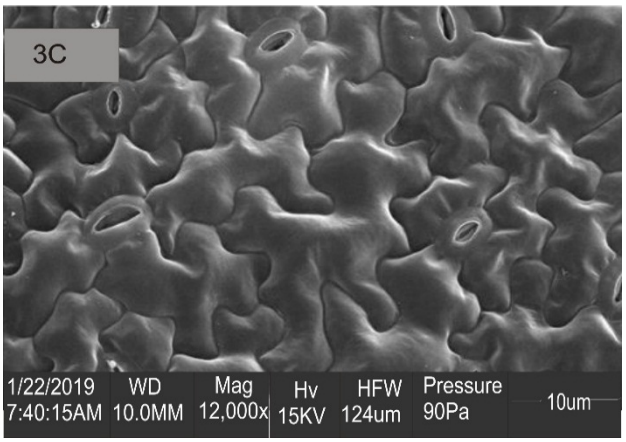

Figure 1 c (ii): Microstructure of SP at PMF-3

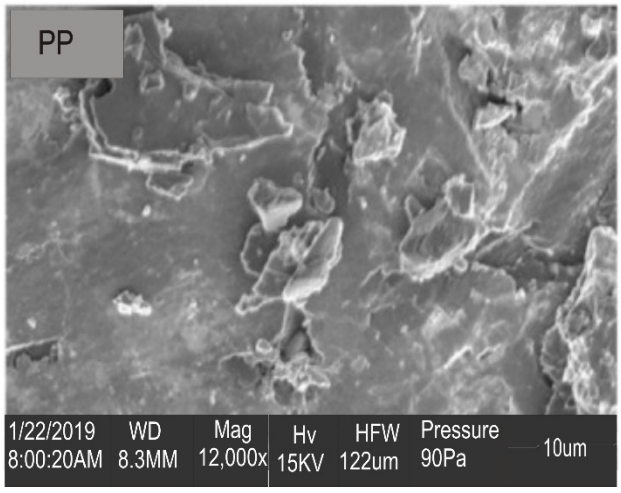

Figure 1d (i): Microstructure of SP for blanched

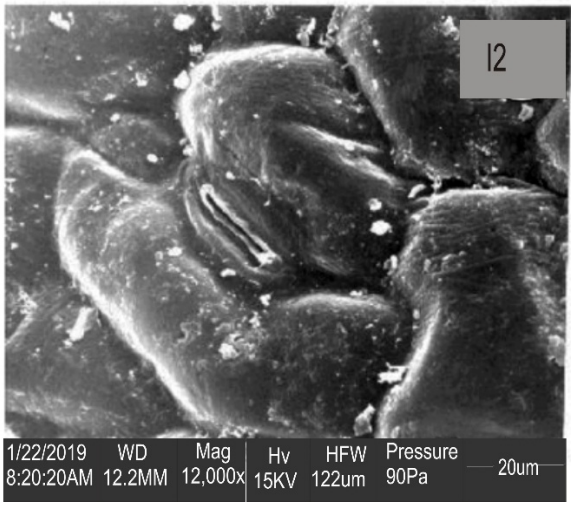

Figure 1d (ii): Microstructure of SP for fresh

3.2. Elemental Distribution of Magnetic Field Pretreated Sweet Pepper (SP)

Figures 2 (a – e) show the elemental distribution of magnetic field pretreated, blanched and fresh samples. The figures present a better understanding of the effect of pretreatments on sweet pepper in the sense that, they quantify the effects of pretreatments on the microstructure by showing different percentages of some elements (Na, K, Ca, Mg and P) present in the product. Also, the figures show the statistical implications of differences noticed among the elements analyzed at 5% probability value. This is indicated with the error bars (I) on each bar representing different pretreatment combination. From the figures, Na values at PMF-2 (2.20%) and PMF-3 (2.20%) are significantly higher than the values obtained for blanched (1.15%) and fresh (1.15%) samples. However, the lowest value of 0.25% for Na is at SMF-2. Also, blanched and fresh samples of SP have same value of 48.80% for K; this value is significantly higher than values obtained at PMF-2 (37.60%) and PMF-3 (35.60%), but not significantly higher than other magnetic field pretreatment combinations. For Ca, 30.30% and 33.00% were obtained at PMF-2 and PMF-3 respectively. These values are significantly higher than 24.22% and 23.14% obtained for blanched and fresh samples respectively. Furthermore, PMF-2 has 12.80% Mg which is only significantly higher than 10.80% obtained each for the blanched and fresh samples. Lastly, SMF-2 has 12.15% of P, and this value is significantly higher than 8.03% and 8.60% present in blanched and fresh samples and other magnetic field pretreatment combinations.

The possible reasons for the variations in elemental distribution might be due to some of the reasons earlier stated under the microstructural characteristics discussion. Also, it might be due to the fact that each element has its own unique characteristics in terms of type bond with other elements, strength of bond and arrangement of their structures within the sweet pepper. As a result, different behavior might be exhibited by each of them when sweet pepper is subjected to magnetic field pretreatment of different types of field (SMF or PMF) with different magnetic field strength and pretreatment time or other types of pretreatment. This might cause chemical reactions leading to adjustment in the values of elements above or below the natural values in the fresh samples. The observations in this study is within the report of Dhawi *et al.,* (2009) that the seedlings of date palm pretreated with static magnetic field strength (10 – 100 mT) for 30 – 360 min showed increase in the concentrations of Ca, Mg, Na, K; however, phosphorus (P) concentration dropped with increase in SMF strengths and time of exposure to the magnetic field.

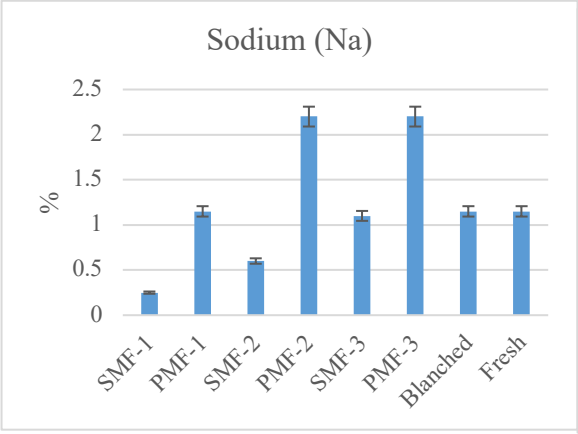

Figure 2 a: Effect of MF pretreatment on Na of SP

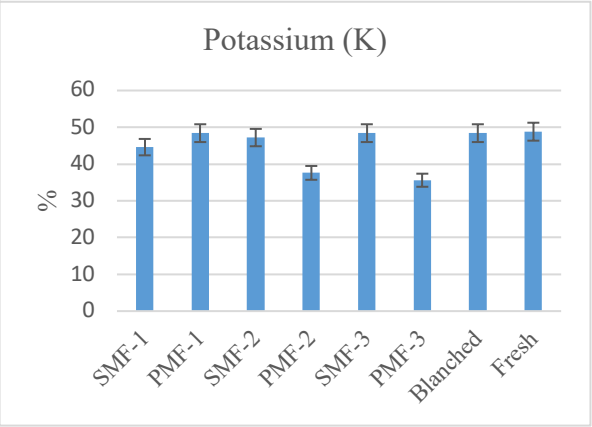

Figure 2 b: Effect of MF pretreatment on K of SP

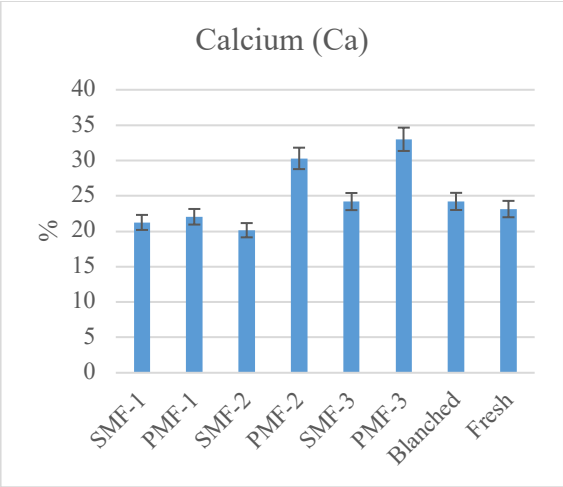

Figure 2 c: Effect of MF pretreatment on Ca of SP

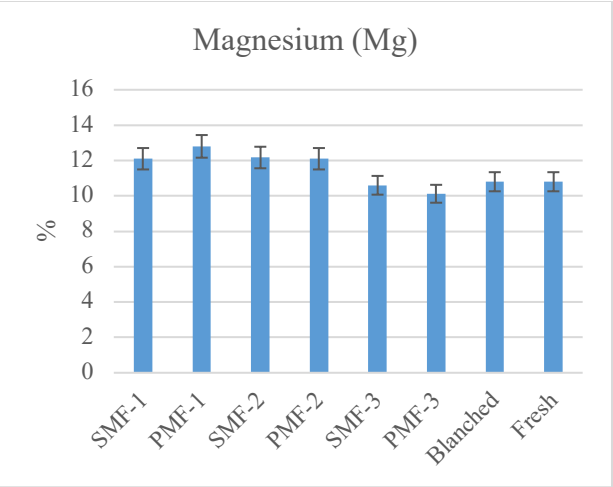

Figure 2 d: Effect of MF pretreatment on Mg of SP

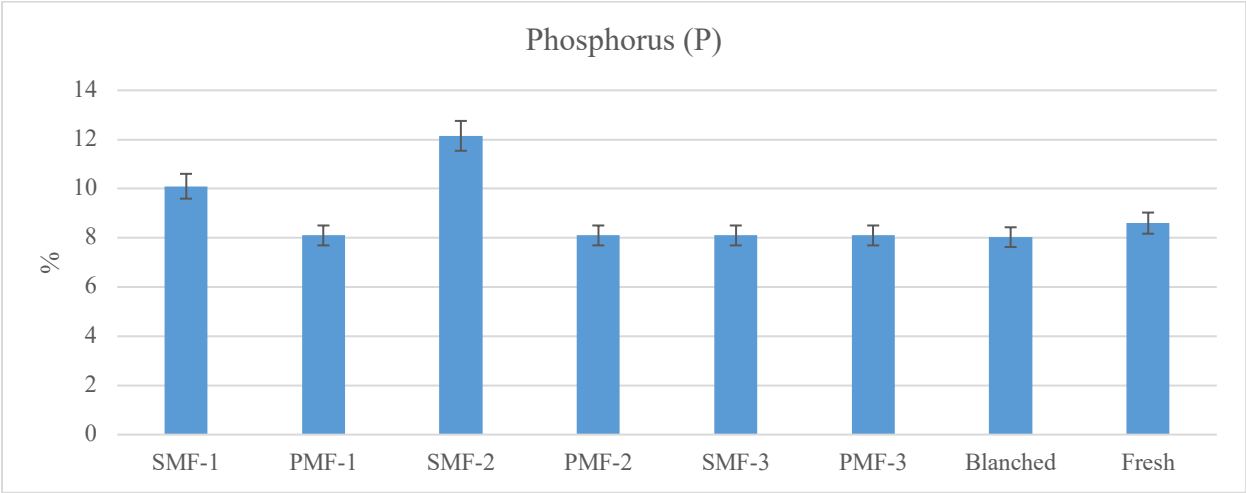

Figure 2 e: Effect of MF pretreatment on P of SP

4. Conclusions

The impact SMF and PMF pretreatments on the microstructures and elemental distribution of sweet pepper in comparison with blanched and fresh samples are not the same. Generally, SMFs exhibited undetached outlooks unlike PMFs that are more of visible segregated microstructures. The elemental analysis established that magnetic field pretreatment at some combination of factors (PMF-2, PMF-3, PMF-1 and SMF-2) led to significant improvement/better retention in values of most elements (Na, Ca, Mg and P) considered than blanched and fresh samples at 5% probability level. Hence, magnetic field pretreatment (which is non-thermal) is more beneficial than blanching (thermal) pretreatment for the processing of vegetables. Further research on the use of higher values of magnetic field strength in combination with other processing factors with the consideration of more micronutrients, macronutrients and phytonutrients is recommended.

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PRE-TREATMENT (OHMIC AND OVEN) EFFECT ON THERMODYNAMIC PARAMETERS OF KIWI DRYING IN MICROWAVE DRYER

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ABSTRACT

In this article, have been investigated effects pre-treatment ohmic and oven on the amount of energy and exergy kiwi fruit drying in a microwave dryer. In the present study, multilayer perceptron (MLP) artificial neural network was selected. The results of the experiments showed that the oven and ohmic time is significant for the energy efficiency and exergy efficiency and specific energy and exergy loss. In total, with increasing ohmic and oven time and microwave power, the amount of energy and exergy efficiency of the microwave dryer would increase. Based on the results obtained, the maximum amount of R2 in a network containing 5 and 10 neurons was R2Oven = 0.9924 and R2Ohmic = 0.9890 in the hidden layer for energy efficiency, R2oven = 0.9930 R2ohmic = 0.9936 10 neuron and 5 neuron (First layer), 10 neuron (Second layer) in the hidden layer for specific energy loss, R2Oven = 0. 9877 and R2Ohmic = 0.9978 for exergy efficiency was observed 5 neuron (First layer) and 5 neuron (Second layer) in hidden layer and for specific exergy loss was best R2 value (R2Oven = 0. 9837 and R2Ohmic = 0.9865) in hidden layer with 10 neuron in first and second layer.

1.Introduction

Kiwi fruit (Actinidia deliciosa) has high vitamin C content. It is a very important fruit species in terms of healthy nutrition due to its low calorie level. Also, it can be stored between 2 and 6 months. Therefore, the shelf life of the kiwifruit can be extended with drying. (Özdemir *et al.*, 2017). Drying has a vital role in postharvest processing. It has always been of great importance for conserving agricultural products and for extending the food shelf life (Azadbakht *et al.*, 2017b)(Deshmukh *et al.*, 2013). Drying is known as the best method to preserve fruits and vegetables. Water removal during drying prevents microorganism evolution and harmful chemical reactions leading to longer storage time(Azadbakht *et al.*, 2017c)(Nikbakht *et al.*, 2014). Drying is one of the oldest unit operations and has recently become widespread in various industries to gain different utilities. There are more than 200 types of dryers and for each dryer, the process conditions, such as drying chamber temperature, pressure, air velocity (if the carrier gas is air), relative humidity and the product retention time, have to be determined according to feed, product, purpose and method (Azadbakht *et al.*, 2018b; Erbay and Icier 2011). Also drying is widely used to preserve porous medium products. It is a complicated process involving

heat and mass transfer between the material surface and its surroundings (Prommas *et al.*, 2010).Microwave drying is a new addition in the existing drying techniques, vs. convective air drying (cabinet, fluidized bed, tunnel), spray, vacuum, foam mat and freeze drying. Microwave is an electromagnetic wave in the frequency range of 300–30000MHz. It is the combination of electrical and magnetic fields, with only the former being engaged in the conversion process when waves interact with the non-magnetic materials. The conversion of microwave energy into heat in the food is because of the presence of water(Sharma and Prasad 2006). Drying temperature and microwave power are the two most important factors in microwave drying of agricultural products. These two factors significantly influence the drying parameters such as drying time, drying curve, drying speed, drying efficiency, and the final product quality. To improve microwave drying, a number of studies have been conducted to investigate the effects of different microwave power levels and drying temperatures, and different prediction models have been established (Li *et al.,* 2010). Drying of fruits and vegetables is one of the most time and energy consuming processes. Drying rate must be accelerated to reduce the drying process and energy consumption without compromising the quality. One of the major obstacles in removing the moisture from the material is the outer layer of the material, the skin. It acts as the major resistance to the moisture transport from interior of the material to surface. Pre-treatment is an essential step before processing of food materials to overcome this problem up to great extent. It has been reported that pre-treatments can accelerate the drying rate by dissociating the wax and forming the fine cracks on the surface of the material for easy moisture removal (Deshmukh *et al.,* 2013). Ohmic treatment is one of the electron heating methods based on the passage of electrical current through a food product having electrical resistance. The electrical energy is converted to heat while the amount of heat generated through the food product is directly related to the voltage gradient and the electrical conductivity. Ohmic heating as an alternate processing method has shown to yield foods with higher quality compared to the conventional heating. This difference is mainly due to its ability to heat materials rapidly and uniformly leading to a less aggressive thermal treatment (Nouroallahi Soghani *et al.,* 2018).

Darvishi *et al*. (2014) analyzed the energy and exergy of white mulberries in the process of drying with microwave dryer and reported that the specific energy loss increases with increasing microwave power. Additionally, energy efficiency was reduced by decreasing the moisture content and microwave power. The best energy and exergy for white mulberry was observed at 100 W microwave power.

Darvishi *et al*. (2016) conducted energy and exergy analysis and modeled Kiwi slices with a microwave dryer and it was found that the energy and exergy efficiency increases with increasing microwave power and decreasing the thickness of kiwi slices. Additionally, this parameter decreases by reducing the moisture content of slices.

Salengke *et al*. (2005) performed an experiment on effect of Ohmic Pre-treatment on the Drying Rate of Grapes and Adsorption Isotherm of Raisins, which Results of this study reveal that the drying rate of the grapes was significantly increased by the ohmic pre-treatment, especially at low electrical frequencies. The effect of the ohmic pre-treatment on equilibrium moisture content of the raisins produced was evident at 0.75 or higher water activities but there was no or limited effect at low to moderate water activities.

Nouroallahi Soghani *et al*. (2018) Performed experiment on Ohmic blanching of white mushroom and its pre-treatment during microwave drying Which showed the results of this experiment blanched sample at low voltage and heating duration consumed the minimum total energy during the drying process.

The objective of this research is the energy performance analyses of energy and exergy of the microwave dryers for drying kiwi slices

under pre-treatment (ohmic – oven) and non-treatment in order to reduce the energy consumption in the microwave dryer and increase the energy and exergy efficiency of the microwave with new processes. In addition, this research used artificial neural network to process the numbers in order to verify the accuracy of the numbers obtained. Additionally, sensitivity coefficient test was used to relate the energy and exergy factors to microwave and pre-treatment.

2. Materials and methods

2.1. Sample preparation

Newly-harvested kiwi fruit were purchased from the local store in Gorgan city of Iran, and were kept in the laboratory at 10 ° C. At the beginning of each experiment, the kiwi was washed and the slices were cut in circular in a thickness of 5 mm and they were weighted. Then, samples were placed in an oven with Temperature at 100 ° C for 3, 5 and 7 min to be pretreated. Also samples were placed in an ohmic heating with voltage 80 for 3, 5 and 7 min to be pretreated. Drying process was performed in a microwave dryer in the Bio System Mechanics Department of Gorgan University of Agricultural Sciences and Natural Resources (Figure 1).

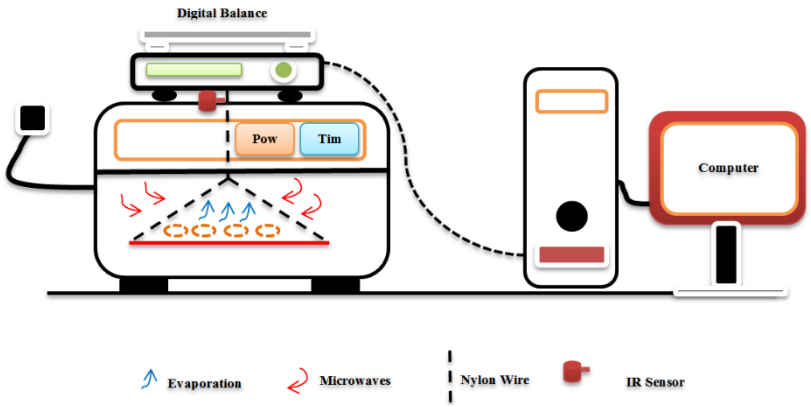

Figure 1. Diagram of microwave drying system

2.2. Experiment method

Slices were pretreated and placed in containers and dried at three powers of 360, 600 and 900 W. The weight of kiwi was measured using a 0.01 mg precision scale. The weight of each sample was measured and recorded at a time interval of 1 minute to reach constant moisture. For each of the treatments, the experiments were repeated three times. The experiment was conducted at a temperature of 20 ° C and relative humidity of 79%. The moisture content of kiwi was also calculated using equation (1) (Yogendrasasidhar and Pydi Setty 2018).

$$MC = \frac{W - We}{W} \qquad (1)$$

2.3. Energy analysis

Energy used in the drying and heating process is important for production processes in the industrial and household sectors. However, the price of this energy is extremely expensive; therefore, there is a strong incentive to invent processes that will use energy efficiently. Currently, widely used drying and heating processes are complicated and inefficient and are generally damaging to the environment. Thus, it is required to have a simplified lower-cost approach replicable in a wide range of situations (Jindarat *et al.*, 2011).

The mass and energy survival in the microwave dryers' chamber is shown in Figure 2. The general relation of mass moisture survival is calculated using Equation (2) (Darvishi *et al.,* 2016).

$$\sum m_{in} = \sum m_{out} \qquad (2)$$

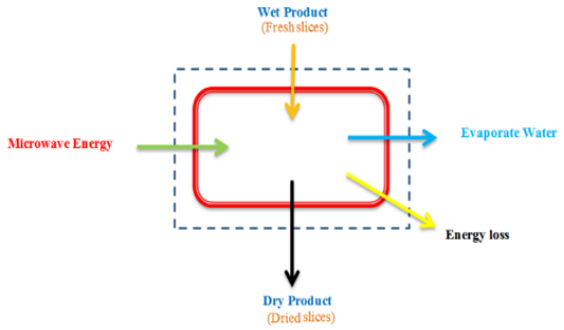

Figure 2. Volume control of microwave system

According to Equation 3, the initial mass of the sample is equal to the amount of water vapor removed and the rate of dried sample mass.

$$m_o = m_{ew} + m_p \qquad (3)$$

The mass of evaporated water is obtained using Equation 4 (Azadbakht *et al.,* 2018a)

$$m_{wt} = m_d(M_0 - M_t) \qquad (4)$$

The protected energy of the sensible heat, latent heat, and the thermal source of the microwave were calculated using Equation 5 and the input energy of the dryer was calculated using Equation 6(Jindarat et al. 2011). In equation 5, the lost energy is$P_{ref} + P_{tra}$. Equation 6 shows the amount of input energy of the microwave. This formula is composed of three parts, including absorbed energy, reflected energy, and passed energy. In equation (6) equals to the absorbed energy of product.

$$P_{in} = P_{abs} + P_{ref} + P_{tra} \qquad (5)$$

$$P_{in} \times t = \left((mC_pT)_{dp} - (mC_pT)_{wp}\right) + \lambda_K m_w + E_{ref} + E_{tra} \qquad (6)$$

The latent heat of the kiwi samples is calculated using Equation 7 (Abdelmotaleb *et al.,* 2009).

$$\frac{\lambda_K}{\lambda_{wf}} = 1 + 23\exp(-40M_t) \qquad (7)$$

The latent heat of free water evaporation has been calculated by Broker et al and using Equation 8 (Darvishi 2017).

$$\lambda_{wf} = 2503 - 2.386(T - 273) \qquad (8)$$

The thermal capacity is a function of the moisture content and can be calculated through Equation 9) Brooker et al. 1992 . (

$$C_P = 840 + 3350 \times \left(\frac{M_t}{1+M_t}\right) \qquad (9)$$

The thermal efficiency of the dryer is calculated using Equation 10 (Soysal et al. 2006).

$$\eta_{en} = \frac{energy\ absorption}{P_{in} \times t} \qquad (10)$$

The specific energy loss was measured using Equation 11 (Darvishi et al. 2014)

$$E_{loss} = \frac{E_{in} - E_{abs}}{m_w}\ or\ E_{loss} = (1 - \eta_{en}) \times \frac{P_{in} \times t}{m_w} \qquad (11)$$

2.4. Exergy analysis

With the onset of the energy crisis, energy and exergy (the maximum useful work that comes from a certain amount of available energy or from the flow of materials) analyses are among the leading thermodynamic research works. In the exergy analysis, the main purpose is to determine the location and amount of irreversible production during the various processes of the thermodynamic cycle and the factors affecting the production of this irreversibility. In this way, in addition to evaluating the performance of various components of the thermodynamic cycle, methods to increase cycle efficiency are also identified (Mokhtarian et al. 2016).

The general exergy equilibrium in the microwave chamber was stated as follows (Darvishi *et al.*, 2016)

$$EX_{in} = EX_{abs} + EX_{ref} + EX_{tra} \quad (12)$$

Exergy loss

$$P_{in} \times t = \left((m \times ex)_{dp} - (m \times ex)_{wp}\right) + ex'_{exap} \times t) + E_{ref} + E_{tra} \quad (13)$$

:

The amount of exergy transmitted due to evaporation in the drying chamber was calculated using Equation 14 (Sarker et al. 2015)

$$ex'_{exap} = (1 - \frac{T_0}{T_p}) \times m_{wv}\lambda_{wp} \quad (14)$$

In formula 14, m_{wv} is calculated using formula 15) Darvishi et al. 2016 (

$$m_{wv} = \frac{m_{t+\Delta t} + m_{wv}\lambda_{wp}}{\Delta t} \quad (15)$$

Specific exergy loss was calculated using formula 16: (Darvishi *et al.*, 2014)

$$ex = C_p[(T - T_0) - T_0 \ln(\frac{T}{T_0})]$$

(16)

Exergy efficiency for each dryer system as the exergy rate used in drying the product to the exergy of drying source supplied to the system is calculated by the Equation 17 (Dincer and Sahin 2004)

$$\eta_{en} = \frac{exergy\ absorption}{P_{in} \times t} \times 100$$

(17)

The specific exergy loss was calculated using Equation 18(Darvishi 2017) .

$$EX_{loss} = \frac{EX_{in} - EX_{abs}}{m_w} \quad (18)$$

In this research, the source of temperature and pressure in the environment was 20 ° C and 101325 Pascal, respectively.

2.5. Artificial Neural Network Modeling

In this research, the artificial multilayer perceptron (MLP) neural network was used for modeling the energy and exergy of the microwave dryer to predict energy efficiency, specific energy loss, exergy efficiency and specific exergy loss by one and two hidden layer and the number of neurons is shown in the table 2 , for data analyses was used Neuro Solution 6 software. Hyperbolic tangent linear activation functions (Equation 19), which are the most common type of activation functions, were used in the in hidden input and output layer. In this paper, the Levenberg-Marquardt algorithm was used to learn the network. Additionally, 80% of the data were used for training, 20% of them were used for testing the network (Testing data) (Table 2). The microwave power and the duration of the ohmic and oven were considered as network inputs and the energy efficiency, specific energy loss, exergy efficiency, and the specific exergy loss were the considered network outputs. Five repetitions were considered to achieve the minimum error rate and maximum network stability as a mean of 5000 Epoch for the network. Error was estimated using algorithm with back propagation error. Statistical parameters including, Root Mean Square Error (RMSE), R2, and Mean Absolute Error (MAE) were calculated for inputs and relationships were calculated using the formulas shown in Table 1.

Table 1. Neural Network Relationships

Formula	Formula Number	Reference
Tanh = $\frac{e^{x}-e^{-x}}{e^{x}+e^{-x}}$	) 19 (	(B. Khoshnevisan, Sh. Rafiee, M. Omid, 2013)
R^2 = 1- $\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{(P_i-O)^2}$	) 20 (	(Azadbakht, Vehedi Torshizi, & Ziaratban, 2016)
r = $\sqrt{1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{(P_i-O)^2}}$	) 21 (	(Azadbakht, Aghili, Ziaratban, & Vehedi Torshizi, 2017)
RMSE = $\sqrt{\sum_{i=1}^{n}\frac{(P_i-O_i)^2}{n}}$	) 22 (	(B. Khoshnevisan, Sh. Rafiee, M. Omid, 2013)
MAE = $\frac{\sum_{i=1}^{n}\lvert P_i-O_i\rvert}{n}$	) 23 (	(Azadbakht, Aghili, Ziaratban, & Vehedi Torshizi, 2017)

Table 2. Optimization values for artificial neural network parameters

Number of hidden layers	Learning rule	Type of activation function	The number of One hidden layer neurons	The number of two hidden layer neurons	Testing data %	Training data %
1	Levenberg Marquardt	Hyperbolic tangent	5	-	20%	80%
1	Levenberg Marquardt	Hyperbolic tangent	10	-	20%	80%
2	Levenberg Marquardt	Hyperbolic tangent	5	5	20%	80%
2	Levenberg Marquardt	Hyperbolic tangent	10	10	20%	80%
2	Levenberg Marquardt	Hyperbolic tangent	5	10	20%	80%
2	Levenberg Marquardt	Hyperbolic tangent	10	5	20%	80%

2.6. Statistical Analysis

The kiwi were dried in microwave at three powers of 360, 600, 900 and three ohmic heating and oven time of 3, 5 and 7 min, and the numbers

obtained were sorted and calculated using the Excel spreadsheet software. All experiments were performed in three replications and the results were analyzed using a factorial experiment in a completely randomized design with SAS statistical software.

3.Results and discussions

The results of analysis of variance of kiwi slices drying in different microwave power at different time Ohmic and oven for energy efficiency, specific energy loss, specific exergy loss, and exergy efficiency are shown in Table 3 and 4. According to the results, microwave power has been significant for energy efficiency, specific exergy loss, exergy efficiency and specific energy loss at 1% level and significant. Additionally, according to Table 3, the results obtained for ohmic time have shown the significance level of 1% energy efficiency, specific exergy loss, exergy efficiency and specific energy .Also, the results obtained for oven time have shown the significance level of 1% energy efficiency, specific exergy loss, exergy efficiency and specific energy.The interaction effect (Microwave power × Ohmic time) of energy efficiency and exergy efficiency at 5% level and non-significance for the specific energy and exergy loss. The interaction effect (Microwave power × Oven time) of energy efficiency and exergy efficiency at 1% and 5% level , Respectively and non-significance for the specific energy and exergy loss Thus, we compared the means with the LSD test, which its results are shown in figure 3 and 6.

Table 3. Analysis of variance of energy efficiency, specific lost energy lost, specific lost exergy and exergy efficiency under different powers and ohmic time

Parameter	Energy efficiency		Specific energy loss		Exergy efficiency		Specific exergy loss	
	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
Microwave power	746.860	183.36**	71.444	203.25**	465.470	109.74**	60.353	145.26**
Ohmic time	658.627	161.69**	2.521	7.17**	459.759	108.39**	23.098	55.59**
Microwave power × Ohmic time	24.988	6.13*	0.353	1ns	22.92	5.41*	0.023	0.06ns
ERROR	3.745		0.851		3.984		0.714	

Table 4. Analysis of variance of energy efficiency, specific lost energy lost, specific lost exergy and exergy efficiency under different powers and oven time

Parameter	Energy efficiency		Specific energy loss		Exergy efficiency		Specific exergy loss	
	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
Microwave power	599.852	198.68**	140.268	241.68**	378.53	89.24**	35.46	85.35**
Oven time	581.791	192.70**	15.470	26.66**	444.288	104.75**	27.92	67.21**
Microwave power × Oven time	31.193	10.33**	1.11	1.92ns	25.01	5.90*	0.696	1.68ns
ERROR	4.073		0.580		4.241		0.415	

3.1. Effect of microwave power and pre-treatment time (ohmic and oven time) on energy efficiency

Based on the Table 3, an interaction effect of power and pre-treatment time on energy efficiency is significant at the level of 1% and Figure 3 shows the interaction of these parameters on energy efficiency. According to the results obtained, energy efficiency increased significantly with increasing microwave power

and pre-treatment time. In addition, based on the results obtained, with increasing the amount of pre-treatment time, the energy efficiency increased significantly and it can be justified by the fact that with increasing the pre-treatment time, the amount of mass reduction in kiwi, led to increase in kiwi dry matter and removed water from kiwi, and that's why it reduces the amount of drying time and the energy efficiency increases. Also, by increasing the amount of ohmic time, the surface hardness of the kiwi has been reduced, which also results in the easier removal of water from the surface of the sample.

The maximum amount of the energy efficiency is observed at power of 900 W and 7 min (57.34%$_{\text{Ohmic}}$ - 55.31% $_{\text{Oven}}$) and the minimum amount energy efficiency is observed in power of 360 W and 3 min (18.77 %$_{\text{Ohmic}}$ - 16.57% $_{\text{Oven}}$). Also, according to the results with Ohmic pre-treatment, the energy efficiency has been 1.97 times, and this value has been 1.90 for oven pre-treatment.

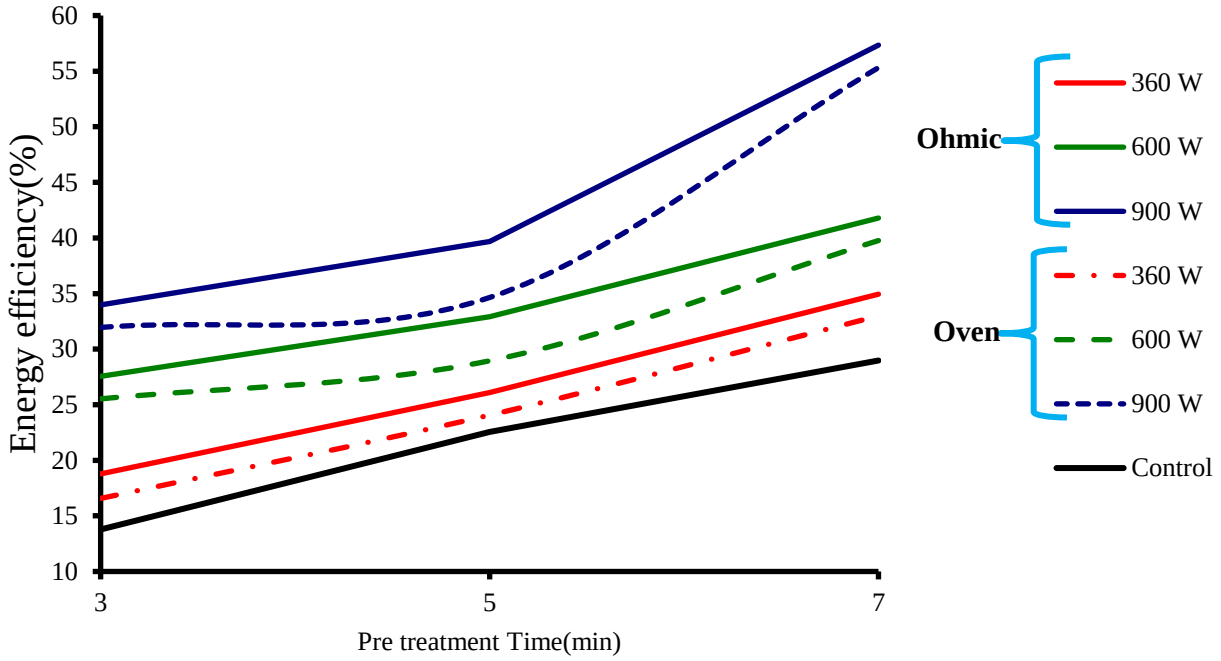

Figure 3. The interaction effect of pre-treatment time (Ohmic–Oven) and microwave power on the energy efficiency

3.2. Effect of microwave power and pre-treatment time (ohmic and oven time) on Specific energy loss

Figure 4 shows the results obtained. The maximum amount of the specific energy loss is observed at power of 360 W (6.457 $_{\text{Ohmic}}$ – 9.983 $_{\text{Oven}}$- 12.663 $_{\text{Control}}$ MJ) and the minimum amount of specific energy loss is observed in power of 900 W (5.495 $_{\text{Ohmic}}$ – 7.45 $_{\text{Oven}}$ – 9.980 $_{\text{Control}}$ MJ). Also, according to the figure, there is no significant difference between 360 and 600 watts, but there is a difference between 900 watts and two other powers. also the maximum amount of the specific energy loss in pre-treatment time is observed at time of 3 min (8.8 $_{\text{Ohmic}}$ – 12.53 $_{\text{Oven}}$ MJ) and the minimum amount of specific energy loss is observed in time of 7 min (3.18 $_{\text{Ohmic}}$ – 4.17 $_{\text{Oven}}$ MJ). As specific energy loss is inversely related to the water removed from the product, by increasing the amount of water removed from the product, the amount of specific energy loss decreases. Also changing the resistance to internal moisture diffusion by altering the microstructure due to physical damage to the sample, and this reduces the amount of drying time, and this cause ,

reduces the amount of energy Specific energy loss(Orikasa et al. 2018).

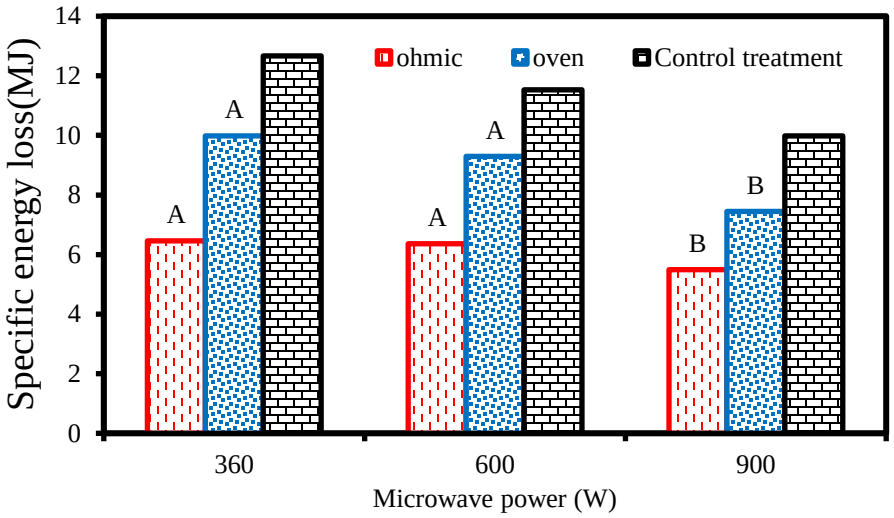

Figure 4. Effect of microwave power on the Specific energy loss

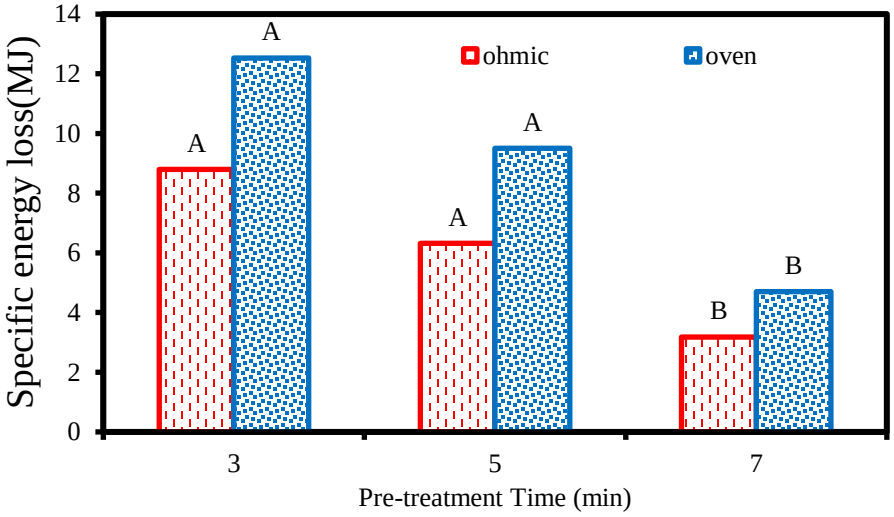

Figure 5. Effect of pre-treatment time (ohmic-oven) on the Specific energy loss

3.3. Effect of microwave power and pre-treatment time (ohmic and oven time) on energy efficiency

Figure 6 shows the interaction of these parameters on exergy efficiency. According to the results obtained, exergy efficiency increased significantly with increasing microwave power and pre-treatment time. The maximum amount of the exergy efficiency is observed at power of 900 W and 7 min (46.737%Ohmic – 42.72% Oven) and the minimum amount exergy efficiency is observed in power of 360 W and 3 min (26.37 $\%_{Ohmic}$ -22.38% $_{Oven}$). Also, according to the results with Ohmic pre-treatment, the exergy efficiency has been 2.128 times, and this value has been 1.946 times for oven pre-treatment.

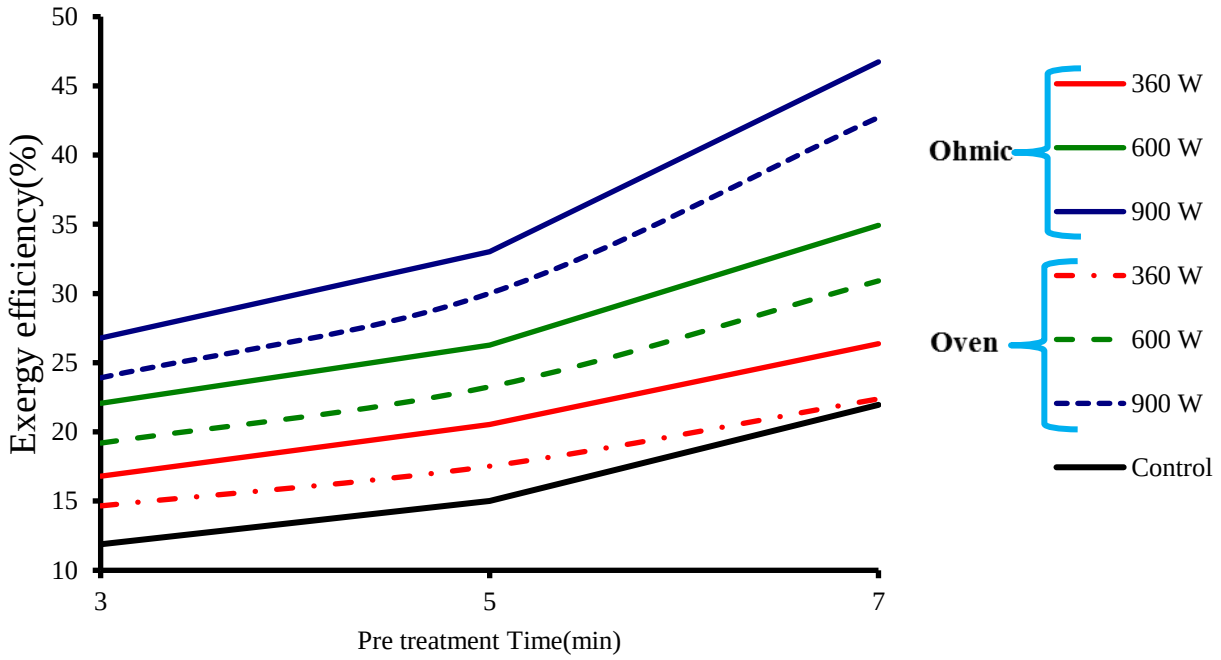

Figure 6. The interaction effect of pre-treatment time (Ohmic–Oven) and microwave power on the exergy efficiency

3.4. Effect of microwave power and pre-treatment time (ohmic and oven time) on Specific exergy loss

Figure 7 shows the results obtained. The maximum amount of the specific exergy loss is observed at power of 360 W (9.973 $_{\text{Ohmic}}$ – 12.23 $_{\text{Oven}}$- 16.52 $_{\text{Control}}$ MJ) and the minimum amount of specific exergy loss is observed in power of 900 W (6.77 $_{\text{Ohmic}}$ – 8.86 $_{\text{Oven}}$ – 12.16 $_{\text{Control}}$ MJ). Also, according to the figure, there is significant difference between 360, 600 and 900 watts. also the maximum amount of the specific energy loss in pre-treatment time is observed at time of 3 min (10.92 $_{\text{Ohmic}}$ – 12.66 $_{\text{Oven}}$ MJ) and the minimum amount of specific energy loss is observed in time of 7 min (5.74 $_{\text{Ohmic}}$ – 8.69 $_{\text{Oven}}$ MJ). Also, given that the amount of Specific exergy loss in oven pre-treatment is more than ohmic pre-treatment, the reason for this could be stated as follows that, ohmic pre-treatment has softened the fruit tissue than oven pre-treatment that this also reduces the drying time and the easier absorption of temperature for the fruit.

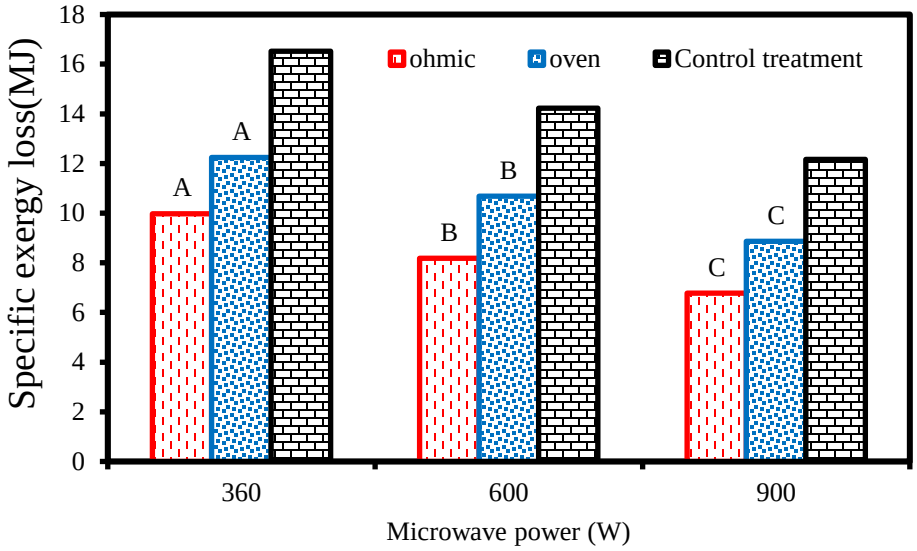

Figure 7. effect of microwave power on the Specific exergy loss

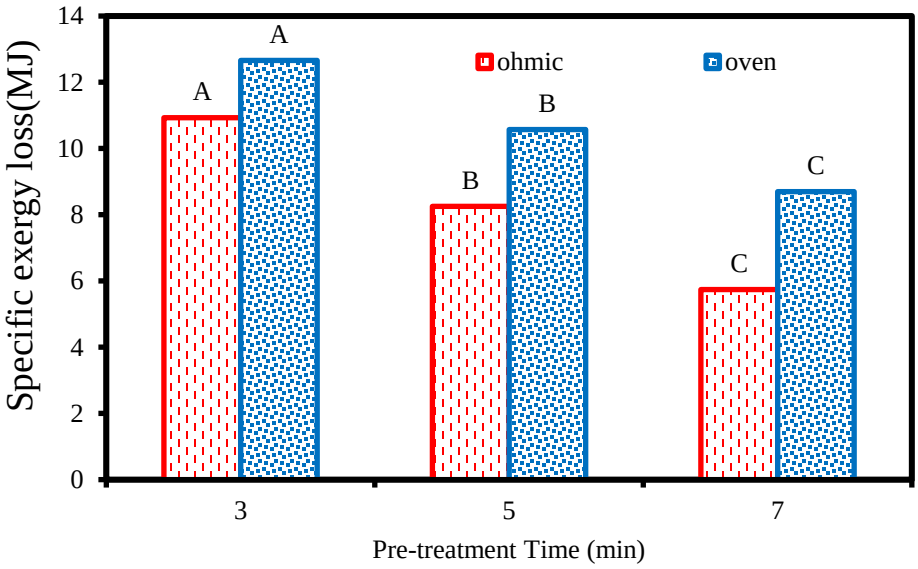

Figure 8. effect of pre-treatment time (ohmic-oven) on the Specific exergy loss

3.5. Artificial neural network

In order to predict energy efficiency, specific energy loss, exergy efficiency, and specific exergy loss, a multi-layered perceptron (MLP) neural network model was used. The duration of kiwi samples placement in ohmic, oven and microwave power were considered as input and energy efficiency, specific energy loss, exergy efficiency, and specific exergy loss were considered as network output. As lower error value was obtained by using the hyperbolic tangent activation function, this type of function was selected as the activation function in the hidden layer and the output. Based on the test method, 80% of the data were used for training and the network could learn the relationships between inputs and outputs well and 20 % of the data were used to test (Table 5).

Table 5. Error values in predicting experimental data using optimal artificial neural network (Oven Pre-Treatment)

			MSE		RMSE		MAE		R	
			Training	Test	Training	Test	Training	Test	Training	Test
Energy efficiency	Two layer	5-5	2.863	6.601	1.6920	2.5692	1.354	2.272	0.9861	0.973
		10-10	2.823	4.914	1.6802	2.2168	1.387	1.902	0.9878	0.9806
		5-10	2.840	5.227	1.6852	2.2863	1.390	2.131	0.985	0.98461
		10-5	2.104	8.108	1.4505	2.8475	1.227	1.98	0.9899	0.9475
	One Layer	5	2.020	11.140	1.4213	3.3377	1.135	3.061	0.99241	0.6287
		10	2.943	4.568	1.7155	2.1373	1.501	1.664	0.988223	0.99161
Specific energy loss	Two layer	5-5	0.481	0.0460	0.6935	0.2145	0.507	0.184	0.979	0.9977
		10-10	0.263	1.788	0.5128	1.3372	0.370	1.13	0.9890	0.8448
		5-10	0.192	1.813	0.4382	1.3465	0.368	0.920	0.9914	0.9517
		10-5	0.3698	0.693	0.6081	0.8325	0.369	0.764	0.98459	0.952
	One Layer	5	0.369	0.956	0.6075	0.9778	0.412	0.817	0.950	0.979
		10	0.180	2.027	0.4243	1.4237	0.362	0.9492	0.993061	0.93085
Exergy efficiency	Two layer	5-5	1.647	18.390	1.2834	4.2884	1.088	3.383	0.98713	0.8876
		10-10	2.931	4.122	1.7120	2.0303	1.389	1.920	0.9793	0.9930
		5-10	2.048	9.049	1.4311	3.0082	1.172	2.319	0.9822	0.959
		10-5	2.618	5.63	1.6180	2.3728	1.411	1.890	0.9769	0.9915
	One Layer	5	2.948	4.689	1.7170	2.1654	1.383	2.072	0.981911	0.90684
		10	1.971	8.568	1.4039	2.9271	1.123	2.358	0.9861	0.89533
Specific exergy loss	Two layer	5-5	0.251	0.6886	0.5010	0.8298	0.408	0.7492	0.9771	0.9151
		10-10	0.193	1.280	0.4393	1.1314	0.332	1.062	0.9831	0.6237
		5-10	0.234	1.088	0.4837	1.0431	0.379	1.0139	0.9797	0.90563
		10-5	0.2812	0.393	0.5303	0.6269	0.444	0.5194	0.97530	0.9365
	One Layer	5	0.2225	0.6991	0.4717	0.8361	0.411	0.7311	0.9709	0.9707
		10	0.235	0.7749	0.4848	0.8803	0.386	0.8517	0.9812	0.8233

The results showed that neural network has 5 neurons in the hidden layer for energy efficiency (R^2 $_{training}$ = 0.9924-RMSE $_{training}$ =1.421 -MAE $_{training}$ =1.135), and 10 neuron in hidden layer for specific energy loss (R^2 $_{training}$ = 0.9930-RMSE $_{training}$ =0.424 -MAE $_{training}$ = 0.362) and the neural network 5 (First layer) and 5 (Second layer) neuron in the hidden layer

for exergy efficiency (R^2 training = 0.9871-RMSE training =1.283 -MAE training =1.088) and the neural network 10 (First layer) and 10 (Second layer) neurons in the hidden layer for specific exergy loss (R2 training = 0.9831-RMSE training =0.439 -MAE training =0.332) can predict energy efficiency, specific energy loss, exergy efficiency, and specific exergy loss in different oven times and microwave powers (table 5).For energy efficiency, the best value of R^2 Test is observed in a network with 10 neurons in one hidden layer and for specific energy loss in two hidden layer with 5 (First layer) and 5 (Second layer) neuron and for Exergy efficiency in two hidden layer with 10 (First layer) and 10 (Second layer) and Specific exergy loss in hidden layer with 5 neuron . Also for a better understanding of the value of R^2, in Figure 9 R^2 value of training data is shown.

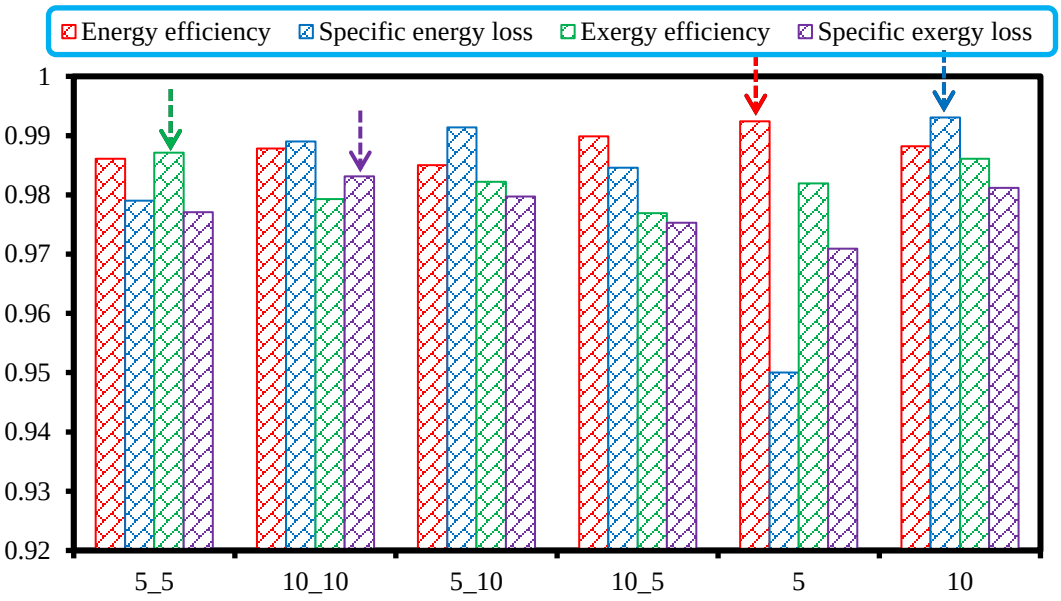

Figure 9. R^2 Value for training data (oven pre-treatment)

Table 6 also shows the best network between input data and data simulated by network for each of neurons in the hidden layer. Lower value of Epoch indicates that the number of neurons in the layer has been able to have learning from the neural network compared to other number of neurons.

As shown in Table 6, the best network for energy efficiency at Training (Run = 1, Epoch = 15) in the 10 (First layer) and 10 (Second layer) neuron state in the hidden layer reaches to constant value after about 15 generations of error, and the best network for the specific energy loss in Training (Run = 1, Epoch = 16) in 5,10 (First layer) and 10,5 (Second layer) neuron in the hidden layer, it reaches to constant value after about 16 generations of errors. For exergy efficiency of Training value (Run = 1, Epoch = 15), it was found in 5, 10 (First layer) and 10, 10 (Second layer) state in the hidden layer, and for the specific exergy loss (Run = 1, Epoch = 16), it was found in the 5,10,5 (First layer) and 10, 5, 5 (Second layer) state in the hidden layer.

Table 6. Some of the best MLP neural network topologies to predict test values (Oven Pre-Treatment)

			EPOCH	RUN
Energy efficiency	Two layer	5-5	19	1
		10-10	15	1
		5-10	18	2
		10-5	17	1
	One Layer	5	20	2
		10	17	3
Specific energy loss	Two layer	5-5	18	5
		10-10	16	2
		5-10	16	1
		10-5	16	1
	One Layer	5	18	4
		10	17	2
Exergy efficiency	Two layer	5-5	17	1
		10-10	15	1
		5-10	15	1
		10-5	18	2
	One Layer	5	19	5
		10	18	1
Specific exergy loss	Two layer	5-5	16	1
		10-10	16	1
		5-10	16	1
		10-5	19	1
	One Layer	5	20	1
		10	19	1

The results table 7 showed that neural network has 10 neurons in the hidden layer for energy efficiency (R^2 $_{training}$ = 0.9889-RMSE $_{training}$ =1.532), and 5 (First layer) and 10 (Second layer) neuron in hidden layer for specific energy loss (R^2 $_{training}$ = 0.9936-RMSE $_{training}$ =0.265 -MAE $_{training}$ = 0.207) and the neural network 5 (First layer) and 5 (Second layer) neuron in the hidden layer for exergy efficiency (R^2 $_{training}$ = 0.9987-RMSE training =1.208 - MAE training =0.939) and the neural network 10 (First layer) and 10 (Second layer) neurons in the hidden layer for specific exergy loss (R2 training = 0.9865-RMSE training =0.401 -

MAE training =0.302) can predict energy efficiency, specific energy loss, exergy efficiency, and specific exergy loss in different ohmic times and microwave powers (table 5).For energy efficiency, the best value of R^2 Test is observed in a network with 5 (First layer) and 5 (Second layer) neuron in hidden layer and for specific energy loss in hidden layer with 5 neuron and for Exergy efficiency in two hidden layer with 10 (First layer) and 10 (Second layer) and Specific exergy loss in hidden layer with 5 neuron. Also for a better understanding of the value of R^2, in Figure 10 R2 value of training data is shown.

Table 7. Error values in predicting experimental data using optimal artificial neural network (ohmic Pre-Treatment)

			MSE		RMSE		MAE		R	
			Training	Test	Training	Test	Training	Test	Training	Test
Energy efficiency	Two layer	5-5	2.868	4.033	1.694	2.008	1.392	1.868	0.9815	0.9902
		10-10	6.433	2.770	2.536	1.664	1.805	1.429	0.979	0.9867
		5-10	2.751	3.502	1.659	1.871	1.350	1.631	0.98706	0.9667
		10-5	2.364	6.389	1.538	2.528	1.337	1.786	0.9880	0.9799
	One Layer	5	2.860	8.899	1.691	2.983	1.398	2.694	0.98701	0.95660
		10	2.347	6.500	1.532	2.550	1.358	2.090	0.9889	0.9705
Specific energy loss	Two layer	5-5	0.087	1.205	0.295	1.098	0.243	0.761	0.9914	0.9513
		10-10	0.565	0.201	0.752	0.448	0.554	0.3395	0.9868	0.9809
		5-10	0.0700	1.404	0.265	1.185	0.207	0.849	0.9936	0.9119
		10-5	0.2476	0.2494	0.498	0.499	0.353	0.4397	0.9781	0.9905
	One Layer	5	0.302	0.1242	0.550	0.352	0.442	0.3327	0.9729	0.99215
		10	0.2496	0.250	0.500	0.500	0.358	0.4115	0.9794	0.9869
Exergy efficiency	Two layer	5-5	1.459	18.66	1.208	4.320	0.939	4.174	0.9978	0.9711
		10-10	3.249	2.922	1.802	1.709	1.529	1.433	0.9849	0.9777
		5-10	3.032	3.553	1.741	1.885	1.423	1.698	0.9790	0.9759
		10-5	2.582	5.64	1.607	2.375	1.358	2.085	0.9804	0.9770
	One Layer	5	2.832	7.939	1.683	2.818	1.332	2.442	0.9807	0.9512
		10	2.322	8.170	1.524	2.858	1.251	2.491	0.9834	0.9661
Specific exergy loss	Two layer	5-5	0.251	0.693	0.501	0.832	0.404	0.749	0.9830	0.9151
		10-10	0.161	1.280	0.401	1.131	0.302	1.0624	0.9865	0.7678
		5-10	0.2347	1.08	0.484	1.039	0.379	1.01	0.9843	0.8173
		10-5	0.281	0.393	0.530	0.627	0.444	0.519	0.9814	0.9415
	One Layer	5	0.2340	0.694	0.484	0.833	0.411	0.702	0.9779	0.9748

		10	0.2225	0.7749	0.472	0.880	0.386	0.851	0.9853	0.9176

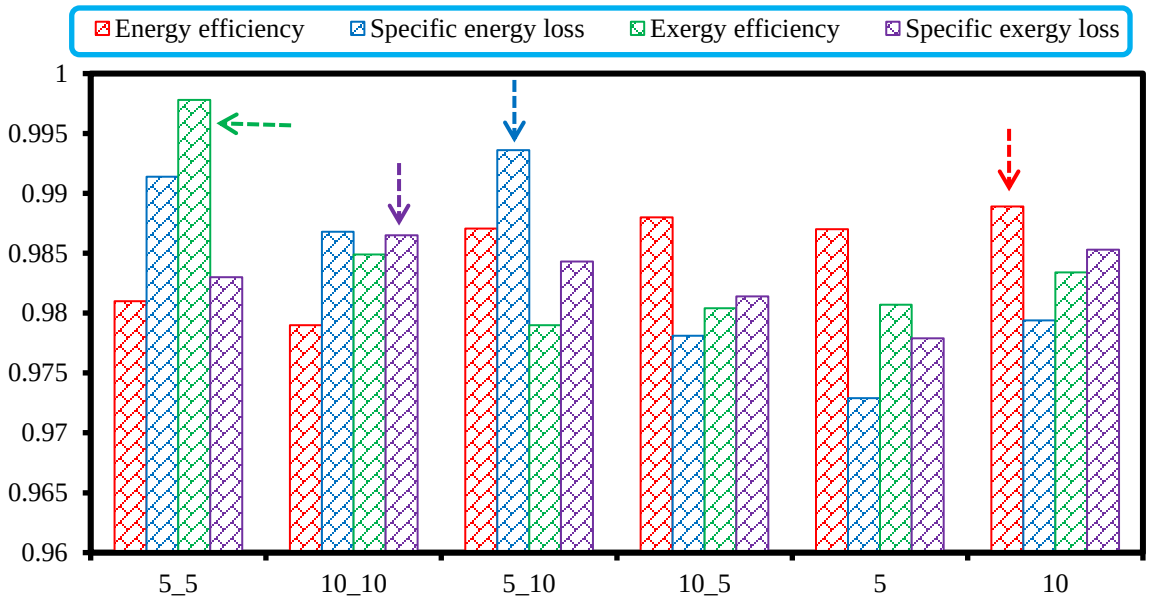

Figure 10. R^2 Value for training data (Ohmic pre-treatment)

s shown in Table 8, the best network for energy efficiency at Training (Run = 1, Epoch = 17) in the 5 (First layer) and 10 (Second layer) neuron state in the hidden layer reaches to constant value after about 17 generations of error, and the best network for the specific energy loss in Training (Run = 1, Epoch = 16) in 10,10 (First layer) and 10,5 (Second layer) neuron in the hidden layer, it reaches to constant value after about 16 generations of errors. For exergy efficiency of Training value (Run = 1, Epoch = 17), it was found in 10 (First layer) and 10 (Second layer) state in the hidden layer, and for the specific exergy loss (Run = 1, Epoch = 13), it was found in the 10 neuron state in the hidden layer.

Table 8. Some of the best MLP neural network topologies to predict test values (Ohmic Pre-Treatment)

			EPOCH	RUN
Energy efficiency	Two layer	5-5	20	1
		10-10	18	2
		5-10	17	1
		10-5	18	2
	One Layer	5	22	4
		10	18	2

Specific energy loss	Two layer	5-5	17	1
		10-10	16	1
		5-10	21	2
		10-5	16	1
	One Layer	5	23	2
		10	18	2
Exergy efficiency	Two layer	5-5	18	5
		10-10	17	1
		5-10	23	3
		10-5	18	2
	One Layer	5	25	2
		10	20	1
Specific exergy loss	Two layer	5-5	20	1
		10-10	16	1
		5-10	19	1
		10-5	18	1
	One Layer	5	16	2
		10	13	1

3.5.1. Sensitivity coefficient for ohmic pre-treatment

As shown in Figure 11 and 12, the ohmic time in a neural network with 5 (First layer) and 10 (Second layer) neurons in the hidden layer was considered as the most effective factor in predicting energy and exergy efficiency and for specific energy and exergy loss, the highest sensitivity was obtained for the ohmic time in the hidden layers with 10 (First layer) and 5 (Second layer). The results of the sensitivity analysis for microwave power are shown in Figure 10. Based on this figure, the highest sensitivity for energy and exergy efficiency was obtained for the microwave power in the one hidden layers with 5 neurons and for specific energy and exergy loss, the highest sensitivity was obtained for the microwave power in the one hidden layers with 10 and in the two hidden layers 10 (First layer) and 10 (Second layer) Respectively.

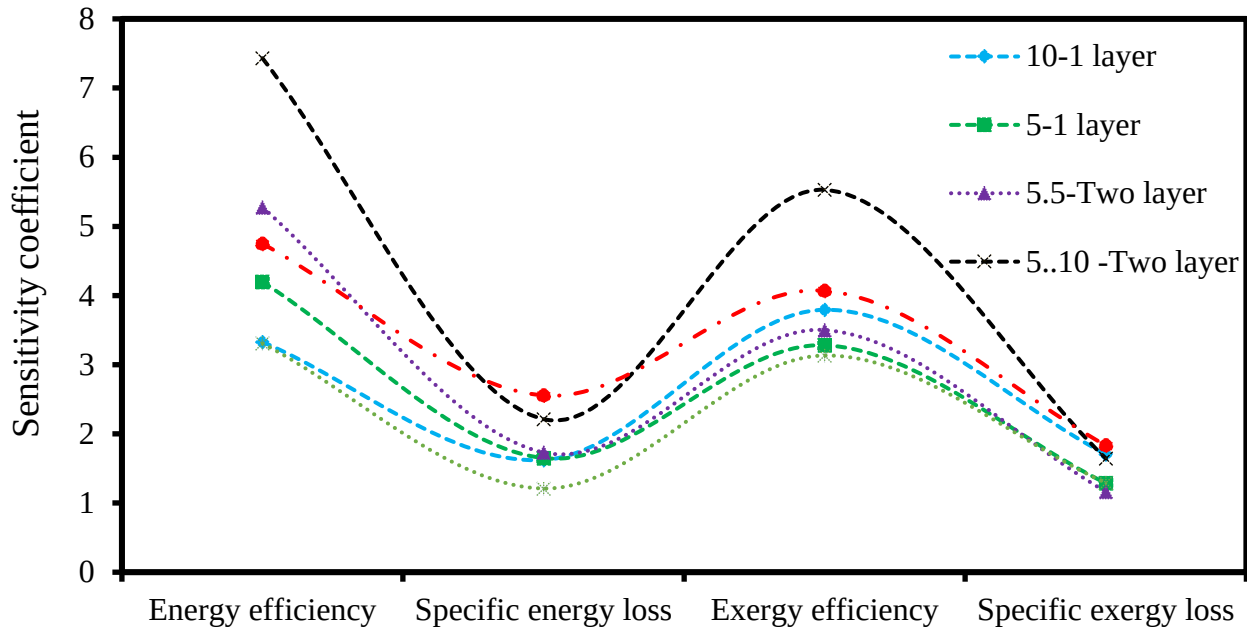

Figure 11. Sensitivity coefficient for ohmic time

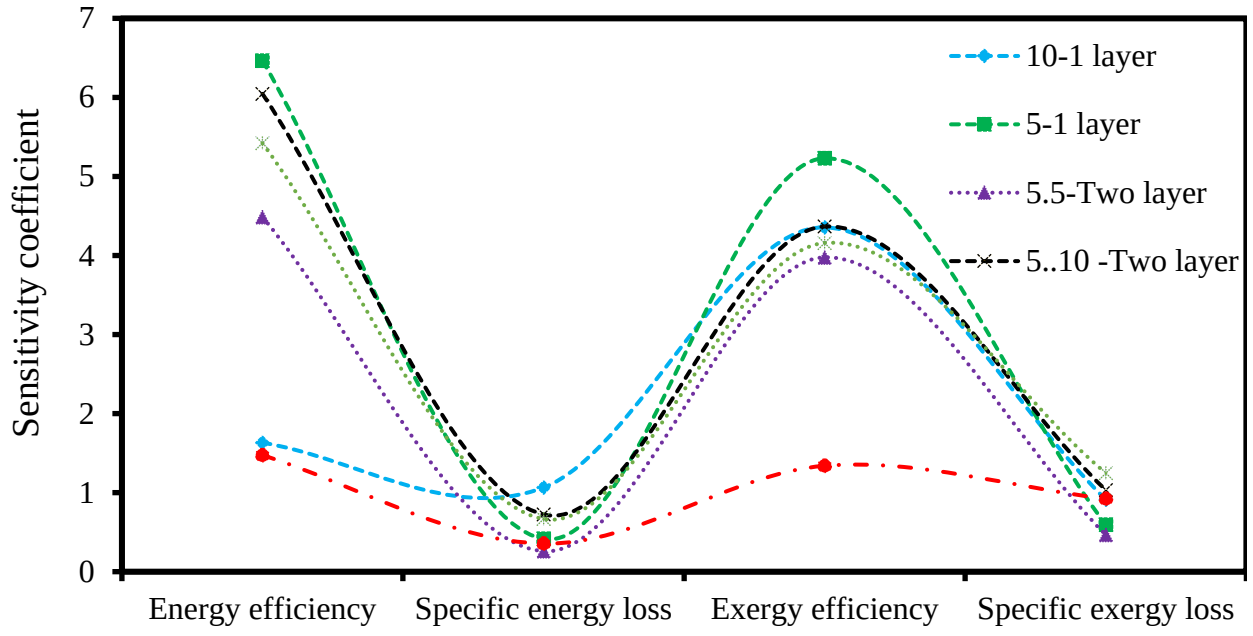

Figure 12. Sensitivity coefficient for microwave power in ohmic pre-treatment

3.5.2. Sensitivity coefficient for oven pre-treatment

As shown in Figure 13 and 14, the oven time in a neural network with 5 and 10 neurons in the hidden layer was considered as the most effective factor in predicting energy and exergy efficiency, Respectively, and for specific energy and exergy loss, the highest sensitivity was obtained for the oven time in the one hidden layers with 10 neuron and two hidden layers with 10 (First layer) and 5 (Second layer) neuron, Respectively. The results of the sensitivity analysis for microwave power are shown in Figure 14. Based on this figure, the highest sensitivity for energy and exergy efficiency was obtained for the microwave power in two hidden layers with 5 (First layer) and 5 (Second layer) neuron and one hidden layer with 10 neuron, Respectively, and for specific energy and exergy loss, the highest

sensitivity was obtained for the microwave power in the two hidden layers with 5 (First layer) and 5 (Second layer) and in the two hidden layers 10 (First layer) and 10 (Second layer) Respectively.

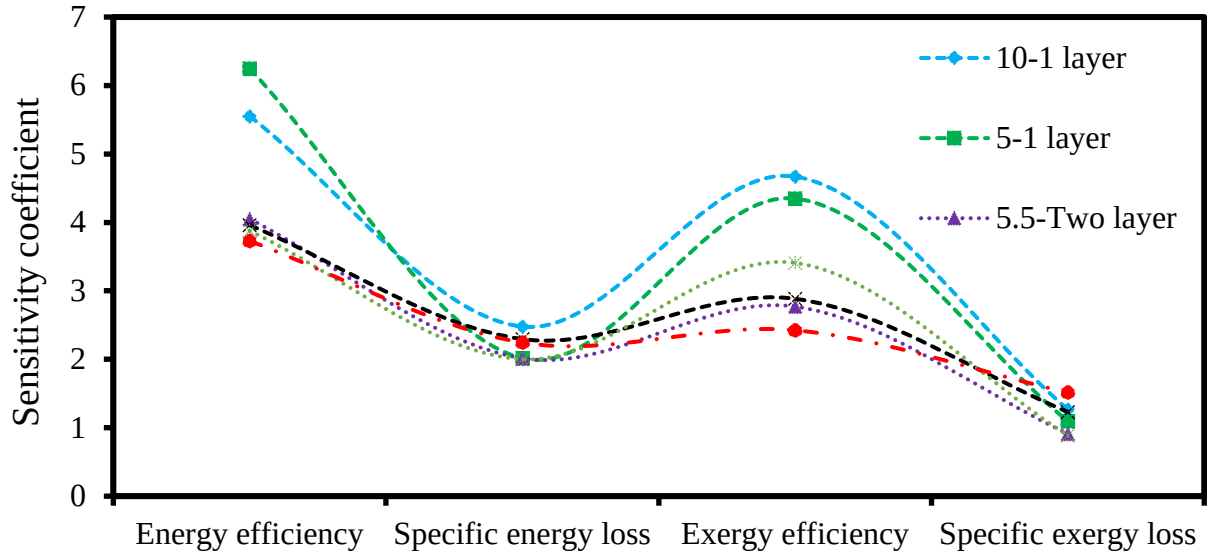

Figure 13. Sensitivity coefficient for oven time

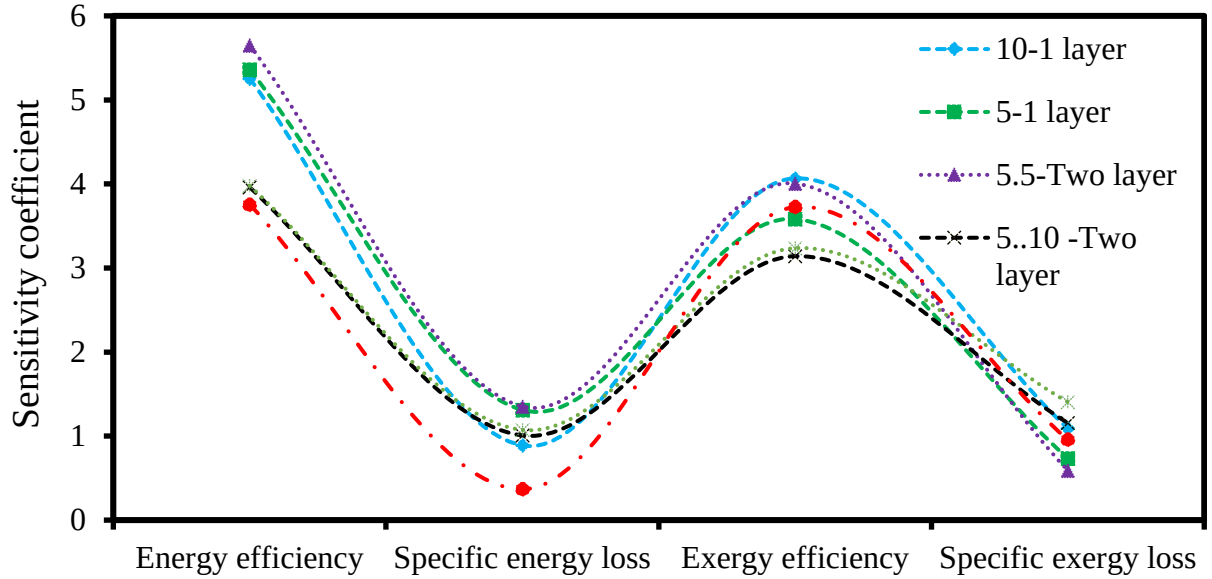

Figure 14. Sensitivity coefficient for microwave power in oven pre-treatment

4. Conclusions

- Microwave power plays an important role in determining the characteristic of kiwi drying. Increasing microwave power increases the energy and exergy efficiency drying, leading to reduced drying time.
- Oven and ohmic pre-treatment has significant effect on energy and exergy loss.
- Ohmic pre-treatment increased the absorption of heat in kiwi, led to an increase in the energy and exergy efficiency during drying.
- Ohmic Pre-treatment has a greater effect on exergy and energy than oven pre-treatment.
- Increasing the ohmic and oven time and microwave power had a significant effect on the amount of energy and exergy.

- Based on the results obtained, ohmic time had more effect on the energy efficiency than the exergy efficiency.
- Also, according to the results with Ohmic pre-treatment, the exergy efficiency has been 2.128 times, and this value has been 1.946 times for oven pre-treatment.
- Also, according to the results with Ohmic pre-treatment, the energy efficiency has been 1.97 times, and this value has been 1.90 for oven pre-treatment.
- Two hidden layer network has shown a higher sensitivity factor for ohmic time and highest sensitivity for power microwave in ohmic pre-treatment was in network by one hidden layer.
- One hidden layer network has shown a higher sensitivity factor for oven time and highest sensitivity for power microwave in oven pre-treatment was in network by two hidden layer.
- Data obtained from network and the initial data obtained from the experiment overlap for the energy efficiency, the specific exergy loss, and the exergy efficiency.
- Given the results obtained for R2, RMSE and Epoch, it can be stated that the neural network has the ability to predict the energy efficiency, specific energy loss, exergy efficiency and specific exergy loss at an acceptable level for kiwi
- To predict the exergy efficiency and specific exergy loss is obtained the best network in a two-hidden layer network.
- To predict the energy efficiency and specific energy loss is obtained the best network in a one-hidden layer network.

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IMPACT OF FREEZING AND DRYING PREPROCESSING ON PIGMENTS EXTRACTION FROM THE BROWN SEAWEED« *PHYLLARIA RENIFORMIS*» COLLECTED IN ALGERIAN COAST

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ABSTRACT

Seaweeds are an excellent source of natural pigments such as chlorophylls and carotenoids that exhibit several bioactive properties fully exploited in food and health products. Due to the high sensitivity and the rapid degradation of pigments, recent researches are now focusing on development of efficient techniques for their extraction, while the sample preprocessing as the main important step attracted less attention. The objective of this study was the evaluation of the effect of freezing and drying preprocessing on pigments quantity, quality and antioxidant activity of the brown seaweed *Phyllaria reniformis*. Pigments were quantified using UV-Visible spectrophotometry and fully characterized by reverse phase high performance liquid chromatography (RP-HPLC). *Phyllaria reniformis* was characterized by a high amount of pigments especially fucoxanthin. Based on UV-visible spectrophotometry results, alga preprocessing before extraction showed a high variability on pigments content. As shown by RP-HPLC freezing preprocessing exhibited the most efficient pigment extraction in term of quantity. While, drying preprocessing demonstrated higher amount of *β*-carotene and pheophytin *a*. The highest and most efficient antioxidant activities were obtained in the frozen samples. The quality, quantity and antioxidant activities of *Phyllaria reniformis* pigments extract was found to be deeply related to the preprocessing step.

1.Introduction

Nowadays marine macro-algae commonly known as seaweeds have been extensively used in food (Durmaz et *al*., 2008; Bocanegra et *al*., 2009; Anis et *al*., 2017), agricultural (Ramya et *al*., 2015), pharmaceutical (Raman and Doble 2015) and cosmetic areas (Fabrowska et *al*., 2015).They represent a natural source of bioactive compounds as they are able to produce a great variety of secondary metabolites such as pigments, flavonoids, polyphenols characterized by several biological proprieties (Lim et *al*., 2002; Kudaa et *al*., 2005; Duan et *al*., 2006; Lordan et *al*., 2011; Vairappan et *al*., 2011; Rajauria et *al*., 2013; Sivaramakrishnan et *al*., 2017).

In the past decade, natural pigments were researched for their safety and health benefits

compared to the synthetic ones. Macro-algae are renewable source of natural pigments such as chlorophylls and carotenoids (Hegazi et *al*., 1998; Durmaz et *al*., 2008).These Pigments have shown many biological activities as antioxidant (Yan et *al*., 1999; Sachindra et *al*., 2007; Hsu et *al*., 2013), anti-obesity (Maeda et *al*., 2007), chemotherapeutic (Hosokawaa et *al*., 2004) and anti-inflammatory activities (Shiratori et *al*., 2005).Brown seaweeds like the other classes of algae are rich on photosynthetic pigments in particular fucoxanthin and chlorophyll *c* (Kumar et *al*., 2017).

Several methods were used for pigments extraction: Conventional or advanced (Kumar et *al*., 2010). For brown seaweed, the conventional method could be lengthy and difficult because of the thalli consistency mainly due to the polysaccharides. Therefore, innovative techniques allow obtaining algae pigments more quickly with higher yield and especially with reduced risk of their degradation. Multiple alternative extraction technologies have been suggested, such as ultrasounds, ultrasound-assisted enzymatic hydrolysis, microwaves, supercritical fluids, pulsed electric fields, high-pressure homogenization and liquid pressurization (Le Guillard et *al*., 2016; Poojary et *al*., 2016; Mittal et *al*., 2017; Zhu et *al*., 2017).

Due to the high instability and easy degradation of pigments, new strategies for samples preprocessing before extraction must be suggested. Acid, enzymes, temperature, heat, light and oxygen, are the most important factors affecting the stability of naturals pigments. Although, many studies on algae pigments extraction and identification have been reported, little information is still available on the relation between pigments content and algae preprocessing. On the other hand, seaweed after their harvest are exposed to degradation, hence, drying and freezing are usually applied to minimize biological compounds degradation and conserve algae for long time.

Against this background, the main purpose of this work was the investigation of the effect of drying and freezing as preprocessing method on seaweed pigments quantity, quality and antioxidants activity. To the best of our knowledge, this is the first report on pigments characterization by spectrophotometer and by RP-HPLC analysis of *Phyllaria reniformis* collected from the Algerian coast and the effect of conservation method on seaweed pigments quality and quantity has also been understated.

2. Materials and methods

2.1. Seaweed collection and preprocessing

The brown seaweed *Phyllaria reniformis* was collected by hand at more than 15 meters depth from Tipaza (Algeria) in June. After a first rinse on-site with sea water, samples were taken to the laboratory in isothermal boxes. Then, all samples were washed for a second time with fresh tap water to remove sand, epiphytes, shells and any sediment. Afterwards, distilled water was used for a third wash.

The fresh alga samples were divided in three parts. One part was dried at 38 ± 1°C for one week, another part was frozen at -18°C for one week and the last part was immediately prepared for extraction. All these steps were performed in low light and as quickly as possible to prevent pigment degradation.

2.2. Extraction of seaweed pigments

The fresh, frozen and dried alga samples were cut into small pieces of 3 to 5 mm and mixed with acetone at a ratio of 1/3 (w/v). Pigments were extracted in an ultrasonic bath in the following conditions: Power 100W and 20Hz for 90 min at 24°C.

Then, all the obtained extracts were filtered and the solvent was evaporated using rotary evaporator at 28°C. The obtained residues were lyophilized and stored at – 20°C in brown glass flasks for later analysis.

2.3. Chlorophylls and carotenoids content

To estimate the chlorophylls and carotenoids contents in samples, 100 mg of dry extract were mixed with 2 mL of solvent and filtered using Nylon microfilter (0.45 μm). The Absorption (Abs) of pigments in the filtrate was recorded in

the range of 350-800 nm by UV-Visible Spectrophotometry (SPECORD 210 PLUS 623F1138, Germany). The content of chlorophyll *a*, *b*, *c*, Fucoxanthin and total carotenoids were calculated according to the equation of Lichtenthaler and Wellburn (1983) and Seely et *al*., (1972).

$$[C_a] = 11.75 \times Abs_{662} - 2.35 \times Abs_{665}$$
$$[C_b] = 18.61 \times Abs_{645} - 3.96 \times Abs_{662}$$
$$[C_c] = (Abs_{631} + Abs_{581} - 0.3Abs_{664})/62.2$$
$$[Fx] = (Abs_{470} - 1.239 \times (Abs_{631} + Abs_{581} - 0.3 \times Abs_{664}) - 0.0275 \times Abs_{664})/141$$
$$[Tot\ Carot] = (1000 \times Abs_{470} - 2.27 \times C_a - 81.4 \times C_{ab})/227$$

Where:

Abs is the absorbance in the specified wavelength

C_a is the concentration of chlorophyll *a*

C_b is the concentration of chlorophyll *b*

C_c is the concentration of chlorophyll *c*

Tot Carot is the total carotenoids

Fx is the fucoxanthin

2.4. High performance liquid chromatography pigments Analysis

The separation of seaweed pigments was conducted by analytical HPLC (Agilent 1100, USA) equipped with UV-Visible detector. The column was C18, 5µm, 150× 4.6 mm. The injection loop size was 20 µl. The used method was inspired from the study of Wright (1991).The column was equilibrated using a gradient of elution of solvent A (methanol: 0.5M ammonium acetate, 80:20 v/v) and solvent B (Acetonitrile: water, 90:10 v/v), solvent C (ethyl acetate). The flow rate was 1mL/min, and the gradient was as follows (minutes; % solvent A; % solvent B; % solvent C): (0; 100; 0; 0), (4; 0; 100; 0), (18; 0; 20; 80), (21; 0; 100; 0), (22;100;0;0), (25; 100; 0; 0). The column equilibration was 10 min. Pigments were detected by recording Abs at 440 nm. All these steps were carried out at room temperature. The obtained HPLC peaks were identified by comparing the retention times with those of standards pigments.

2.5. Pigments standards

The authentic standard pigments Chlorophylls (*a, b,* c_1+c_2 and c_3), Chlorophyllides *a* and fucoxanthin were obtained from *Sargassum vulgare* by semi preparative HPLC and to confirm the purification, each pigment was chromatographed in the analytical column C18 and C8. The same gradient was used with a flow rate of 5mL/min. Each pigment was collected at the outlet of the detector, isolated immediately from solvent by evaporation. Pheophytin *a* was obtained by acidification of chlorophyll *a* with 1M of hydrochloric acid (HCl) (Wright 1991). The identification of separated pigments was confirmed from their visible spectral absorption and compared with the literature (Pereira et *al*., 2014). Visible spectra were obtained with UV-Visible Spectrophotometer (SPECORD 210 PLUS 623F1138, Germany). *β*-Carotene was purchased from Sigma Aldrich.

2.6. DPPH radical scavenging activity

The pigment extracts antioxidant activity was evaluated using a modified method previously described by (Menaceur et *al*., 2013) and (Hazzit et *al*., 2009) 25µL of each sample at different concentrations (from 0 to 100 µg/mL dissolved in methanol) were added to 1975 µL of 2,2-diphenylpicrylhydrazyl (DPPH) solution (0.0024%) and incubated for 30 min in the dark at room temperature. The Abs was measured at 517 nm with UV-Visible spectrophotometer (SPECORD 210 PLUS 623F1138, Germany). Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) were used as standard and all measurements were done in triplicates. The DPPH radical scavenging activity was calculated using the following equation:

$$Scavenging\ activity\ (\%) = (Abs_b - Abs_s\ /\ Abs_b) \times 100$$; Where Abs_s is the sample Abs after 30 min and Abs_b is the sample Abs before reaction. The concentration providing 50% inhibition (IC_{50}) of samples was calculated

using the graph by plotting inhibition percentage against concentration.

2.7. Statistical Analysis

All the analysis was run in triplicate. The data are presented as Mean ± Standard error. The Statistical Package for Social Science (SPSS Version: 20) was used for the analysis. One-way analysis of variance (ANOVA) was performed and comparison of data for significant differences (p-value ≤ 0.05) was made with Tukey's HSD test.

3. Results and discussions

3.1. UV-Visible absorption spectra of pigments extract

Chlorophylls and carotenoids represent the major group of photosynthetic pigments found in plants and in algae. Each group has multiple types of pigment that can be identified by the specific wavelength. Pigments absorb on only specific wavelengths of visible light while reflecting the others; the reflected light is color. The set of wavelengths absorbed by a pigment is its absorption spectrum.

Figure 1 shows the absorption spectrum of the obtained pigment extracts recorded from 350 to 800 nm. All the pigments extracts absorb mostly in the blue (between 400 and 500nm) and red (between 600 and 700nm) visible spectral regions. A high Abs was observed in pigments extract obtained after freezing preprocessing followed by that obtained after drying preprocessing while the low Abs was recorded in the extract of fresh alga.

The broad absorption in the blue and red regions is probably due to the presence of carotenoids, chlorophyll *a* and chlorophyll *b* in the three pigments extracts. Each pigment has unique Abs spectra, whereas carotenoids absorb visual light broadly in the blue spectral range from 400 to 500 nm, whilst chlorophyll *a* and chlorophyll *b* absorb with narrow bands maximally in the blue (near 430 and 453nm) and red (near 662 and 642nm).

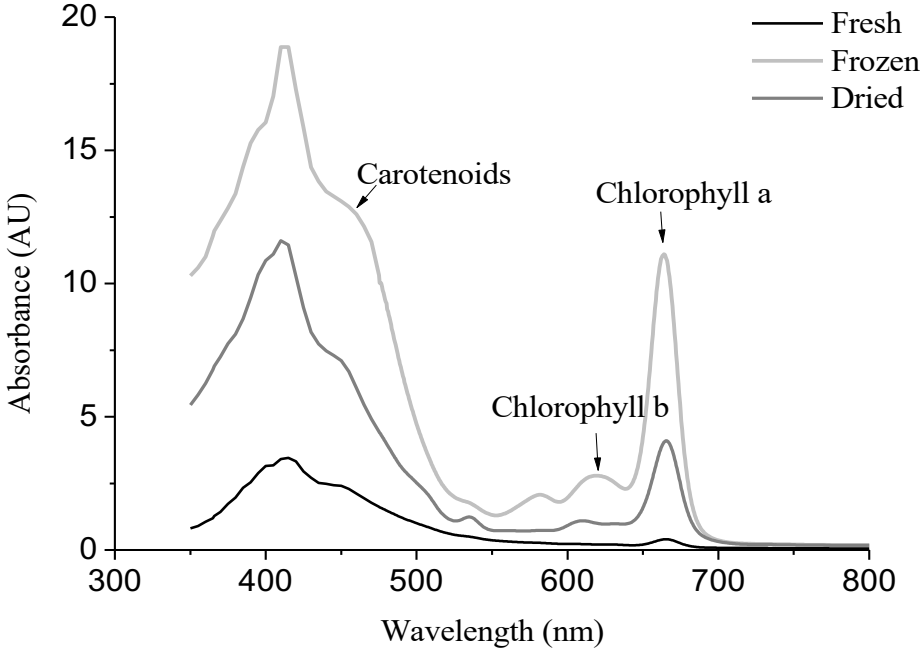

Figure 1. Absorbance spectra of pigments extract from fresh, frozen and dried *Phyllaria reniformis.*

3.2. Chlorophylls and carotenoids contents

Chlorophylls and carotenoids content in the three pigment extracts (dry, fresh and frozen) were determined by UV-Visible spectrophotometry and presented on figure 2 for Chlorophyll *a*, *b* and *c* and figure 3 for fucoxanthin and total carotenoids.

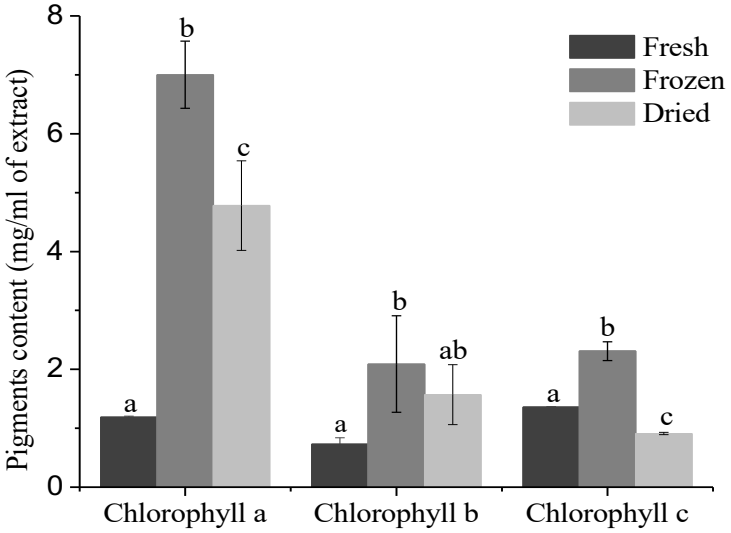

Figure 2. Chlorophylls content in pigments extracts obtained from the fresh, frozen and dried brown alga *Phyllaria reniformis* (Mean ± SD). Within any given pigment, bars with different letters indicate significant differences between alga preprocessing types (p-value ≤ 0.05, Tukey's HSD test)

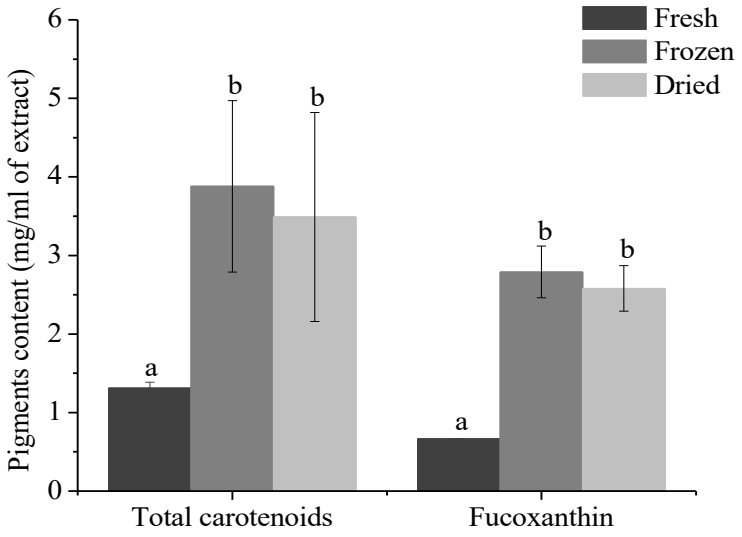

Figure 3. Total carotenoids and fucoxanthin content in pigments extracts obtained from the fresh, frozen and dried brown alga *Phyllaria reniformis* (Mean ± SD). Within any given pigment, bars with different letters indicate significant differences between alga preprocessing types (p-value ≤ 0.05, Tukey's HSD test)

Results showed a variability of quantities for each pigment in relation to the applied preprocessing. For Chlorophyll *a*, *b*, fucoxanthin and total carotenoids, the highest amount were reported in frozen sample extract (7.00 ± 0.57, 2.09 ± 0.82, 2.79 ± 0.33, 3.88 ± 1.09 mg/mL respectively) followed by the dried one (4.78 ± 0.76, 1.57 ± 0.51, 2.58 ± 0.29, 3.49 ± 1.33 mg/mL respectively) and the lowest amount of these pigments were found in the fresh sample (1.19 ± 0.01, 0.73 ± 0.11, 0.66 ± 0.00, 1.31 ± 0.07 mg/mL respectively).

The highest value for chlorophyll *c* was found in the frozen extract (2.31 ± 0.16 mg/mL) followed by the fresh alga extract (1.31 ± 0.36 mg/mL), however the lowest have been demonstrated in extract obtained after drying preprocessing (0.91 ± 0.02mg/mL).

The analysis of variance showed a significant effect of preprocessing on pigments contents (*p*-value ≤ 0.05). However, the pairwise comparisons using Tukey's HSD test revealed that for both, total carotenoid and fucoxanthin, no significant difference was found between drying and freezing preprocessing, but for chlorophylls (*a* and *c*) it was statistically significant. This test revealed also a significant difference between fresh and frozen samples extracts in chlorophylls (*a*, *b*, *c*), fucoxanthin and total carotenoid.

The freezing preprocessing of alga before pigments extraction gave the highest yields; this is probably due to the degradation of the thalli by freezing effect. Whilst the drying preprocessing revealed also an important pigments yield compared to the fresh one, but chlorophyll *c* was underestimated due to the low water content in the dried alga.

The use of UV-Visible spectrophotometry for quantitative determination of chlorophylls and carotenoids is complicated. Due to a similarity in the Abs spectra of some pigments, there could be an underestimation or an overestimation, therefore concentration of total chlorophylls and total carotenoids could accurately be estimated, however individual pigment concentration was difficult to be resolved (Thrane et *al*., 2015).

Furthermore, the determination of the pigment content may have unfair value due to the formation of new products such as pheophytins and chlorophyllides resulting from pigment degradation and having similar wavelength absorption to the original pigment. For that reason, the high content of chlorophyll *a* may be related to chlorophyll *c* that was abounded in brown seaweeds, and it may result to their degradation to pheophytin *a* and chlorophyllide *a*. Moreover, the accuracy of UV-Visible spectrophotometric method is also affected by other facts such as the solvent used for extraction, the type of sample, the sample preprocessing and also the spectrophotometer used (Haryatfrehni et *al*., 2015; Ritchie 2018).

3.3. HPLC analysis

HPLC is considered as an efficient method for measuring pigment concentrations in plant and algae. This technique can resolve most chlorophylls and carotenoids, including their degradation products such as pheophytins (Mantoura and Llewellyn 1983)

Table 1 lists the photosynthetic pigments separated of samples extracts and their retention times. Figure 4 shows typical chromatograms (A, B, C) resulting from RP-HPLC analysis of pigments extracts from respectively fresh, frozen and dried alga samples.

For each sample a good resolution of the major pigments was achieved. Twelve peaks indicating pigments were resolved, as shown in table 1. At the polar end of the chromatogram, chlorophyllides *a*, chlorophyll *c3*, and chlorophylls *c1*, *c2* were almost resolved, however in the central region of the chromatogram, fucoxanthin, trans-neoxanthin and two unidentified components were presented; while at the non-polar end of chromatogram, chlorophylls *a* and *b*, pheophytin *a* and *β*-carotene were resolved.

Table 1. Photosynthetic pigments of *Phyllaria reniformis* extract (Fresh, Frozen, Dried)

Peak	Retention time (min)	Pigment	Fresh	Fozen	Dried
0	1.55	Solvent	+	+	+
1	3.92	Chlorophyllide *a*	+	+	+
2	4.84	Chlorophyll *c3*	+	+	+
3	5.68	Chlorophyll *c1*, *c2*	+	+	+
4	7.38	Fucoxanthin	+	+	+
5	8.48	Trans-neoxanthin	+	+	+
6	8.78	UNK*	+	+	+
7	9.34	UNK	+	-	+
8	14.38	Chlorophyll *b*	+	+	-
9	14. 96	Chlorophyll *a*	+	+	+
10	15.08	Chlorophyll *a*	+	+	+
11	16.73	phaeophytins	+	+	+
12	17.50	*β* Carotene	+	+	+
a	6.46	UNK	-	-	+
b	16.25	UNK	-	-	+
c	17,11	UNK	-	-	+

UNK: unknow

In all, chromatogram of fresh alga (Figure 4.A) was dominated by fucoxanthin, followed by chlorophyll *a*, then chlorophyll *c1+c2*. These three pigments are the main pigments in brown algae, which impart a greenish brown color to the algae (Kadam et *al*., 2013).Chlorophyll *c1* and *c2* are only found in phaeophyceae (Rowan 1989). Smaller amounts of chlorophyllides *a*, chlorophyll *c3*, chlorophyll *b*, pheophytin *a* and *β*-carotene were also resolved. The same resolution was found in the frozen alga sample (Figure 4.B) but with higher peaks.

Chromatogram of the dried pre-proceeded sample (Figure 4.C) shows also a high amount of fucoxanthin and chlorophyll *a*, and a lower amount of chlorophyll *b*, with lower peak intensity in comparison to that of freezing preprocessing but superior to that of the fresh sample. However, in the same sample, chlorophyll *c1+c2* was less abundant compared to the fresh and frozen ones, this might be caused by the high polarity of chlorophylls *c*. Another possible reason may be the percentage of water missing during solvent extraction by aqueous acetone in the dried sample which may lead to a lower diffusion of chlorophylls *c* than that in both fresh and frozen samples. Therefore, drying sample before extraction might be suitable for extraction of hydrophobic compounds (nonpolar) probably because of the lower water content.

β-carotene is completely hydrophobic hence, it was presented by the highest intensity peak compared to fresh and frozen samples. According to Seely et *al*., (1972) dimethyl sulphoxide (DMSO) a more polar solvent was shown to extract much of the chlorophyll *c* and fucoxanthin from the intact thalli of brown algae, while subsequent extraction with acetone rapidly removes most of the chlorophyll *a* and *β*-carotene.

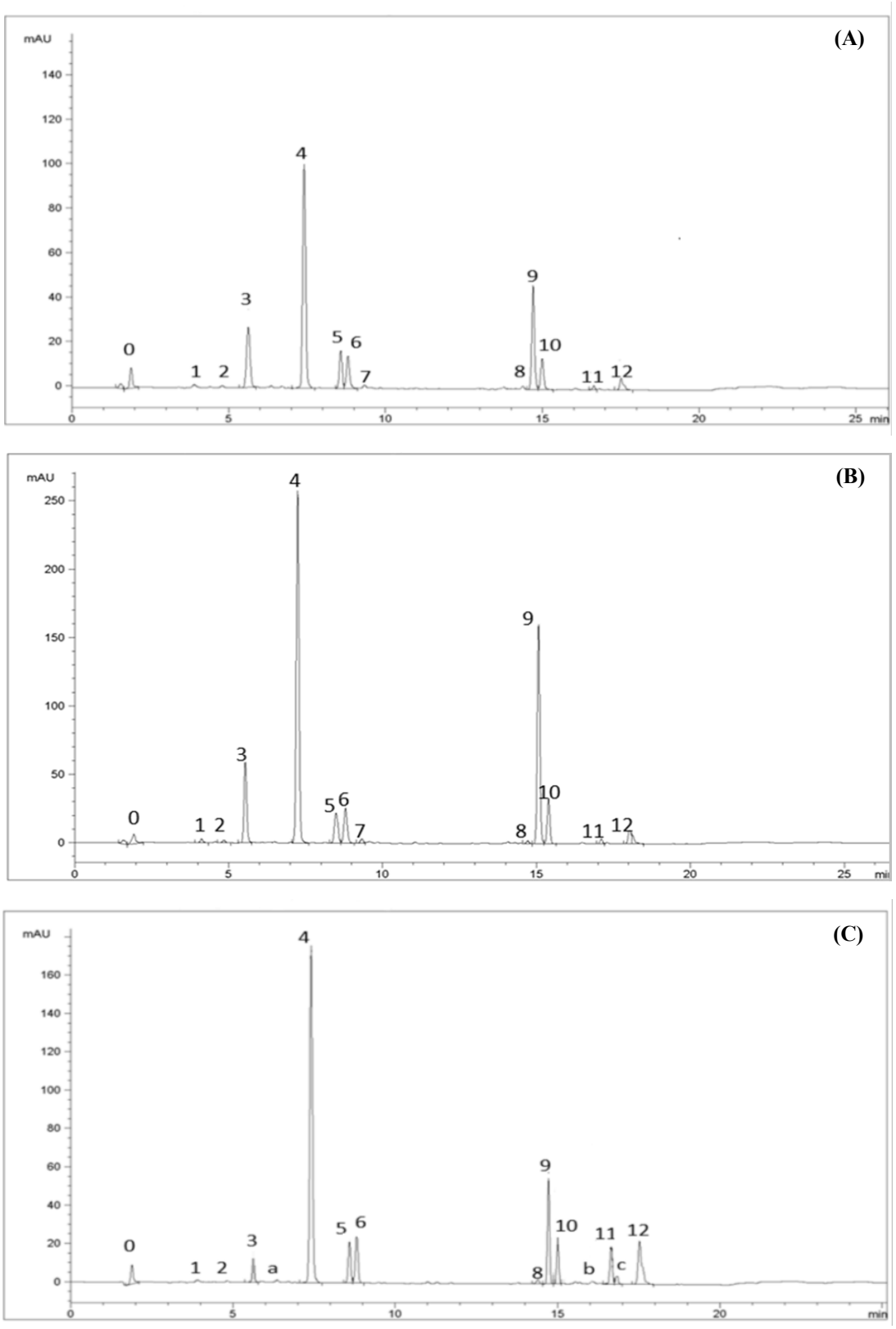

Figure 4. Chromatogram separation of pigment extracts from the fresh (A), the Frozen (B) and the dried (C) brown algae *Phyllaria reniformis*.

Similar result was observed for pheophytin *a*, a hydrophobic pigment highly present in the dried sample probably due to the degradation of chlorophyll *a*. When the chlorophylls were exposed to heat or acidic conditions, the magnesium ion is lost from their structure and the resulting molecule was (pheophytin) which exhibits olive-green color (Mohamed et *al*., 2012). Other traces of pigments are present in the dried sample chromatogram such as the unknown (peaks a, b, c) which may indicate that a little degradation had occurred.

Based on these results it can be assumed that *Phyllaria reniformis* drying before pigments extraction can lead to a selection of pigments especially *β*-carotene. However, Hynstova et *al*., (2018) concluded that the processing of *Chlorella vulgaris* and *Spirulina platensis* dried powder will lead to a decrease *β*-carotene content, probably due to heat or light exposure. The study of Tang and Chen (2000) on the stability and degradation of freeze-dried carotenoids powder showed that the amount of *β*-carotene and lutein decreased with increasing storage temperature. Several researchers demonstrated that carotenoids tend to decrease with increasing drying time due to oxidation and isomerization (Anguelova and Warthesen 2000; Karabuluta et *al*., 2007). According to Chan et *al*., (1997) the nutritional composition including pigments of seaweed *Sargassum hemiphyllum* is greatly affected by different drying methods.

3.4. Antioxidant activity

In comparison with red and green seaweeds, brown seaweeds are characterized by higher antioxidant potential. Several researches demonstrated that brown algae extracts and especially algae pigments are comparatively similar or superior to synthetic antioxidants due to the presence of carotenoid and fucoxanthin (Tutour et *al*., 1998; Sudhakar et *al*., 2013; Kosanić et *al*., 2019). Moreover, chlorophylls, pheophytins and carotenoids are known to act as antioxidants to prevent oxidative DNA damage and lipid peroxidation (Lanfer-Marquez et *al*., 2005; Heo et *al*., 2008; Sindhu et *al*., 2010; Hsu et *al*., 2013). In the present study the antioxidant abilities of pigments extracts were evaluated by scavenging of DPPH radical. The scavenging effect increased with the increasing sample concentrations as shown in figure 5.

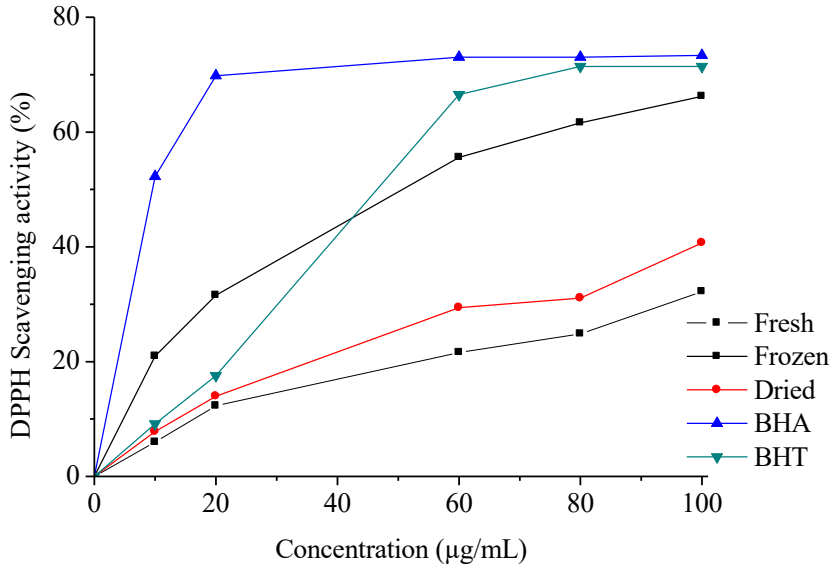

Figure 5. Free radical-scavenging capacities of reference antioxidant (BHA, BHT) and pigments extracts obtained from the fresh, frozen and dried brown alga *Phyllaria reniformis,* (Mean ± SD)

All pigment extracts from fresh, dried and frozen alga exhibited antioxidant activity. The frozen sample extract (at a concentration exceeding 60µg/mL) showed significant activity almost similar to BHA and BHT. In the same sample, the maximum alga pigment extract concentration used (100µg/mL) exhibited more than 80% of radical inhibition while those extracted from the dried and fresh sample extracts showed lower activities 50.82% and 32.17%, respectively.

The effectiveness of antioxidant properties is inversely correlated with their IC_{50} values representing the concentration of extracts at which they scavenge the 50% of the DPPH solution. The lower the IC_{50} value of an antioxidant the higher would be its free radical scavenging power. Figure 6 displays comparison of the IC_{50} values of BHA and BHT as standards with those of pigments extracts.

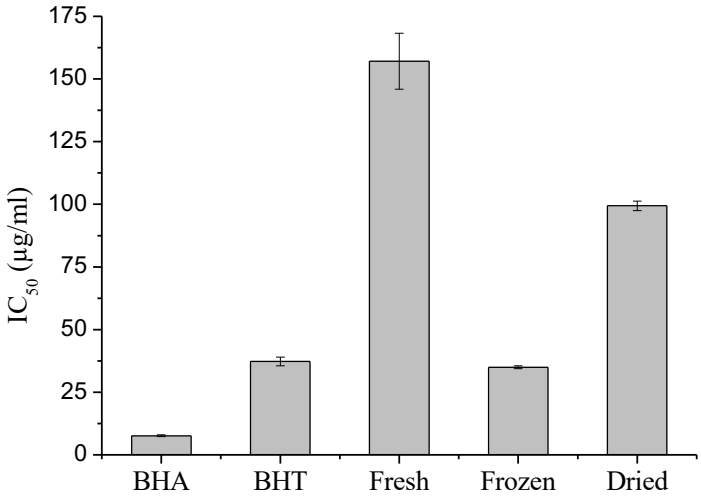

Figure 6. DPPH (IC_{50}) values of reference antioxidants (BHA, BHT) and pigments extracts obtained from the fresh, frozen and dried brown alga *Phyllaria reniformis*, (Mean ± SD)

Pigments extract obtained after frozen preprocessing was the most efficient by the lowest IC_{50} values of 34.96±0.6 µg/ml among all extracts and BHT reference antioxidant, and it was less efficient compared to BHA reference antioxidant. A low antioxidant activity was observed in the fresh sample extract with 157.09 ±11.14 µg/ml of IC_{50} value. While the IC_{50} of the dried sample extract was 99.39±1.90µg/ml. The analysis of variance showed a significant difference between the pigments extracts (*p*-value ≤ 0.05). This difference is probably due to the effect of the preprocessing of the alga before pigments extraction.

4. Conclusions

Marine algae are an excellent source of biologically active compounds for pharmaceutical, food, cosmetic sectors. Seaweed could be exploited as a good source of natural pigments. Consequently, for appropriate pigments extraction method, the preprocessing step of algae before extraction remains the most important because of the highest sensitivity of pigments. This study showed the effect of drying and freezing preprocessing on quantity, quality and antioxidant activity of pigments extracts. Based on the obtained results from the spectrophotometric determination of chlorophylls and carotenoids and their separation by chromatography method (RP-HPLC), the freezing preprocessing of alga was

the most efficient technique to isolate high level of chlorophylls and carotenoids. The drying preprocessing gave also a fairly large amount of pigments compared to fresh alga especially for hydrophobic pigments such as *β*-carotene. This may be due to the small content of water in sample, in spite of that, drying could contribute to a loss of pigments justified by the presence of pheophytin *a* probably produced after chlorophylls degradation and other pigments traces. According to DPPH scavenging activity results, *Phyllarira reniformis* could constitute a natural source of antioxidant substances of high importance. The highest activity was obtained in frozen sample extract. To sum up, this study offers to *Phyllarira reniformis* the opportunity to be used as a natural source of biocompounds in different fields, because of its richness in antioxidant pigments especially fucoxanthin. On the other hand, and from an economical point of view, freezing preprocessing is an appropriate method for pigments extraction with high efficiency. Freezing before pigments extraction could be employed to recover more pigments from algae in term of quality, because of alga thalli degradation, while the drying preprocessing led to the extraction of higher contains of the most stable pigments such as *β*-carotene. The choice of the suitable preprocessing technique before pigments extraction could direct the researcher to a specific pigment.

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COMPARISON OF CHEMICAL COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF PEKIN DUCK AND CHERRY VALLEY DUCK EGGS

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ABSTRACT

The aim of this study was to determine and compare chemical composition and physicochemical properties of duck eggs obtained from the two common duck breeds in Croatia, the Pekin duck and Cherry Valley duck. A total sample of 120 eggs (60 eggs of each duck breed) was collected from one year old free range raised ducks. The Cherry Valley duck eggs were significantly heavier than Pekin ducks and had higher percentage of albumen, while the Peking duck eggs had higher percentages of yolk and shell. The crude protein and total ash contents in the yolk were recorded to be significantly higher at Cherry Valley duck eggs, while crude fat and carbohydrate contents were significantly higher at Pekin duck eggs. The Cherry Valley duck eggs had significantly higher crude protein content in the albumen, while Pekin duck eggs had significantly higher carbohydrate content. Total ash content in the egg shell was significantly higher at Pekin duck eggs. No significant differences of albumen and yolk pH were observed. The Pekin duck egg yolks had higher intensity of the red color.

1. Introduction

Eggs are sources of protein, fats and micronutrients that play an important role in basic nutrition (Miranda *et al*., 2015). Egg consumption differs widely among countries, with per capita consumption being high among the developed countries (Ayim-Akonor and Akonor, 2014). Hen and duck eggs are the most commonly eaten eggs, and are highly nutritious (Kaewmanee *et al*., 2009). The duck eggs has become increasingly more important in the world because of its nutrition and less capital input is required to produce it. In several countries of the Far East duck eggs are produced and consumed in large quantities by the local population for many years, but duck eggs are usually not consumed in the countries in America and Europe in the second half of the 20th century, mostly due to the potential Salmonella risk (Huang and Lin, 2011). However, in the first part of the 21st century, there has been an increase in the use of duck eggs. The study of Owen *et al*. (2016) has shown that Salmonella spp. was detected only in two of 145 samples of ducks eggs, similar to that found in hen eggs.

Ducks produce larger eggs with more nutrients and contain relatively less water and higher percentage of proteins and fats in the yolk, albumen and total contents of egg as compared to hen eggs (Etuk *et al*., 2012). Duck eggs also have a relative higher percentage of egg yolk compared with other avian eggs. This favors duck eggs when the products utilize the egg yolk instead of whole egg (Huang and Lin, 2011). Duck eggs provide plenty of complete, high-quality protein (which includes all amino acids essential for humans) and supply many substances with biological functions beyond basic nutrition (Lopez-Fandino *et al*., 2007).

Duck breeding has also received immense attention due to its higher profitability compared to other poultry species, mainly due to higher feed conversion ratios (El-Soukkary *et al*., 2005). Market demand is progressively focused on high quality products (organic or others sustainable production under quality control) which requires free range conditions. One of the reasons is that intensive production conveys a negative picture of the welfare of the animals, and the products are generally considered low quality (Huang *et al*., 2012). According to that demand, an increasing number of family farms in Croatia starting with duck raising in the backyard under free range conditions where the number varies from a few dozen to a few hundred ducks.

Knowledge of the egg chemical composition and the physicochemical properties of its individual components can increase the potential applications in the food industry and can also enhance our understanding of various biological processes (Raikos *et al*., 2006). This knowledge should be also useful for interpreting the changes that occur during egg storage and during pasteurizing, drying and freezing (Powrie and Nakai, 1999).

A few studies were found in the literature about chemical composition and the physicochemical properties of Pekin duck eggs, but there is scarity of literature about chemical composition and the physicochemical properties of Cherry Valley duck eggs. The aim of this study was to determine and compare chemical composition and physicochemical properties of duck eggs obtained from the two most common duck breeds in Croatia, the Pekin duck and Cherry Valley duck.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Duck eggs used in this study were collected from two family farms located within a circle of 60 km of the Zagreb, capitol of Croatia. On the first farm located near Krizevci (latitude 46° 01’ N, longitude 16° 32’ E) Pekin ducks were breeding, and on the second farm located near Ivanic Grad (latitude 45° 43’ N, longitude 16° 23’ E) Cherry Valley ducks were breeding. Both farms have annual production of about hundred ducks. On both farms ducks are free range raised and fed with combined forage based on cereals and with kitchen waste. Ducks spend only the night in a closed object, while during the day they are on the fenced area with allowed access to open water. According to size and housing system those farms are similar to most duck farms from that part of Croatia. Eggs were randomly collected during May 2018 from one year old female ducks. A total sample of 120 eggs was evaluated, consisting of 60 eggs collected from each duck breed.

2.2. Methods

2.2.1. Egg weight and composition

To evaluate the total egg weight, eggs were separately weighed on a precision electronic balance reading to 0.01 g. Before weighing the yolk, the chalazae were carefully removed from the yolk and the yolks were separated from the albumen. All yolks were also rolled on a paper towel to remove adhering albumen. The shells were carefully washed and dried for 48 h in a drying oven at 21°C and then weighed. Albumen weight was determined by subtracting yolk weight and shell weight from the original egg weight. Using the individual weight of each egg and its components, albumen percentage (albumen weight/egg weight x 100), yolk percentage (yolk weight/egg weight x 100) and shell percentage (shell weight/egg weight x 100) were calculated.

2.2.2. Egg chemical composition

The basic chemical composition of egg albumen, yolk and shell was analyzed by the standard methods of AOAC (1980). The dry matter content was determined by drying a sample at 100°C until constant weight according to AOAC method 925.30. The crude protein content resulted from total nitrogen content assessment via the Kjeldahl method, according to AOAC method 925.31. The total nitrogen content was multiplied by 6.25, which generated the crude protein content. Total lipids

as crude fat content was determined by AOAC method 925.32. The total ash content was assessed via incinerating at 550 ºC in accordance with AOAC method 900.02. The carbohydrate content was calculated as the difference between 100% and the sum of the percentages of water, crude protein, crude fat, total ash and crude fibre. The egg shell calcium content was determined by complexometric method and phosphorus content was determined by spectrometric method.

2.2.3.Egg physicochemical properties

The pH of egg yolk and albumen were measured by using a digital pH meter Mettler Toledo SevenMulti (Mettler-Toledo GmbH, Greifensee, Switzerland). For measurement of yolk color according to CIE (1986) L* a* b* color system a Minolta Chroma Meter CR-310 (Minolta Camera Co. Ltd, Osaka, Japan) was used. The L* value indicates the lightness, representing dark to light (0–100). The a* (redness) value indicates the degree of the red-green color, with a higher positive a* value indicating more red color. The b* (yellowness) value indicates the degree of the yellow-blue color, with a higher positive b* value indicating more yellow color. Subjective yolk color was determined using the Roche Yolk Color Fan (DSM Nutritional Products, Kaiseraugst, Switzerland) by one person.

2.2.4. Statistical analysis

Statistical data analysis was done with the SAS software (SAS Institute, 2004). The results were expressed as mean value ± standard deviation (SD) of 60 measurements for egg chemical and physicochemical properties for each duck breed. The significance of differences between the values of observed parameters was assessed by analysis of variance (ANOVA). The Fisher's least significant difference (LSD) test was used to compare the means and differences were considered as significant at the level of probability $P<0.05$.

3. Results and discussions

3.1. Egg weight and composition

The total weight and composition of Pekin duck and Cherry Valley duck eggs are presented in Table 1. The total weight of the Cherry Valley duck eggs and the weight of egg components (albumen, yolk and shell) was significantly higher at Cherry Valley duck eggs ($P<0.05$). There is deficit of technical information and data in the scientific literature about physical and chemical characteristics of Cherry Valley duck eggs, so results obtained in this study were compared with the characteristics of Pekin duck eggs published by other authors. The average weight of Pekin duck eggs observed in this study (71.91 g) was higher than weight of Pekin duck egg reported by Balkan and Biricik (2008) with 69.51 g, but other authors reported higher values like 77.57 g (Kralik *et al*., 2015), 80.7 g (Kokoszynski *et al*., 2007), 82.1-83.8 g (Onbasilar *et al*., 2007), 82.8-86.7 g (Okruszek *et al*., 2008), 91-45-95.56 g (Biesiada-Drzazga *et al*., 2014) 91.89 g (Al-Obaidi and Al-Shadeedi, 2016) and 97.31 g (Yuan *et al*., 2013). Statistical analysis revealed that significant differences were also appeared in the components percentage. The albumen percentage was significantly higher ($P<0.05$) at Cherry Valley duck eggs, while yolk and shell percentages were significantly higher ($P<0.05$) at Pekin duck eggs. The albumen percentage of Pekin duck eggs observed in this study (52.19%) was close to albumen percentage of Pekin duck eggs reported by Ipek and Sozcu (2017) in range 52.7-54.5% and in range 52.21-53.44% reported by Biesiada-Drzazga *et al*. (2014), but lower than albumen percentage of Pekin duck eggs of 53.51% reported by Balkan and Biricik (2008) and 55.35% reported by Al-Obaidi and Al-Shadeedi (2016). The yolk percentage of Pekin duck eggs observed in this study (35.16%) was higher than yolk percentage of Pekin duck eggs of 34.06% reported by Balkan and Biricik (2008) and 32.26% reported by Al-Obaidi and Al-Shadeedi (2016), but lower than yolk percentage of Pekin duck eggs in range of 39.2-40.8% reported by Okruszek *et al*. (2008). According to Ipek and

Sozcu (2017), the quantity of yolk and albumen in duck eggs is altered depending on changes in egg weight. The shell percentage of Pekin duck eggs observed in this study (12.65%) was close to shell percentage of Pekin duck eggs of 12.39% reported by Al-Obaidi and Al-Shadeedi (2016), but higher than shell percentage of Pekin duck eggs in range 8.9-9.9% reported by Ipek andnd Sozcu (2017) and 8.91-9.12% reported by Okruszek *et al*. (2008). The considerably higher shell percentage of Pekin duck eggs in range 13.55-14.61% was reported by Biesiada-Drzazga *et al*. (2014).

Table 1. Comparison of total weight and composition of Pekin duck and Cherry Valley duck eggs

Parameter	Sample	
	Pekin duck	**Cherry Valley duck**
Total weight (g)	71.91 ± 5.14^{a}	94.23 ± 4.89^{b}
Albumen weight (g)	37.58 ± 3.87^{a}	51.72 ± 4.06^{b}
Albumen percentage (%)	52.19 ± 3.08^{a}	54.85 ± 2.29^{b}
Yolk weight (g)	25.26 ± 2.67^{a}	31.21 ± 1.93^{b}
Yolk percentage (%)	35.16 ± 3.32^{a}	33.17 ± 2.26^{b}
Shell weight (g)	9.07 ± 0.54^{a}	11.30 ± 0.81^{b}
Shell percentage (%)	12.65 ± 0.73^{a}	$^{11.98} \pm 0.35^{b}$

Values are averages of 60 samples ± standard deviation
Values in the same row followed by different letters are significantly different (P<0.05)

3.2. Egg chemical composition

Analysis of chemical composition of the egg yolk showed significant differences between Pekin duck and Cherry Valley duck eggs (Table 2). The crude protein and total ash contents in yolk were recorded to be significantly higher (P<0.05) at Cherry Valley duck eggs, while crude fat and carbohydrate contents were significantly higher (P<0.05) at Pekin duck eggs. No significant differences were observed in dry matter percentage values between Pekin duck and Cherry Valley duck eggs.

Table 2. Comparison of yolk chemical composition of Pekin duck and Cherry Valley duck eggs

Parameter	Sample	
	Pekin duck	**Cherry Valley duck**
Dry matter, %	55.40 ± 0.89^{a}	55.33 ± 0.37^{a}
Crude protein, %	15.76 ± 0.35^{a}	17.29 ± 0.52^{b}
Crude fat, %	35.90 ± 0.75^{a}	34.46 ± 0.70^{b}
Total ash, %	1.92 ± 0.15^{a}	2.71 ± 0.22^{b}
Carbohydrate, %	1.82 ± 0.28^{a}	0.87 ± 0.25^{b}

Values are averages of 60 samples ± standard deviation
Values in the same row followed by different letters are significantly different (P<0.05)

The yolk dry matter percentages of both Pekin duck and Cherry Valley duck eggs observed in this study (55.40% and 55.33%, respectively) were close to yolk dry matter percentage of Pekin duck eggs in range 54.8-55.5% reported by Ipek and Sozcu (2017), 55.14% reported by Balkan and Biricik (2008) and 55.8% reported by Sekiguchi *et al*. (1979), higher than dry matter percentage of Pekin duck eggs in range 50.4-51.0% reported by Okruszek *et al*. (2008) and lower than dry matter percentage of Pekin duck eggs in range

56.27-57.68% reported by Onbasilar *et al*. (2011). Egg polyfunctionality in food systems is correlated, to a high extent, with its chemical composition and more specifically with its protein content (Raikos *et al*., 2006). Protein is an essential component of human diet which is needed for the replacement of tissue and supply of energy (Bashir *et al*., 2015).

According to Kaewmanee *et al*. (2009), duck egg yolk was rich in protein and had a high content of lipids or fat. Proteins present in egg are distributed among the egg white and yolk, whereas lipids are mainly concentrated in the yolk (Abeyrathne *et al*., 2013). The crude protein content in yolk of Pekin duck eggs observed in this study (15.76%) was significantly lower (P<0.05) in comparison to Cherry Valley duck eggs (17.29%), and also lower than protein content in yolk of Pekin duck eggs in range 17.33-17.70% reported by Onbasilar *et al*. (2011), but within range 15.56-16.21% reported by Okruszek *et al*. (2006). The crude fat content in yolk of Pekin duck eggs observed in this study (35.90%) was significantly higher (P<0.05) in comparison to Cherry Valley duck eggs (34.46%) and also higher than fat content in yolk of Pekin duck eggs in range 28.59-30.86% reported by Okruszek *et al*. (2006). According to Pikul (1998), the fat content in the duck egg is 2.5 percent higher than that in the hen egg and for this reason the water content in the duck egg is lower than that in the hen egg. The yolk ash percentage of Pekin duck eggs observed in this study (1.92%) were lower than ash content in yolk of Pekin duck eggs in range 2.51-2.67% reported by Onbasilar *et al*. (2011).

The albumen chemical compositions of Pekin duck and Cherry Valley duck eggs are presented in Table 3. The Cherry Valley duck eggs had significantly higher (P<0.05) dry matter and crude protein content, while Pekin duck eggs had significantly higher (P<0.05) carbohydrate content. No significant differences were observed in total ash content between Pekin duck and Cherry Valley duck eggs. The albumen dry matter percentages of Pekin duck eggs observed in this study (12.11%) was close to albumen dry matter percentage of Pekin duck eggs in range 12.2-12.7% reported by Ipek and Sozcu (2017) and 12.3% reported by Sekiguchi *et al*. (1979), but lower than range 12.44-13.65% reported by Onbasilar *et al*. (2011) and 13.66% reported by Balkan and Biricik (2008).

Table 3. Comparison of albumen chemical composition of Pekin duck and Cherry Valley duck eggs

Parameter	Sample	
	Pekin duck	**Cherry Valley duck**
Dry matter, %	12.11 ± 1.26^{a}	13.21 ± 0.26^{b}
Crude protein, %	10.35 ± 1.12^{a}	11.54 ± 0.28^{b}
Total ash, %	0.68 ± 0.05^{a}	0.70 ± 0.04^{a}
Carbohydrate, %	1.19 ± 0.13^{a}	0.97 ± 0.19^{b}

Values are averages of 60 samples ± standard deviation
Values in the same row followed by different letters are significantly different (P<0.05)

Water content of yolk is much lower than of albumen because of the important lipid content (Roca *et al*., 1984). According to Balkan and Biricik (2008), water proportion of Pekin duck eggs is similar to that of other domestic duck forms and mallard. According to Kaewmanee *et al*. (2009), protein is the major constituent of duck egg albumen solids while the amount of lipid in albumen was negligible. The crude protein content in albumen of Pekin duck eggs observed in this study (10.35%) was close to albumen protein content of Pekin duck eggs reported by Okruszek *et al*. (2006) in range 10.43-10.84%, while Onbasilar *et al*. (2011) reported higher protein content in range 11.39-12.51%. The albumen ash content of

Pekin duck and Cherry Valley duck eggs observed in this study (0.68% and 0.70%, respectively) were lower than albumen ash content of Pekin duck eggs in range 0.97-1.07% reported by Onbasilar *et al*. (2011). The ash content gives a measure of total amount of inorganic compounds like minerals present in a food (Bashir *et al*., 2015). According to Kaewmanee *et al*. (2009), duck egg albumen had a lower content of ash than had egg yolk and this is confirmed in this study. This study also revealed that Cherry Valley duck eggs contain significantly more ash in the yolk than Pekin duck eggs what is an indication that Cherry Valley duck eggs contain more minerals. On the contrary, Pekin duck eggs contain significantly higher content of carbohydrate in the yolk and albumen than Cherry Valley duck eggs.

The comparison of egg shell chemical composition of Pekin duck and Cherry Valley duck eggs is presented in Table 4. No significant differences were observed in egg shell dry matter percentage values between Pekin duck and Cherry Valley duck eggs. The crude protein content in egg shell was recorded to be significantly higher ($P<0.05$) at Pekin duck eggs, while total ash content was significantly higher ($P<0.05$) at Pekin duck eggs. Most of the egg proteins are present in the egg yolk and albumen, while the egg shell contains the rest of the proteins, 9.46% and 7.22% in Pekin duck and Cherry Valley duck egg shell, respectively. According to Al-Awwal and Ali (2015), the average eggshell contains 89.9-91.1% ash content. The results obtained in this study are slightly out of that range, Pekin duck eggs contain 89.21% and Cherry Valley duck eggs contain 91.83% in average total ash in egg shell. Calcium is the major component in an eggshell and there is also a small amount of phosphorus and magnesium and trace amounts of other micro elements (Shwetha *et al*., 2018). In this study Cherry Valley duck egg shell had significantly ($P<0.05$) higher calcium content than Pekin duck egg shell, while no significant differences were observed in phosphorus content.

Table 4. Comparison of egg shell chemical composition of Pekin duck and Cherry Valley duck eggs

Parameter	**Sample**	
	Pekin duck	**Cherry Valley duck**
Dry matter, %	98.68 ± 0.42^a	99.05 ± 0.07^a
Crude protein, %	9.46 ± 0.76^a	7.22 ± 0.33^b
Total ash, %	89.21 ± 0.42^a	91.83 ± 0.32^b
Calcium, g/100 g	33.18 ± 0.83^a	34.56 ± 0.49^b
Phosphorus, g/100 g	0.17 ± 0.01^a	0.18 ± 0.01^a

Values are averages of 60 samples ± standard deviation
Values in the same row followed by different letters are significantly different ($P<0.05$)

3.3.Egg physicochemical properties

Physicochemical properties of Pekin duck and Cherry Valley duck eggs are presented in Table 5. One of the most important interior egg quality characteristics is the pH value of the albumen and yolk, and these correlate with embryo development during the incubation period. An albumen pH between 8.2 and 8.8 is optimal for embryo development (Walsh, 1993). Brake *et al*. (1997) reported that optimal yolk pH was about 6.0. In this study, no significant differences of albumen and yolk pH were observed. The pH of the albumen of both duck breed eggs was found to be slightly higher than above optimum range, 8.89 and 8.92 for Cherry Valley duck and Pekin duck eggs, respectively. Ipek and Sozcu (2017) reported similar albumen pH of Pekin duck eggs 8.8-8.9, but some authors reported higher albumen pH of Pekin duck eggs: 8.93-9.05 (Okruszek *et al*., 2006), 8.94-8.99 (Okruszek *et al*., 2008), 9.00 (Kralik *et al*., 2015) and 9.02 (Onbasilar *et al*.,

2007). The lower pH value of Pekin duck egg albumen was reported in range 8.06-8.70 by Kokoszynski *et al*. (2007), 8.10-8.52 by Biesiada-Drzazga *et al*. (2014) and 8.29-8.35 by Yuan *et al*. (2013). In this study yolk pH was determined as 6.03 and 6.12 for Cherry Valley duck and Pekin duck eggs, respectively. The higher yolk pH of Pekin duck eggs was reported by Kralik *et al*. (2015) 6.16 and in range 6.25-6.31 by Okruszek *et al*. (2008) and 6.32-6.93 by Okruszek *et al*. (2006). The lower yolk pH of Pekin duck egg albumen was reported in range 5.40-5.75 by Biesiada-Drzazga *et al*. (2014), 5.87-6.00 by Onbasilar *et al*. (2007) and 5.9-6.0 by Ipek and Sozcu (2017).

Table 5. Comparison of physicochemical properties of Pekin duck and Cherry Valley duck eggs

Parameter	**Sample**	
	Pekin duck	**Cherry Valley duck**
Albumen pH	8.92 ± 0.04^{a}	8.89 ± 0.04^{a}
Yolk pH	6.12 ± 0.08^{a}	6.03 ± 0.07^{a}
Yolk color L	69.72 ± 0.94^{a}	70.90 ± 1.96^{a}
Yolk color a	19.02 ± 1.83^{a}	15.60 ± 2.15^{b}
Yolk color b	69.86 ± 0.89^{a}	71.18 ± 2.56^{a}
RYCF scale	10.79 ± 0.80^{a}	9.07 ± 1.10^{b}

Values are averages of 60 samples ± standard deviation
Values in the same row followed by different letters are significantly different ($P<0.05$)

The color of the egg yolk is an important quality feature of the egg yolk, being attributed to the high quality of eggs and the products made of eggs (Dvorak *et al*., 2010). Color of egg yolk is also an important factor in consumer's acceptance of a product. Desirable egg yolk color varies between markets, but yellow to golden colors are usually considered as an indication of better egg quality (Kljak *et al*., 2012). The measurement of egg yolk color according to CIE (1986) L* a* b* color system showed that no significant differences were observed in this study for the yolk lightness L* value between Pekin duck eggs and Cherry Valley duck eggs (69.72 and 70.90, respectively) and also for the yolk yellowness b* value (69.86 and 71.18, respectively). The Pekin duck egg yolks were characterized by a significantly higher ($P<0.05$) redness a* value (19.02) than Cherry Valley duck egg yolks (15.60). The similarly L* values for the Pekin duck egg yolk lightness were reported in range 68.59-70.48 by Okruszek *et al*. (2006) and in range 70.1-71.0 by Okruszek *et al*. (2008). The same authors reported considerably lower redness a* value and yellowness b* value for the Pekin duck egg yolks than values observed in this study. Okruszek *et al*. (2006) reported redness a* value in range 1.06-3.24 and yellowness b* value in range 38.44-40.99, while Okruszek *et al*. (2008) reported these values in ranges 1.89-3.21 and 42.9-45.1, respectively. The comparison with these values showed that Pekin duck egg and Cherry Valley duck egg yolks tested in this study had higher intensity of the red and yellow color.

In this study the egg yolk color was also determined by the Roche Yolk Color Fan (RYCF) scale. RYCF scale is a common tool used to determine yolk color, but these determinations are highly subjective which makes them difficult to compare with determinations made in different conditions (Kljak *et al*., 2012). Marked differences exist in the preference of egg yolk color hue between the consumers in various European countries. Consumers in Germany, Netherlands, Spain, and Belgium prefer egg yolk color with the values 13-14 of RYCF scale, in France, south England, and Finland with the values 11-12 of

RYCF scale, and in Ireland, north England, and Sweden with the values of 8-9 RYCF scale (Bovskova *et al*., 2014). On the other hand, US consumers prefer egg yolk color with the values of 7-10 RYCF scale (Galobart *et al*., 2004). According to Sencic and Butko (2006), Croatian consumers prefer egg yolks with intensive golden, almost orange color with 10 to 12 value on RYCF scale. In this study, the average RYCF value for Pekin duck egg yolks (10.79) was significantly higher ($P<0.05$) than average RYCF value for Cherry Valley duck egg yolks (9.07), which showed that Pekin duck egg yolks had more intensive color. Similar RYCF value for Pekin duck egg yolks 9.56 was reported by Kralik *et al*. (2015), while much lower RYCF values in range 5.67-6.90 were reported by Biesiada-Drzazga *et al*. (2014).

4. Conclusions

Based on the obtained results in this study, it can be concluded that duck breed had significant influence on egg chemical composition. Statistically significant differences ($P<0.05$) between Pekin duck and Cherry Valley duck eggs were observed in egg yolk, albumen and shell chemical composition. The Cherry Valley duck eggs had significantly higher crude protein content in yolk and albumen. The Pekin duck eggs had significantly higher crude fat content in yolk, carbohydrate content in yolk and albumen and total ash content in the egg shell. There were not so many differences in egg physicochemical characteristics between two duck breeds. No significant differences of albumen and yolk pH were observed, but Pekin duck egg yolks had more intensive color. The results obtained in this study could be useful for food industry when selecting eggs as ingredients for different products.

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STUDY OF BLURRING AND HYSTERESIS OF PHASE TRANSFORMATIONS OF MILK FAT BY TRANSIT CALORIMETRY METHOD

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ABSTRACT

The so-called effective heat capacity of dairy products, which may include enthalpy changes due to the heat of phase transformations of individual components of the product is used in the technological calculations; the use of the additive rule in this case can lead to the significant errors. The use of the transit calorimetry method described in this article gives an opportunity to deepen the knowledge on the blurring phase transition in dairy products, to clarify information on technological and thermophysical characteristics of the products, to establish a connection between them, to reduce the expenses of energy and material resources per unit of finished products, to detect counterfeit dairy products. The result of the paper is to determine the probable quantitative characteristics of the heat capacity of milk fat of total phase, and the fraction of solid phase due to the phase transformations, their blurring and hysteresis.

1. Introduction

Technologies for processing, transporting and storing of dairy products require a large amount of different kinds of energy and material resources. Timely and accurate information on the technological and thermophysical characteristics of raw materials, intermediates and finished products during the recipe, technological and process calculations, as well as directly during production contributes to the reduction of expenses of energy, raw materials and materials per unit of finished products.

Milk fat (MF) is the main nutrient and energy component of all whole dairy products, its content in the product is of the main technological characteristics. The existing standardized method for determining the mass fraction of fat in milk, dairy products and canned foods – Gerber's acid method (GOST 5867–90) – requires a certain qualification of a laboratory assistant (the human factor largely influences the accuracy of determination) and significant expenses of time and resources (Ivanova, 2000). Therefore, search for methods of express-determination of the proportion of fat and other components of dairy products, for example, on the basis of establishing connections between technological (fat, moisture, acidity, etc.) and thermophysical (heat capacity, thermal conductivity, temperature conductivity, viscosity, etc.) product characteristics is of immediate interest.

Heat capacity is a fundamental thermophysical characteristic for dairy products; there are practically no isothermal or adiabatic processes in this field, when the concept of heat capacity is meaningless and isobaric processes prevail. The heat capacity of the cream should be taken into account, as its temperature changes even when calculating formally mechanical processes, such as the transportation of cream to butter-making equipment.

Heat is a typically extensive characteristic of a process, its relation to a change in body

temperature due to the absorption of this heat by it, that is, the heat capacity of the body, can also be considered an extensive characteristic, but no longer of the process, but of this body. An additive heat capacity for a substance that is a mixture of components that do not react with each other in a chemical reaction is used in practical calculations.

The so-called effective heat capacity of dairy products, which may include enthalpy changes due to the heat of phase transformations (PT) of individual components of the product is used in the technological calculations; the use of the additive rule in this case can lead to the significant errors. PT occurs this time in a certain temperature range unlike "pure" substances, in addition differently – the product is heated or cooled. In the second half of the last century, this phenomenon was recorded for various substances, including those that remain solid crystalline during the PT, it (phenomenon) is used to study different characteristics of a substance and their interaction with each other. The theory of blurring phase transformations by B. N. Rolov (Rolov and Yurkevich, 1983) is in the basis of these works.

Blurring phase transition (BPT) is the transformation of one condensed phase into another under the conditions of some interval of the actual parameter (temperature, density, concentration, etc.) by definition. According to B. N. Rolov, each thermodynamic characteristic of a complex system can be represented as the sum of the normal part caused by the microstructure of the system and the abnormal part due to the phase transformation. It is a kind of thermodynamic formalism which main task is to give general regularities of change of the abnormal parts irrespective of their specific mechanism.

Phase transformations of a complex nature occur in dairy products during the technological processing, mainly due to the main components of milk fat – triglycerides (modern name – triacylglycerides), the difference in melting point and solidification of which can reach 15–20 K (Belousov, 1984) under the same rate of change of product temperature. Thus, these PT are typical blurring transformations. The use of the transit calorimetry method described in this article gives an opportunity to deepen the knowledge on BPT in dairy products, to clarify information on technological and thermophysical characteristics of the products, to establish a connection between them, to reduce the expenses of energy and material resources per unit of finished products, to detect counterfeit dairy products.

2. Materials and methods

The theory of phase blurring transformations (Rolov and Yurkevich, 1983) is widely used in the study of solid-state crystals, solid solutions, etc., that is, when changes in temperature lead mainly to orientational and structural changes in the studied samples, so it is common to call these changes as phase transitions rather than transformations (Aliev, 2007; Yegorov *et al.*, 2008; Yegorov *et al.*, 2009; Yegorov *et al.*, 2013). Such PT are called second-order transitions (PT-2). With regard to dairy products, phase transformations in milk fat occur mainly with a change in aggregate state - melting or crystallization (PT-1), although PT-2 also takes place.

In general, PT of seven genera are considered in the solid state physics (Aliev, 2007). PT-3 takes place in ferroelectrics, where temperature rise causes disturbance of the ordered arrangement of dipole moments. PT-4 is a spontaneous magnetization of ferromagnets. If PT-3 and PT-4 occur simultaneously, it is PT-5. PT-6 accompanies superconductivity and superfluidity under extremely low temperatures. PT-7 only takes place in some semiconductors. Phase transitions from PT-3 to PT-7 are considered only if electric and magnetic fields are applied to the appropriate materials.

From a thermodynamic point of view, many physical characteristics of a substance, including heat capacity at the moment of its PT should go to infinity. In real technological processes, the heat capacity c, J/(kg·K) reaches some peak at the moment of PT and then decreases to a new

value – this is the so-called lambda-transformation. The peak is smoothed for BPT, the point value T_o of the PT temperature expands to a certain interval. The dependence of *c(T)* before and after PT may be different: zero, positive or negative. There is no jump *c(T)* at T_o point, but there is a jump up or down. This dependence is depicted in Figure 1 as positive before and after T_o for the perfect jump, real PT-1 or PT-2, as well as with the upward jump for BPT.

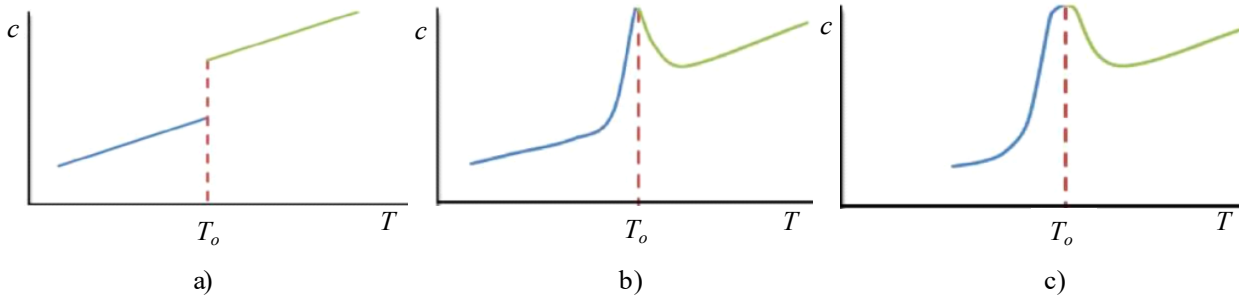

Figure 1. Dependence of heat capacity on temperature under the condition of PT: a) perfect transformation; b) lambda-transformation; c) blurring phase transformation

From a technological point of view, the difference between PT-1 and PT-2 is that the additional energy is released or absorbed during PT-1 and no during PT-2.

Ratio between PT-1 and PT-2 in molecular crystals of various organic compounds was investigated in the works (Yegorov *et al.*, 2008; Yegorov *et al.*, 2009; Yegorov *et al.*, 2013) by one of the methods of differential-and-thermal analysis (DTA) – by differential scanning calorimetry (DSC). We recall that this group of crystals includes crystals of proteins, nucleic acids, binary compounds (CO_2 and H_2O). Polymorphic transformations also belong to PT of molecular crystals, except typical PT-1 – melting, sublimation. All these PTs occur as a rule without breaking the integrity of the molecules. The temperature hysteresis of heat capacity was established for all the test materials, that is, T_{on} during the samples heating did not coincide with T_{oo} of the samples cooling.

The change of endo- and exopeaks in the work (Yegorov *et al.*, 2008) was used to find out that partial melting, that is PT-1, and polymorphic PT-2 can occur in solid alkano-alcohols, as well as for the quantitative analysis of the temperature dependence of the heat capacity of these compounds on the temperature. If alcohols with different lengths of finite groups of molecules were the samples in (Yegorov *et al.*, 2008), then diols in (Yegorov *et al.*, 2009), dicarboxylic acids of the same gradation in (Yegorov *et al.*, 2013), conclusions are similar. Information is also given in (Yegorov *et al.*, 2009) on the change of enthalpy and entropy *s*, J/(kg·K) of the samples, connection between the value of heat capacity peak Δc with heat of PT was established in (Yegorov et al., 2013; Andrianov et al., 1988).

DTA methods are widely used in the determination of Δc, Δh, Δs of loose, viscous and liquid substances. These methods were also used in the study of milk fat, in particular polymorphism phenomena using endopic on thermograms, but the connection between endopic form and "polymorphic characteristics" appeared to be doubtful (Belousov, 1984).

In recent years, DTA methods and other kinds of thermal analysis have been used to evaluate the quality of canned milk (Budanina *et al.*, 2015), to ascertain the connections between the composition of fatty acids of individual MF fractions and their PT temperature (Wang *et al.*, 2017) , for the use of MF stearin fraction as a moderator of omega-3 acids oxidation (ω-3), which are among the most important nutrients

(Li *et al.*, 2017), and to establish a connection between heat and PT temperature of individual triacylglycerides (Tolstorebrov *et al.*, 2014).

In general, DTA methods have numerous drawbacks. The keyword in DTA and DSC is "differential", that is all measurements are relative, so their errors are
doubled. The thermal resistance of the sample affects not only the overall thermogram level but also the peaks shift, that is the temperature hysteresis of PT (Yegorov *et al.*, 2008). The internal geometry of the sample, especially for the crystalline samples, as well as the external geometry, that is, the volume, surface and the ratio between them influences the reproducibility of the experiments in addition to scanning speed and sample weight.

The study of PT in milk fat with the aim to specify the effects of polymorphic transformations was performed by using DTA, XRSA (X-ray structural analysis), infrared spectral characterization and magnetic resonance spectroscopy, but no single point of view was found on the nature of this effect (Belousov, 1984; Small, 1986).

A significant contribution to the study of the ratio of the solid and liquid phases of fat in dairy products was made by the dilatometric method, since the difference in the specific volumes of these phases is significant. P. Walstra found that the fraction of solid phase *x* in milk fat (MF) may differ markedly at the same temperature, depending on whether the MF is heated or cooled, that is there is PT hysteresis (Figure 2) (Walstra and van Beresteyn, 1975). Similar hystereses were determined during the cooling and heating of the cream, but the balance "solid–liquid MF" required more time (Belousov, 1984).

Works of (Huliaiev-Zaitsev and Tyshchenko, 2003; Tyshchenko, 2002; Upadhyay *et al.*, 2017) were dedicated to detection and prevention of dairy products falsification because of the use of non-dairy fats by the value of Reichert-Messli number and the difference of refractive indexes.

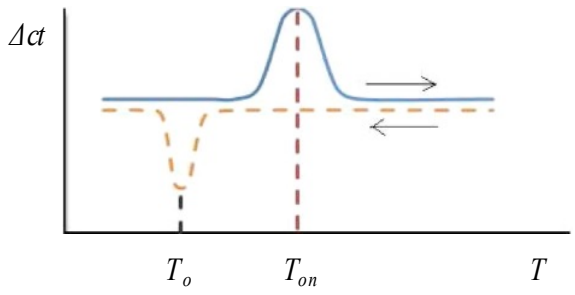

Figure. 2. Temperature hysteresis of the heat capacity of MF

The values of the effective heat capacity of dairy products required for technological calculations were determined by traditional calorimetric methods (direct heating, mixing, etc.) by various researchers, including L. Riegel, K. Becker, V. Leidenfrost, G. Cook, Yu. Olenev, and H. Tverdohleb (cit. (by Fedorov and Pakhomov, 1973) and (Ginzburg *et al.*, 1980)). The differences in heat capacity data increase with the growth of MF in the products, and they can reach one and a half – two times for pure MF. However, information on PT blurring and their hysteresis during heating or cooling is absent everywhere (except for those mentioned above (Small, 1986; Walstra and van Beresteyn, 1975).

These differences can be explained not only by the different composition of dairy products, the content of MF in them in different regions, various seasons, feeding rations and conditions of animals keeping. MF is the most complex of fats of animal and vegetable origin by chemical composition. It is approximately 98 % composed of triacylglycerides, which are composed of more than 140 fatty acids, more than half the weight of MF are saturated acids (from 50.3 to 73.8 %), the rest are unsaturated, which in turn are divided into mono- and polyunsaturated. The physical properties of triacylglycerides depend not only on the ratio of these acids, but also on how the radicals of the acids are located in the triglyceride. For example, the melting points of triacylglycerides range from –75 °C for tributyrin to +68 °C for stereodipalmitin. The ability of triacylglycerides to dissolve into each other time in solid state of

MF and to form mixed crystals during transformation to solid state, their ability to become supercool by 15–20 K against the crystallization point leads to BPT during the thermal (heating or cooling) processing of dairy products in addition to the aforementioned polymorphism, that is the ability of triacylglycerides of the same composition to form different crystal structures. The mechanical processing of the dairy product and its pre-exposure under certain temperature conditions also promotes the formation of BPT.

A relatively new method of thermal analysis – modulated differential scanning calorimetry (MDSC) is recommended for determining thermal capacity in the paper of (Phinney *et al.*, 2017) dedicated to the possibility of predicting the thermophysical characteristics of new foods depending on the content of major components (fats, hydrocarbons, proteins, fiber, ash and water). At the same time, the sinusoidal heating mode is imposed on the main heating of the sample with linear velocity, which makes it possible to separate the product's own heat capacity from the additional one at the expense of the PT.

The purpose of this work is to develop a method for determining PT and their quantitative characteristics, as well as the heat capacity of MF.

3. Results and discussions

3.1. The method of transit calorimetry and its development

This method was developed on the basis of thermometry created in Ukraine and its use in the study of various technological processes (Fedorov, 1974). The heat meter is a plate with a thickness of 1–2 mm and a diameter or side of a square of 8–20 mm, where the junctions of differential thermocouples in the amount of 300–1500 pieces/cm^2 are led out on the surface. This makes it possible to measure the heat flux density q, W/m^2 passing through the thermometer up to 3 %. The inertia of such heat flow sensors is 5–30 s, which allows recording any changes in heat load on the product sample.

The scheme of transit calorimetry (TCM) (Figure 3) includes heat meters 1 and 2 with thermocouples on the surfaces of sample 3 and sources of supply or removal of heat 4 and 5.

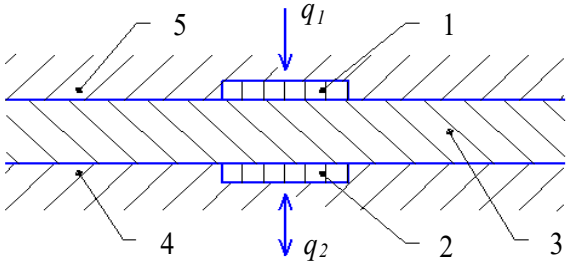

Figure 3. The scheme of transit calorimetry

Thermostatic cameras (TC), or electric heater (E), infrared source (IS) are used as the sources of heat supply, (TC) or thermoelectric (semiconductor) thermostat (TE) for heat removal. Heat supply occurs from above to prevent convection in the liquid sample.

Each experiment begins with a stationary mode which is fixed by the signals of heat meters and thermocouples. The cycle is formed by the perturbation of the mode, q_1 begins to differ from q_2, which forms a closed area upon the establishment of a new stationary mode that is proportional to Q, J/m^2 – the thermal energy accumulated by the sample layer for the transient mode during τ:

$$Q = \int_{\tau} (q_1 \pm q_2) dt. \qquad (1)$$

Figure 4 shows the forms of closed surfaces, proportional to Q for the most commonly used combination of TC-TC sources and possible ratios of the initial q_n and the final q_k of the heat flux density in steady-state modes. The signals q_2 in Figure 4 are given with the " – " sign for clarity.

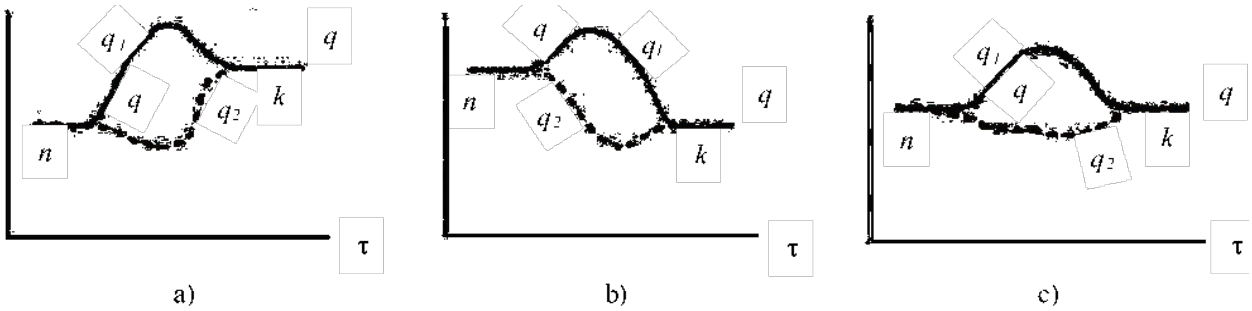

Figure 4. Forms of transient modes (cycles) of TCM

The Q value during one cycle (that is why the method is often called the "cycle method") is proportional to the enthalpy change of the Δh sample. The following formula is used to calculate the amount of heat Q_o spent for the process conducting if there is no PT in the product during the technological process:

$$Q_o = cm\delta t, \tag{2}$$

where δt is the change of the average temperature of the product per the process, and if PT are significant, then:

$$Q_o = m(h_2 - h_1), \tag{3}$$

where h is the enthalpy of the product, its change takes into account both the change of t and the heat of PT. The beginning of the h reference is not fundamental. $h_0 = 0$ at $t = -25$ °C is most often taken for dairy products. The differential equation of thermal conductivity, which is expressed by the enthalpy change is used to derive the calculated formula of the effective heat capacity (Karslow and Eger, 1984):

$$P\frac{dh}{d\tau} = \frac{\partial}{\partial x}\left(\lambda\frac{dt}{dx} + \lambda\frac{dt}{dy} + \lambda\frac{dt}{dz}\right) + q_v, \tag{4}$$

where q_v, W/m^3 is the volume density of the heat flux (due to PT), ρ, kg/m^3 is the product density.

The thermal conductivity λ, W/(m·K) of dairy and other labile products is highly dependent on the temperature, but (4) can be simplified by using the Kirghoff substitution (Karslow and Eger, 1984):

$$Q = \frac{1}{\lambda_{mid}}\int_{t_1}^{t_2}\lambda dt, \tag{5}$$

where t_1 and t_2 are the initial and final temperatures of the product, λ_{mid} is the average λ during the experiment. We get the following by substituting (5) to (4) and simple transformations:

$$\frac{P}{\lambda_{mid}}\frac{dh}{d\tau} = \nabla^2\theta + \frac{q}{\lambda_{mid}}. \tag{6}$$

We obtain the calculated equation for Δh by assigning q to the unit of the sample thickness l and taking into account the actual (measured) heat accumulation q_1+q_2:

$$\Delta h = \frac{1}{\rho\tau}\int_{\tau}(q_1 + q_2)d\tau. \tag{7}$$

It is necessary to know the average temperature increase of the sample over the δt cycle to determine c:

$$c = \frac{\Delta h}{\delta t}. \tag{8}$$

The found c value is assigned to the average calorimetric temperature of the sample for the $\bar{t}$ cycle:

$$\bar{t} = t_1 \pm \frac{\int_\tau (t_2 - t_1)(q_2 + q_1)d\tau}{2\int_\tau (q_2 + q_1)d\tau}. \qquad (9)$$

But in most cases there is $\bar{t} = 0.5(t_1 + t_2)$.

The final steady-state mode can be used as the initial one for the next cycle, so the *c(t)* dependence can be obtained by one experiment over a rather wide temperature range, which is especially important for labile dairy products.

The method of cycles is complex because it is possible to determine the thermal conductivity of the λ sample, W/(m·K) in each stationary mode:

$$\lambda = \frac{ql}{\Delta t}, \qquad (10)$$

where $q_1 = q_2 = q$, and its temperature conductivity a, m^2/s:

$$\alpha = \frac{\lambda}{c\rho}. \qquad (11)$$

This method was used in the study of effective thermal and physical characteristics (TPC) of various foods: beef and pork meat, cattle blood, nonfat greaves, salo (cured pork fat), fruits and vegetables, grain and green malt (Fedorov, 1974; Fedorov, 1987) in addition to dairy products (fat-free milk, dairy cheese, unsweetened condensed milk, cream, etc.).

Work experience with this method allowed minimizing the error in determining the effective heat capacity by optimizing the thickness of l sample (the first independent factor) and the shape of the closed area of the cycle. The measurement accuracy is influenced by this second factor F, it was determined by the height of the figure and its length, that is, $\tau_2 - \tau_1 = \Delta\tau$ – cycle duration, hence $F = Q/\Delta\tau$. The error itself was taken as the response function, that is, the estimate of the root mean square error of S. Molten milk fat in the temperature range of +10...+50 °C was selected as the samples.

The planning and realization of the experiments of the full factorial experiment and then the orthogonal composite plan were carried out; the optimum was determined analytically and graphically, only the optimal values of F = 8.8 kW/m and l = 3.75 mm were obtained.

Microsensors – thermometers with thermocouples with a 5 mm thickness of dough between them in the middle of a flat pastry piece were placed in the direction of heat flow in the process of baking bread during the study of heat-and-mass transfer. The sensors were perforated for free passage of moisture or steam through them.

During the first half of the process, the signals from both thermometers (1 and 2, Figure 5) were close to zero, then they formed a closed figure. The signals from the thermometers were then practically the same (curve 3). This made it possible to determine the heat capacity of the crumb directly in the baking process; it was $c\rho$ = 0.86 MJ/(m^3·K), which is in good agreement with the reference data (Ginsburg *et al.*, 1980) for the crumb of scone products.

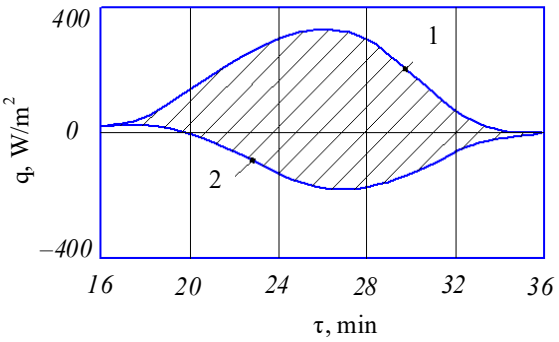

Figure 5. TCM of the fancy pastry in the process of baking

Similar information was obtained during baking of biscuit partly baked product, thermometers with thermocouples were located one (1) on the upper surface of the pastry piece, the second (2) – 5 mm below it (Figure 6). The signal of the thermometer 1 initially increases sharply, then it drops when the pastry warms, the signal of the thermometer 2 is slow, and then becomes the same. The volumetric heat capacity

of the pastry at an average temperature of 39 °C was $c\rho$ = 1.2 MJ/(m^3·K), which also corresponds to the reference data (Ginsburg *et al.*, 1980).

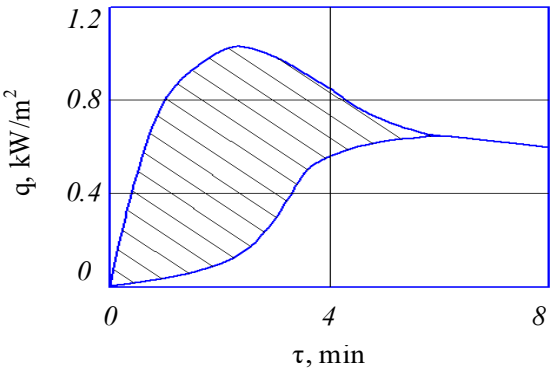

Figure 6. TCM of the biscuit pastry in the process of baking

The TCM method was upgraded by introducing a quasi-stationary or regular mode of the second kind of PP-2 of heating or cooling of the sample in order to continuously obtaining the information needed to determine the heat capacity and other TPC. The heat flux density on both surfaces of the sample was kept constant for this, so that, $q_1 > q2$. Then the temperatures of these surfaces will increase in time linearly, with $t_1 > t_2$ (Figure 7), the theory of the method is outlined in (Fedorov, 1987).

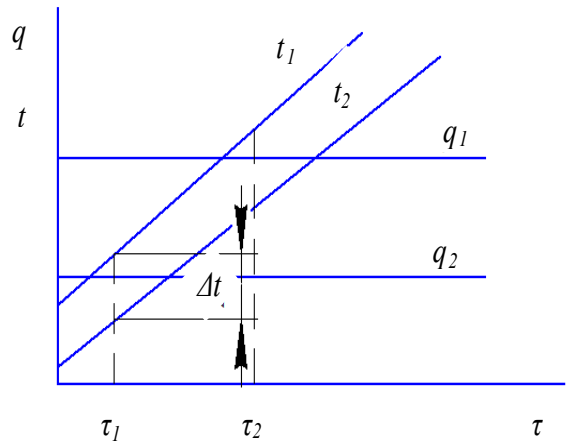

Figure 7. Change in time *q* and *t* during PP-2

The calculated formulas for the volumetric heat capacity $c\rho$ and the thermal conductivity λ have the form:

$$c\rho = \frac{q_1 - q_2}{uh}; \tag{12}$$

$$\lambda = \frac{q_1 + q_2}{2\Delta t}, \tag{13}$$

where $\Delta t = t_1 - t_2$; $u = \Delta t/(\tau_2 - \tau_1)$.

Methods of cycles and PP-2 are implemented at the same equipment, it is advisable to combine them, if significant PT occur at separate temperature intervals.

The possibility of minimizing the error during PP-2 is presented in (Fedorov, 1987), the results of:

$$0.17 \le \frac{q_1 - q_2}{q_1 + q_2} \le 0.30. \tag{14}$$

3.2. Results of the study of blurring and hysteresis of the characteristics of milk fat

According to Rolov's theory, the structure of any whole milk product can be represented as a mixture which ratio between the components depends on the type of the product. It is a mixture of liquid and solid (crystalline) fat for MF (McGee, 1988). This makes it possible to show the effective heat capacity *c* as the sum of intense (own) c_b and additional one by the heat of PT of heat capacity c_f:

$$c = c_b + c_f. \tag{15}$$

c_f is meant the amount of heat that emits or absorbs 1 kg of MF in a single interval of temperature change. The total heat of PT (J/kg) can be defined as the integral of c_f in temperature:

$$L = \int_{t_n}^{t} c_f dt. \tag{16}$$

Studies have shown that c_f may exceed c_b for dairy products with a high content of MF; concerning L, then this value should not depend on the mode parameters of the technological process and its direction (heating or cooling). The value of c_f, on the contrary, can significantly depend on these parameters.

TPC and hysteresis of MF characteristics were studied on MF samples with an iodine value of 37.0 and a moisture content of not more than 0.3 %. A wide range of temperatures (from minus 60 °C to plus 80 °C) was ensured by the use of different designs of TPC devices; the E-TC scheme was used in the range of –60 °C...+20 °C supplemented with copper fins which was immersed in the Dewar flask with liquid nitrogen. The intensity of heating load on the sample and its temperature was changed by regulating the proportion of nitrogen boil-off. At positive temperatures, the E-TC scheme was supplemented with the TC-TC scheme with plates with thermometers and thermocouples connected with each other with the gap as the sample thickness. Fat or other pasty or liquid product is placed between the plates (this is a sample) and around them. The use of such joint cassettes allows the sample to be removed from the heat treatment device without disturbing the structure, and to study several samples at a given mode at the same time. Both types of devices were used in the zone of active PT. The error in determining the effective heat capacity was not higher than ±5 %.

Figure 8 shows the results of study c in the range of t = –60...+80 °C under the condition of slow cooling of the sample at a rate of 0.003 K/s, as well as under the condition of heating at the same rate of rapidly pre-cooled sample.

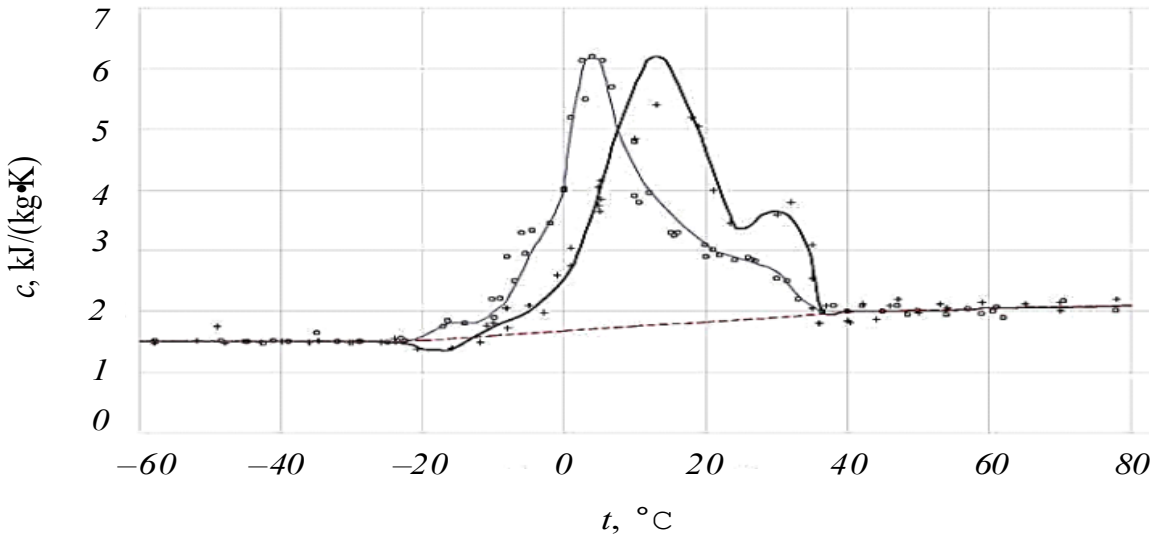

Figure 8. Determination of the effective thermal capacity of MF by the TCM method: "+" – heating, "o" – cooling

The heat capacity of the solid MF is not almost independent of the temperature and it is c = 1.468 ± 0.009 kJ/(kg·K) in the range of –72...–25 °C. A slight decrease of c compared to the solid state was observed during heating from –20 to –10 °C, it is probably due to the appearance of PT with an exothermic effect. In the –25...+37 °C range, there are two peaks: 6.3 kJ/(kg·K) at 15 °C and 3.7 kJ/(kg·K) at 30 °C and one peak during cooling – 6.53 kJ/(kg·K), almost the same as during heating, but shifted by 10 K to the left, that is at +5 °C. This hysteresis occurs throughout the PT range. Additional studies of MF with iodine numbers of 30.9 and 41.9 showed that the magnitude of the peaks shift depends on the

content of unsaturated acids in the fat. This dependence results in complexes with different energy being formed during melting and curing.

The heat capacity of completely melted fat does not depend on whether it is heated or cooled (Figure 8), it depends on the temperature linearly and in the temperature range of +37…+80 °C:

$$c = 1.7613 + 0.00586 \pm 0.156. \quad (17)$$

A sufficiently large value of error means that BPT have significant values in fully melted fat. The use of TCM for studies of dairy products with different contents of MF has shown that the more water in the product, the less blurring of PT, and they disappear for skim milk.

This method makes it possible to obtain a value for the proper conducting of the technological process, characterization – the content of the solid phase m_m in fat:

$$x = m_m / m, \quad (18)$$

where m is the mixture mass of solid and liquid fat.

Until now, the value of x was determined indirectly by calorimetric or dilatometric methods, since direct phases separation is a long process and is not suitable for production control. Both methods predict extrapolation of dependences *h(t)* or *v(t)* of the solid and liquid fat in the PT area. Since both of these dependencies are nonlinear and the extrapolation is linear, the error in determining x by both methods lies in the range of 13–25 %.

The TCM method gives the possibility to reduce these errors drastically. If we integrate (14) over a temperature in the range from the lower limit of PT t_h to the upper limit of t_b, we obtain:

$$\int_{t_h}^{t_b} c dt = \int_{t_h}^{t_b} c_b dt + \int_{t_h}^{t_b} c_f dt. \quad (19)$$

The left part (19) is the enthalpy difference $h_b - h_h$ at the range of PT $t_b - t_h$. Studies have shown that this difference does not depend on the chemical composition of MF and the method of sample preparation – hysteresis is located in the middle of the range of $t_b - t_h$, the first summand of the right part (19) can be calculated through the heat capacity of the solid c_s and liquid c_l parts:

$$c_f = xc_s + (1-x)c_l. \quad (20)$$

By integrating (18) over t, we obtain that the first summand of the right part is not practically independent on the form of function *x(t),* hence it is clear that the second summand equal to L must be invariant, which confirms the conclusions of other researchers that L can be considered a constant value. The diagram $c_f(t)$ for MF with iodine number of 37 was constructed using (17) and linear interpolation into the BPT area (Figure 9). Determining the area under the lines $c_f(t)$ under the conditions of heating and cooling of these samples, as well as fat with other iodine numbers gave a value of L close to 84 kJ/kg.

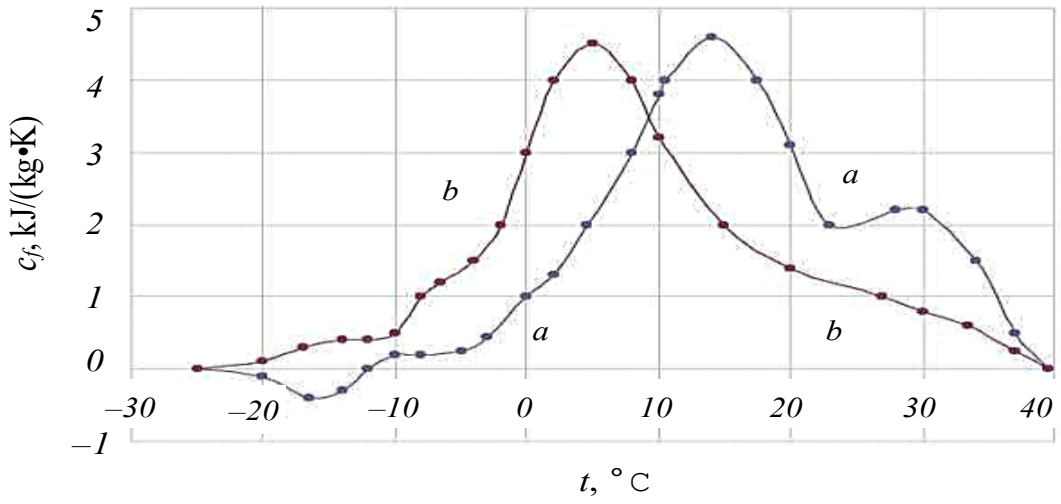

Figure 9. Diagram $c_f(t)$ for MF in PT area: a – heating, b – cooling

The fraction of hardened fat x at any t from t_h or t_k required for the technological calculations is possible to calculate by using the dependence $c_f(t)$ from Figure 9:

$$x = \int_{t_\rho}^{t} \frac{c_f dt}{L} = L\int_{t}^{t_k} \frac{c_f dt}{L}. \qquad (21)$$

You can also use the dependences $c_b(t)$ and $h_k(t)$ to determine $x(t)$:

$$x = \frac{h_k - h_t - \int_t^{t_k} c_{bdt}}{h_k - t_\rho - \int_{t_\rho}^{t_k} c_b dt}. \qquad (22)$$

Figure 10 shows the dependence $x\,(t)$ for MF with iodine number of 37, as well as $h(t)$ with $h = 0$ at $t = –25$ °C currently accepted in the dairy industry, that is, $h–x–t$ diagram in the field of phase transformations. You can determine the fraction of hardened fat x using this diagram, also the amount of heat that must be removed from 1 kg of fat to change x for a given value from x_1 to x_2, that is $\delta h = h_{x1} – h_{x2}$.

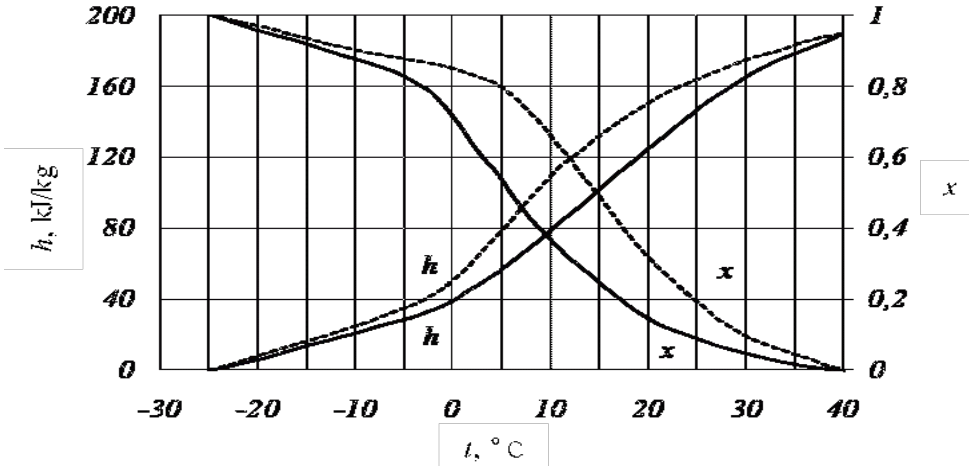

Figure 10. Diagram $h–x–t$ for MF in PT area: "–" – heating, "--" – cooling

Statistical processing of all the data obtained from the blurring and hysteresis of phase transformations of milk fat using TCM was performed by the methods of mathematical planning of experiments. The main tasks of this processing were to establish a reliable probability and to calculate the magnitude of the PT hysteresis during heating (or mechanic processing) of milk fat, to calculate the PT blurring for these processes as a maximum difference of the heat capacity values within one process, as well as to smoothen experimental data.

Both types of TCM – the method of cycles and the method of quasi-stationary mode, – in accordance with the objectives of their implementation in this work, were carried out as a one-factor experiment, and the aforementioned minimization of the error of TPC measurement of food products by the methods of TCM – was carried out as a multifactorial experiment. This gives the possibility to solve not only interpolation but also optimization problems using TCM methods. The data obtained in this work were used to process the results of the processes study connected with the inversion of heat fluxes during heat treatment of food products (Fedorov *et al.*, 2014).

$\gamma = 0.95$ was taken as a reliable probability γ of the results of the statistical processing taking into account the magnitude of the main permissible error for the devices implementing TCM, as well as the ratio of the dynamic properties of the devices with the PT dynamics of milk fat. All the accumulated information from BPT of milk fat obtained by the TCM method was processed in order to solve three main tasks. It was necessary to establish the facts of a statistically significant difference between the sample data of heating and cooling, and vice versa – the belonging of this data of each sample to one group, in spite of BPT. The third task was to smooth out the experimental data, because, as it is clear from the above, there can be no two derivatives at each point of the generalizing curves, but only one, in other words, the lambda-PT for MF is impossible.

The Fisher-Snedecor and Student's distributions, respectively, were used to solve the first two problems, and the method of the moving average (Walkenbach, 2015) was used for the third task. Computer processing was performed using the programs of F and t-tests, respectively, as well as the Moving in the middle program (Walkenbach, 2015).

Both null hypotheses with a reliable probability of 0.95 were confirmed, the results of processing are shown in Figure 9 and 10, as well as in Table 1 and 2. The curves in Figure 5 and 6 did not require the smoothing of the sensor signals. Tables 1 and 2 are compiled by the program of reducing the number to three significant figures.

Table 1. Characteristics of milk fat

Temperature	***t*, °C**	**–60**	**–50**	**–40**	**–30**	**–20**	**–10**	**0**
Heat capacity, heating	*c*, kJ/(kg·K)	1.49	1.49	1.49	1.49	1.32	1.70	2.72
Heat capacity, cooling	*c*, kJ/(kg·K)	1.49	1.49	1.49	1.49	1.68	2.14	3.96
Blurring, heating	*P*, kJ/(kg·K)	0.12	0.22	0.10	0.10	0.14	0.24	0.18
Blurring, cooling	*P*, kJ/(kg·K)	0.16	0.14	0.08	0.06	0.10	0.08	0.06
Hysteresis	*H*, K	0	0	0	0	10.0	6.0	6.2
Temperature	***t*, °C**	**+10**	**+20**	**+30**	**+40**	**+50**	**+60**	**+70**
Heat capacity, heating	*c*, kJ/(kg·K)	5.74	4.72	3.82	1.98	2.02	2.1	2.24
Heat capacity, cooling	*c*, kJ/(kg·K)	4.16	3.06	2.76	1.98	2.02	2.16	2.24
Blurring, heating	*P*, kJ/(kg·K)	0.78	0.42	0.22	0.10	0.10	0.08	0.18
Blurring, cooling	*P*, kJ/(kg·K)	0.20	0.32	0.08	0.10	0.08	0.12	0.18
Hysteresis	*H,* K	10.0	9.2	14,0	0	0	0	0

Table 2. Characteristics of milk fat in PT area

Temperature	*t*, °C	–20	–10	0	+10	+20	+30
Heat capacity due to PT, heating	*c*, kJ/(kg·K)	–0.15	0.12	0.98	3.12	2.70	1.82
Heat capacity due to PT, cooling	*c*, kJ/(kg·K)	0.10	0.42	2.48	2.60	1.22	0.66
Enthalpy of heating	*h*, kJ/kg	6.20	20.4	40.8	80.0	128	168
Enthalpy of cooling	*h*, kJ/kg	8.00	20.6	51.4	109	138	178
Fraction of solid fat, heating	*X*	0.98	0.94	0.88	0.72	0.12	0.02
Fraction of solid fat, cooling	*X*	0.09	0.96	0.94	0.71	0.12	0.09

4. Conclusions

The probable quantitative characteristics of the total heat capacity of milk fat, and due to the phase transformations, the fraction of solid phase, their blurring and hysteresis were determined for the first time. According to the research results, it is desirable to replace and supplement the information on this subject in the reference editions.

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SURVIVAL OF *ESCHERICHIA COLI* O157:H7 ON RAW MATURE GREEN TOMATOES DURING STORAGE TEMPERATURE ABUSE

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ABSTRACT

Tomatoes are important agricultural commodity, which are often consumed fresh without final pathogen elimination step. Being harvested as mature green fruit with further ripening, their shelf life can be greatly increased after harvesting. It is important to immediately cool down harvested fruit to 15ºC to avoid decay and optimize storage. The purpose of the current study was to evaluate survival of five-strain *Escherichia coli* O157:H7 cocktail on the undamaged surface of green mature tomatoes during 4-day storage at 25ºC, 15ºC, and temperature abuse conditions, such as slow ramping from 25ºC to 15ºC over duration of the experiment. Pathogen numbers declined 1.5 log units from theoretical inoculation level of 6.8 $\log_{10}$ cfu/mL of rinsate to 5.3 $\log_{10}$ cfu/mL upon 90 minutes inoculum drying, and significantly continued to decline during storage at both 25ºC and 15ºC, as well as temperature abuse conditions, resulting in final counts of 1.5, 2.4, and 2.6 $\log_{10}$ cfu/mL on day 4 for 25ºC, 15ºC, and ramp, respectively. The fastest decline was observed in 25ºC stored tomatoes. Placing tomatoes immediately into 15ºC incubator, or gradually decreasing storage temperature over a 4-day period, preserved the state of viability of *E.coli* O157:H7 comparing to other treatment.

1.Introduction

Based on the data from the United States Department of Agriculture, tomatoes are among the most popular vegetables in the United States, with 29.6 pounds consumed per person, including 58% eaten as canned product, in 2016 alone (USDA-ERS, 2016). *Salmonella*-associated tomato outbreaks were observed on numerous occasions (CDC 2002; Cummings *et al*. 2001; Croby *et al.* 2004). Natural microflora on the surface of raw tomatoes include variety of groups of bacteria, including Gram positive, Gram negative, pathogenic, opportunistic pathogens, and non-pathogenic (Tokarskyy and Korda, 2019a).

It is a common knowledge that enteric pathogens may be introduced onto tomatoes through irrigation water, bird droppings, wash water, handling by workers, or contaminated surfaces (Beuchat and Ryu, 1997). Tomatoes are commonly eaten fresh with no processing steps present to eliminate bacterial pathogens, such as cooking (Tokarskyy *et al.*, 2009) and irradiation (Schilling *et al.*, 2009). It was noted that pathogen will grow in the tomato even at ambient temperature, if introduced through wounds, stem scars, and abrasions (Zhuang *et al.*, 1995; Das *et al.*, 2006; Shi *et al.*, 2007). However, researchers agree that counts of pathogenic Gram-negative enteric bacteria will decline on undamaged surface over time, depending on bacterial species, strain, resuspension medium, humidity, and tomato storage temperature (Tokarskyy *et al.*, 2018; Tokarskyy and Korda, 2019b; Tokarskyy and Schneider, 2019). Because of *E. coli* O157:H7 not being implicated in tomato-related poisoning to the best of our knowledge so far,

most research related to tomato safety was done with *Salmonella* spp. Hirai (1991) mentioned that *Salmonella* spp. have better survival rates after drying on the surfaces, comparing to *Escherichia coli*. For example, Lang *et al*. (2004) showed that spot-inoculated tomatoes with *Salmonella* spp. or *E. coli* O157:H7 showed counts decline by 2.20 and 3.17 log units, respectively, after twenty four hours inoculum post-drying. A few studies have shown possibility for *Salmonella* Montevideo to colonize and grow on the surface of healthy undamaged tomatoes (Zhuang *et al*., 1995; Ituriaga *et al.,* 2007), but those records might be due to the presence of microabrasions on the surface where pathogen could have been introduced, or possibility of the pathogen introduction onto the stem part during inoculation via complete immersion. Earlier we showed that low contamination levels of *E. coli* O157:H7 do not persist on the surface of mature green, breaker stages, or pink tomatoes, if abovementioned surface is intact or bruised, at 15ºC and 25ºC (Tokarskyy *et al.,* 2018), while high level contamination may stay longer, depending on tomato storage temperature, diluent for pathogen resuspension, and humidity (Tokarskyy and Korda, 2019b).

It is not feasible, due to economic and marketing reasons, as well as due to mass production, to harvest tomatoes as table-ripe red fruit in the United States. Therefore, they are harvested as "mature green" with further ripening, either naturally, or using ethylene gas (Kader *et al.,* 1978). Such techniques, as lower temperature storage (less than 20ºC, but above 12.5ºC) and low oxygen storage (4%), delay ripening and make tomatoes available over longer period of time. Inaba and Chachin (1989) noted that both the respiration rate and the ethylene production were suppressed in green mature tomatoes stored at 5, 10, and 35ºC, but not at 15 or 25ºC, and fruit injury was obvious at 40ºC. However, Batu (2003) wrote, that 15ºC, but not 13ºC, was suitable for certain variety of mature green tomatoes storage to improve keeping quality without influencing flavor and further maturation into red fruit. Additionally, Mulholand *et al.* (2003) wrote, that "heat pulses" of 22.2ºC to 25.9ºC over a 3-day or 7-day periods significantly increased fruit defects and yields in green mature tomatoes in the week immediately following the end of a heat-pulse treatment. A three-day heat-pulse with a mean temperature of 23.0°C was sufficient to cause a 10% loss of fruit classified as class I (Mullholand *et al.,* 2003). Therefore, it is important to cool down green mature tomatoes to 15ºC as soon as possible after harvesting to increase shelf life of the product without damaging flavor and quality during ripening in the future.

The objective of the current study was to determine survival rates of *E. coli* O157:H7 at high contamination level for four days on the surface of unwashed and undamaged green mature tomatoes stored at room temperature (25ºC), cool temperature (15ºC), and during storage temperature abuse conditions (25ºC to 15ºC gradual ramp within four days). The hypothesis was that slower cooling may influence *E. coli* O157:H7 adaptation and cause better survival of the pathogen on the tomato surface.

2. Materials and methods

2.1. Rifampin preparation

Rifampin (rif, Fisher Scientific, BP26795) 0.4 grams was dissolved in 40 mL methanol (HPLC grade, Fisher Scientific), resulting in 10,000 ppm rif stock solution, filter-sterilized (0.2 micron nylon filter, Fisher Scientific), and stored refrigerated (4°C) in the darkness for no longer than one month. Rifampin was added to the cooled autoclaved Difco™ tryptic soy agar (TSA, Becton, Dickinson, and Co) or Bacto™ tryptic soy broth (TSB, Becton, Dickinson, and Co.) in order to yield 100 ppm final rifampin concentration, such as 0.1 mL rif stock to 10 mL TSB tube, or 10 mL rif stock to 1,000 mL TSA medium.

2.2. *E. coli* O157:H7 culture preparation

Two rifampin-resistant (200 ppm) strains of *Escherichia coli* O157:H7 (MDD20, MDD326) and two rifampin-sensitive strains (MDD19 and MDD 327NA), were kindly provided by Dr. Michelle Danyluk's lab (University of Florida, USA). Rifampin-sensitive strain ATCC 35150 was bought directly from American Type Culture Collection (Manassas, WI). Rifampin-sensitive strains were mutated to induce rifampin resistance by transferring a pure culture from TSA plates (37°C, 24 hours) to 10 mL TSB-rif 5ppm broth (37°C, 24 hours), followed by sequential transfer of 0.1 mL aliquot to TBS containing 10, 20, and 40 ppm rifampin. Turbid cultures (40 ppm rif) were streaked on TSA-rif200 plates (37°C, 24 hours), and a single colony was transferred to TSB-rif200 broth to confirm growth. Five rif-resistant *E. coli* O157-H7 strains were maintained on TSA-rif80 ppm slants at 4°C with bi-weekly transfers to fresh TSA-rif80 slants.

For the experimental protocol, five strains were streaked on TSA-rif100 plates (37°C, 24 hours), followed by three consecutive one loopful transfers to 10 mL TSB-rif100 tubes (37°C, 12 hours, 12 hours, and 18 hours). A pathogenic cocktail (10 mL, 10^9 cfu/mL) was prepared by mixing 2 mL of each culture from the third broth. The cocktail was centrifuged (4,300g, 10 minutes, Sorvall RC-5B centrifuge, DuPont Instruments) and washed once in 10 mL Dulbecco A phosphate buffered saline (PBS, Oxoid, Hampshire, England), followed by final centrifugation (4,300g, 10 minutes) and re-suspension in 10 mL 0.1% peptone (Bacto peptone, Becton Dickinson and Co, Sparks, USA). Concentration of inoculum was confirmed by serial dilutions in Buffered Peptone Water (BPW, Becton, Dickinson, and Co.) and pour plating using TSA-rif100 (37°C, 24 hours).

2.3. Tomato preparation, inoculation, and storage for temperature abuse study

Green mature tomatoes variety Florida 47, unwashed and unwaxed, were acquired for each replication from DiMare Co. (Ruskin, Florida, USA). Each tomato was dry rubbed using clean nitrile gloves before inoculation studies to normalize microflora within tomatoes in the same set.

For each replication, thirty nine mature green tomatoes were inoculated with 0.1 mL of pathogenic cocktail as 10 spots of equal size around blossom end each (10^8 cfu/tomato). Three sets of four tomatoes plus one tomato for immediate sampling were left uninoculated and served as negative controls. The procedure was carried out in a biosafety hood and tomatoes were allowed to dry for 90 minutes before moving into 25°C, 15°C, and temperature ramping incubator (see Figure 1 for schedule). A shallow pan with water was placed in each incubator to humidify environment, while temperature and humidity were recorded for four days with 10-minute sampling intervals (Hobo® U12 data logger, Onset Computer Corp, Pocasset, MA). Sets of three inoculated and dried tomatoes with one negative control tomato were tested immediately after drying (day 0, 90 minutes dry), and sampled on day 1, day 2, day 3, and day 4 from each incubator.

2.4. *E. coli* O157:H7 recovery from tomatoes

To recover pathogen, a single tomato was transferred to 20 mL BPW in a stomacher bag and vigorously manually shaken for 30 seconds, rubbed for 30 seconds, and shaken again for 30 seconds. The rinsate was either plated directly or serially diluted in 9 mL BPW tubes before plating using pour plate method and TSA-rif100 medium. The plates were incubated for 24 hours at 37°C before counting.

2.5. Statistical analysis

E. coli O157:H7 survival on tomatoes (three replications) was analyzed using multifactorial ANOVA with two factors – storage day (day 1, day 2, day 3, day 4) and storage temperature (15°C, 25°C, and ramp) influencing bacterial counts. If influence of factors or their combination was significant ($p<0.05$), means were separated using Fisher LSD procedure.

Average temperature for each datapoint for all replications for ramping temperature over 4-day sampling period with 10-minute intervals with overall standard deviation for each datapoint were calculated. Relative humidity values were averaged for all datapoints for each replication for 25°C, 15°C, and ramping temperature over 4-day sampling period with 10-minute intervals with overall standard deviation for each replication calculated.

3.Results and discussions

3.1.Physical monitoring of storage conditions

Results for continuous ramp temperature monitoring over time in temperature-abused inoculated tomatoes and negative controls are shown in Figure 1. Relative humidity in storage incubators are shown under Figure 2 footnote.

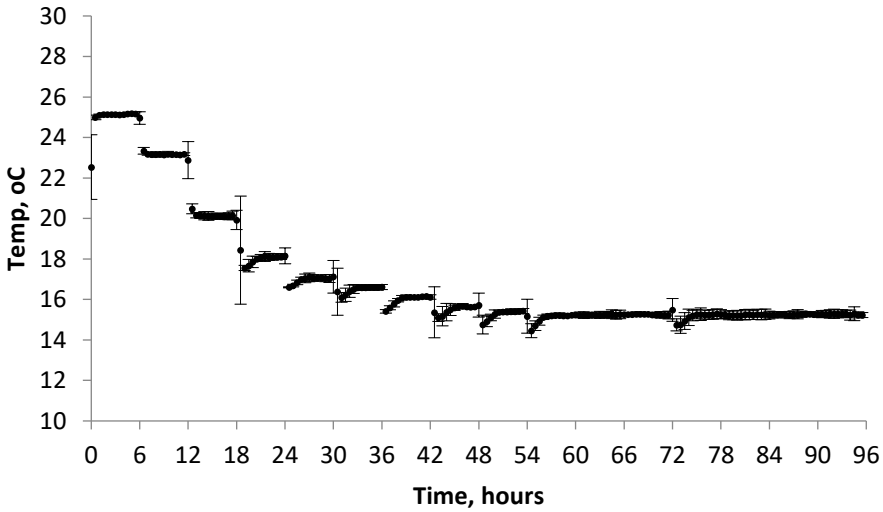

Figure 1. Temperature changes in simulating tomato temperature abuse incubator (25°C to 15°C decrease within four days). Average values of each datapoint for three replications (combined) with standard deviation included.

3.2. *E. coli* O157:H7 enumeration

As expected, *E. coli* O157:H7 numbers declined 1.5 log units from theoretical inoculation level of 6.8±0.1 SD $\log_{10}$ cfu/mL of rinsate to 5.3±0.1 SD $\log_{10}$ cfu/mL upon 90 minutes drying, and continued to decline rapidly during storage at both 25°C and 15°C, as well as temperature abuse conditions, resulting in final counts of 1.5, 2.4, and 2.6 $\log_{10}$ cfu/mL on day 4 for 25°C, 15°C, and ramp, respectively (Figure 2).

There was a significant influence of both factors, storage day and storage conditions, as well as their interaction, on *E.coli* O157:H7 counts ($p<0.05$, Figure 2). It appeared that the biggest decline was observed at 25°C on day 4, suggesting that cool conditions might have preservation effect on the bacterium. Interestingly, final pathogen counts under ramp conditions (25°C to 15°C) were not significantly different from the cool storage (15°C) on day 4 ($p>0.05$), suggesting that both fast cooling and slow cooling support survival, while higher temperature storage (25°C) accelerate bacterial die-off. Similarly, Lang *et al.* (2004) showed that *E. coli* O157:H7 counts in 5% horse serum on the dried spot-inoculated tomatoes decreased 1.07 logs after 1 hour drying and 3.17 logs 24 hours post-drying from initial 7.22 $\log_{10}$ cfu/tomato. Møretrø *et al*. (2010) showed that twelve Shiga-toxin producing *E. coli* strains, each analyzed separately, declined upon dessication in Brain Heart Infusion broth (BHI) on the stainless steel (type 304) from 6-7 logs to

3-5 logs on day 1 and 2-3.5 logs on day 7. Follow-up studies comparing BHI and water, 12°C and 20°C, 70% RH and 80% RH, showed beneficial effect of BHI, 12ºC, and 70% air relative humidity for *E. coli* survival. It can be argued that microorganisms in the dried up inoculum survive better at lower humidity (no metabolic activity) compared to high humidity, as well as at lower temperature, because at otherwise conditions exhausted stationary culture, still metabolically active, slowly dies off.

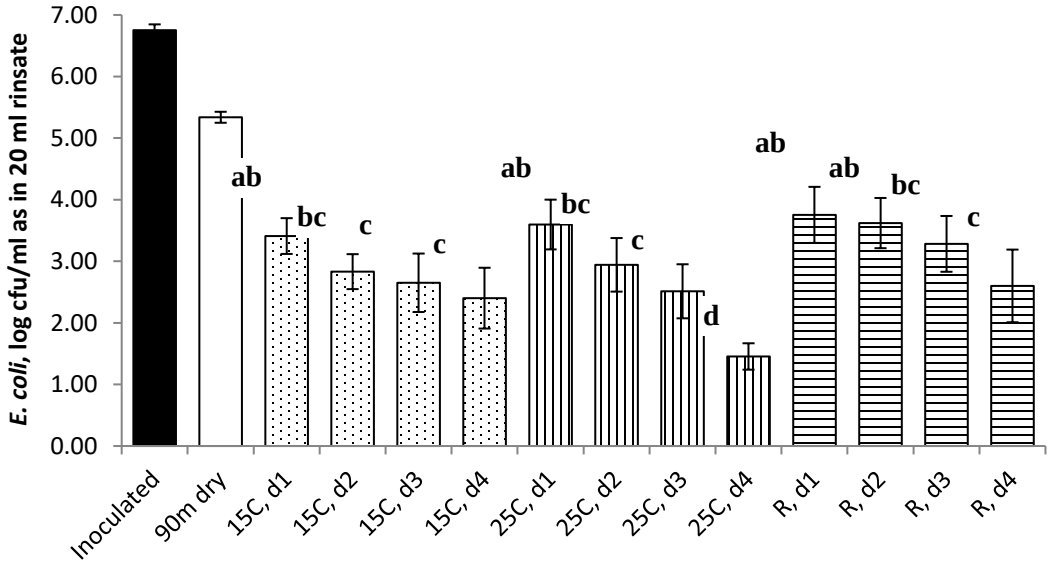

Figure 2. Recovery of *E. coli* O157:H7 from inoculated tomatoes either immediately after drying (90 min dry), or after storage for four days (d1-d4) at different temperatures (*15ºC, **25ºC, ***ramp). Counts expressed as $\log_{10}$ cfu per mL recovered from 20 mL rinsate. Inoculated level calculated theoretically based on stationary culture concentration and is shown for reference. Means with the same letters are not significantly different ($p>0.05$).

*(Relative humidity, 15ºC. Replication 1: 34.8±3.3%. Replication 2: 43.4±6.6%. Replication 3: 44.6±6.5%)
**(Relative humidity, 25ºC. Replication 1: 58.8±3.6%. Replication 2: 59.4±3.8%. Replication 3: 59.5±4.1%)
***(Relative humidity, ramp. Replication 1: 62.6±1.5%. Replication 2: 61.1±1.4%. Replication 3: 63.4±2.1%)

4. Conclusions

To summarize, *E. coli* O157:H7 did not survive well on the intact surface of tomatoes at 25ºC, but lower temperature at 15ºC might stimulate pathogen survival.

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DEVELOPMENT AND CHARACTERIZATION OF ANTIOXIDANT RICH WHEATGRASS CUPCAKE

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ABSTRACT

The optimum formulation for production of an Indian traditional baked wheatgrass cupcake was determined using response surface methodology. Effects of amount of ingredients such as wheatgrass powder (5-15%), and baking time (15–35 min) on the antioxidant potential (total phenolic content, total flavonoid content, % DPPH radical scavenging activity and vitamin C), mineral (Iron) and sensory attributes (overall acceptability) of cakes were investigated. Significant regression models which explained the effects of different percentages of wheatgrass powder, and baking time on all response variables were determined. The coefficients of determination, R^2 of all the response variables were higher than 0.83. Based on the response surface and superimposed plots; the basic formulation for production of baked wheatgrass cupcake with desired sensory quality was obtained by incorporating with 5% of wheatgrass powder, and 35 minutes of baking time. Optimized formulation was analyzed for its nutritional composition, antioxidant properties and anti-nutritional factors. The optimized formulation could be recommended to all the age group but especially for children, lactating mothers and geriatric population due to its high antioxidants, iron, calcium, and fiber content.

1. Introduction

Wheatgrass is an integral part of traditional Indian medicinal system. The young grass of common wheat plant is known as wheat grass *(Triticum aestivum*) belongs to family poaceae. Wheatgrass is rich source of antioxidants, vitamins (A, C, E known as an antioxidant), minerals (ca, mg, iron, zinc etc.), fiber and bioactive compounds (chlorophyllin, qurecitin, apegenine and rutin). The foremost constituent of Wheatgrass is chlorophyll. Chlorophyll constitutes about 70% of total chemical constituents of Wheatgrass (Swati et al. 2010). Chlorophyll which is presence in wheatgrass has almost chemically comparable to hemoglobin. It has been various pharmacological potential, to have blood building activity (Marwaha et al., 2008), anti cancer activity (Dey et al.,2006), anti ulcer activity (Ben-Arye 2002), anti diabetic potential (Chauhan et al., 2014), anti arthritic potential, anti inflammatory and anti aging potential (Smith et al., 2000). It is believed that pharmacological potential of wheatgrass is due to its high nutrient content and presence of bioactive compounds, which makes it a medicinal plant for the treatment of various diseases and life threatening conditions (Walters et al., 1992). With such enormous health benefits, the present study was conducted to optimize the formulation of wheatgrass cupcake of rewarding sensory attribute, nutritional properties and antioxidant content of the developed wheatgrass cupcake.

Cupcake is known to be one of the most expedient and accepted bakery product in the world (Udeme et al., 2014) . During the past,

many experiments were conducted to improve the nutritional value of cupcake like fiber rich, sugar free, antioxidant rich cupcake and fat free cupcake. Now days the renewed costumer's interest in the consumption of nutritious healthy and natural food products that leads to various health benefits.

Therefore, the concentrations used in making the cupcake with incorporation of wheat grass powder has been an important factor in developing a new product with less cost and other more benefits such as improving the aesthetic value, nutritional density, antioxidant and fibre content of cupcake. In this context, the main idea of this work was to develop sustaining and functional food products (Tripathi et al. 2017). The aims of this study were i) optimization of the developed food product. ii) To evaluate the proximate composition, iii) antioxidant potential iv) Anti nutritional factors of the developed product.

2. Materials and methods

2.1. Procurement of the raw material

Wheatgrass seeds for the research were purchased from local market of Allahabad, India and grown in controlled conditions at the laboratory of Centre of Food Technology, University of Allahabad, India. All the other required ingredients like refined wheat flour, sugar, milk, butter, baking powder, and coco powder were purchased from local market of Allahabad. All the chemicals used in analyses were of AR grade.

2.2. Cultivation of wheatgrass

For growing wheatgrass, Superior fine quality wheat was procured from local market of Allahabad, and cleaned properly. The wheat grains were soaked in cold water for 12 hours. After 12 hours of soaking the water was strained and the soaked grains were tied in wet woven cotton cloth and hung for a period of 12 hours. After 12 hours of germination, the germinated wheat was sowed in a shady place. Since wheat can grow in all temperatures, shady place is preferred to avoid excess nutrient loss due to exposure to direct sunlight. The sowed seeds started to grow and on the seventh day, the grass reached the length of 15 to 18cm which was then harvested.

2.3. Preparation of wheatgrass powder

Fresh and whole wheatgrass leaf was washed with water, and dried in a cabinet tray dryer (Chemida, Mumbai, India) at 55 ± 2°C for 8 h. The dried material was ground to powder using a high speed mixer (Sumeet Domestic Plus, M/s. Sumeet, Nashik, India), passed through BS 72 (220 μm) mesh and dehydrated whole wheatgrass powder was obtained. The powder was packed in metallized polyester polyethylene (MPE) laminate pouches (12 μm metallized polyester, 7.5 μm polyethylene) laminated pouches of size 14×12 cm^2 were used for packing and stored at 4°C for further chemical analysis and application studies.

2.4. Experimental design

Response Surface Methodology (RSM) was used to determine the experimental design and optimal ingredient level in preparation of wheatgrass cupcake. RSM is an important tool for optimization, which reduces the number of experimental runs needed to provide sufficient information for statistically acceptable results. A three factor central composite design CCD was used to design the experiments comprising of two independent variables including the wheatgrass powder (5-15 g), and baking time (15-35 minutes) Table 1. The effects of these variables were seen on the responses variables total phenolic content, total flavonoid content, % DPPH scavenging activity, vitamin C content, Iron content and overall acceptability. The experimental sheets of 13 variants with different ratio of independent variables were generated. The response variables to be estimated were entered in the sheet. This data was subjected to analysis of variance (ANOVA) one-way analysis and regression coefficients (R^2) to get the optimum response. Coefficient of determination (R^2) values should be close to 1. The predicted R^2 value should be in reasonable agreement with adjusted R^2 (Bunkar et al.,2012). R^2 can be defined as the ratio of explained

variation to the total variation, which was a measure of the degree of fit (Chan et al.,2009).

2.5. Formulation of the product

2.5.1. Preparation of the wheatgrass cupcake

A cake batter recipe containing 100% refined wheat flour,100% sugar, 25% shortening (butter), 9% cocoa powder, 3% salt and 5% baking powder(all percentages are given on a flour weight basis)was used in the experiments. Amount of water added to the batter was 27% of the overall formulation. Wheatgrass powder (5-15%) was mixed in the proportions as obtained in the experimental design to form different formulations. A cake batter containing no wheatgrass powder was used as control (Deora et al., 2014). During preparation of the cake, first, dry ingredients (refined wheat flour, baking powder, salt and wheatgrass powder) were mixed thoroughly. In a separate cup, sugar and butter were mixed, and then melted shortening was added and mixed for 1min at 85rpm by using a mixer (KitchenAid,5K45SS,St.Joseph,MI,USA).
Then, dry ingredient mix and water were added simultaneously to this mixture and mixed first for 2 min at 85 rpm, then for 1min at 140 rpm and finally for 2min at 85rpm.. In cupcake molds, cake samples of 100 g each were baked in microwave oven at 180±5 ºC for 30 minutes (Jerome et al., 2019). Wheatgrass cupcake was packed in paper/ foil/ polyethylene (PFP) pouches prior to sensory, proximate, antioxidant and anti-nutritional analysis. The data for formulations along with responses were analyzed using statistical software (Design-Expert 7.0.0) of the best-fit design to obtain the optimized compositions.

2.6. Sensory Evaluation

The sensory evaluation of the wheatgrass cupcake (13 formulated combinations) was performed by 20 semi-trained panelists from the Department of Food Science and Technology, University of Allahabad, India. The sensory evaluation was conducted using the seven-point hedonic scale as described by Watts, Ylimaki, and Jeffery (1989). The food samples were randomized, coded with three-digit random numbers and each sample was presented with different number. The randomized order of the sample was presented once at a time to each panelist. Panelists were asked to evaluate the coded samples for each sensorial parameter including color, aroma, texture, flavor, and overall acceptability based on their degree of liking (1 = dislike very much; 2 = dislike moderately; 3 = dislike slightly; 4 = neither like nor dislike; 5 = like slightly; 6 = like moderately; 7 = like very much).

2.7. Nutritional analysis

The moisture content of the wheatgrass cupcake was determined by drying at 105 °C until a constant weight was attained as per (AOAC 2005). The micro Kjeldhal method was employed to determine the total nitrogen and the crude protein content (Nx6.25) (AOAC 2005). Crude lipid was estimated by extraction with petroleum ether (60–80 °C), with a soxhlet apparatus and ash contents were determined as per (AOAC 2005). Dietary fiber was estimated using acid and alkaline digestion method. Ash and carbohydrate contents were determined by (AOAC 2005) method. Vitamin C was determined by titrimetric method (Ranganna 2005). Calcium content was estimated by precipitating it as calcium oxalate and titrating with standard potassium permanganate solution; iron content was estimated using colourimetric method using UV–Visible spectrophotometer (Shimadzu, UV-160A model) at 480 nm (AOAC, 2005). Phosphorous content was analyzed by developing colour using ammonium molybdate and 2, amino −6, naphthol sulphonic acid. The blue colour developed was read at 650 nm in UV–Visible spectrophotometer and expressed as phosphorus mg/100 g. The percent carbohydrate content and the energy value were calculated by difference using the following equations:

$$\%\ Carbohydrate = [100 - (Moisture + Total\ ash + Protein + Fibre + Fat)] \quad \text{Eq.1}$$

Energy (kcal/100g) = 4 (% Protein + % Carbohydrate) + 9(% Fat) Eq.2

2.8. Antioxidant analysis

2.8.1. Total Phenolic Content (TPC)

Phenolic compound concentration in the extract was estimated by a colorimetric assay, based on procedures described by Singleton and Rossi with some modifications (Singleton and Rossi 1965). Briefly, 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction tube was kept in the dark for 60 minutes after which the absorbance was measured at 765 nm (Thermo Scientific, model-Evolution 600). Gallic acid was used to calculate the standard curve (0.01– 0.4 mM). The results are expressed as mg of gallic acid equivalents/g of extract (GAEs).

2.8.2. Total flavonoid content (TFC)

Aluminum chloride colorimetric method was used for flavonoid determination (Bahorun et al. 1996). 1 ml of sample methanolic extract was mixed with 1 ml of 2% aluminum chloride. The absorbance of the reaction mixture was measured at 430 nm with a spectrophotometer (Thermo Scientific, model-Evolution 600). A calibration curve was prepared using a standard solution of quercetin (0.05- 0.5 mg/ml). Final results are expressed as mg quercetin equivalents/g (QE) of sample.

2.8.3. Radical scavenging activity

The DPPH radical was used to measure the free radical scavenging activity of extracts by the method of Blois et al 1956. Sample extracts were taken and 3 mL of a 0.1 mmol/L methanolic solution of DPPH was added to the aliquots of sample extracts of product and standards. DPPH solution (3 mL) along with methanol (100µL) was used as a negative control. All the reaction mixtures were incubated for 20 min in dark. DPPH radical inhibition by the samples was measured at 517 nm against the blank (methanol). The inhibition percentage for scavenging DPPH radical was calculated according to the equation:

% decolorization = Control Absorbance - Sample Absorbance/Control Absorbance × 100

2.8.4. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing ability of the extract was estimated by the method of Pulido et al., 2000. The FRAP reagent was prepared by mixing 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6). 900 µL of FRAP reagent was mixed with 10 µL of aliquot of sample extracts and incubated at 37ºC. After incubation, ferric reducing ability of sample extracts was measured at 595 nm. The results were expressed as µmol/L Fe (II) equivalents/mg extract.

2.8.5. Reducing capacity

The reducing power ability of the extract was evaluated by the method described of Oyaizu et al., 1986. The reaction mixture contained 1.0 mL of product extract (2–10 mg/mL), 2.5 mL of 1% potassium ferricyanide and 2.5 mLof 0.2 mol/L sodium phosphate buffer. The mixture was incubated at 50°C for 20 min and the reaction was terminated by the addition of 2.5 mL of 10% trichloroacetic acid, followed by centrifugation at 3000 r/min for 10 min. 2.5 mL of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicates increased reducing power of the sample. Ascorbic acid was used as standard.

2.8.6. Metal ion chelating activity

The chelating activity of the sample was determined by the method of Dinis et al., 1994. 500 µL of samples were added to 100 µL solution of 2 mmol/L $FeCl_2$. The reaction was initiated by the addition of 400 µL of 5 mmol/L ferrozine and incubated at room temperature for 10 min. Absorbance of the samples was then measured spectrophotometrically at 562 nm against the blank (deionized water). A lower

absorbance of the reaction mixture indicated a higher Fe^{2}+ chelating ability. The control contained all the reagents except sample. Gallic acid and ascorbic acid was used as standard.

2.9. Anti-nutritional analysis

2.9.1. Tannin

Tannin content in optimized product was determined by Folin-Denis method as described by Sadasivum and Manickam (2005). Color intensity was measured at 700 nm after 30 minutes of incubation period. Standard graph was prepared using 0-100 µg tannic acid. Tannin content of the samples was calculated as per cent (%) tannic acid from the standard graph.

2.9.2. Phytate

Phytate content is determined by colorimetric method as described by Sadasivam and Manickam (2005). 3% TCA was used for extracting phytate and was precipitated as ferric phytate, which was then converted into ferric hydroxide, and soluble sodium phytate by adding sodium hydroxide in boiling condition. Hot nitric acid was added to it and solution was diluted. Colour of solution was developed using potassium thiocyanate and its intensity was read immediately at 480nm.The absorbance of iron content so determined was used for calculating phytate phosphorus content assuming a constant 4 Fe: 6 P molecular ratio in the precipitate. Ferric Nitrate was used to make standard curve.

2.9.3. Trypsin inhibitor

Trypsin inhibitor (TI) activity of sample was determined according to the method of Kakade et al.,1974, as modified by (Rackis et al. 1981) using BAPNA (N-a-Benzoyl-DL-Arginine p-nitroanilide) as a substrate.

2.10. Statistical Analysis

The data obtained was analyzed statistically for analysis of variance (ANOVA) using completely randomized design with least significant difference (LSD) at $P < 0.05$ using Design Expert 7.1 statistical software package.

3.Results and discussions

In this study, antioxidant rich wheatgrass cup cake was prepared from natural ingredients to yield products with specific functional properties. The proximate composition of wheatgrass cupcake clearly showed that optimized formulation is rich in calcium (273mg/100g), iron (9.25mg/100g), dietary fiber (12.43%) and energy (433.3 kcal), which fulfills approximately one third nutritional requirement of school going children. The optimized edible product of wheatgrass cupcake was developed using Central Composite Design with minimum possible number of points (Table 1).

The experimental design with different independent variables and respective responses along with the coded variables for the product is given in (Table 2).

Table 1. Levels of dependent vaibles for optimized wheatgrass cupcake.

Variables	Units	(-1) Low level	(+) High level	(-) Alpha	(+) Alpha
Wheatgrass powder	(g)	5	15	2.92893	17.0711
Baking time	(Minutes)	15	35	10.8579	39.1421

Table 2. Experimental data for antioxidant rich wheatgrass cupcake response variables such as wheatgrass powder (g) and baking time (min).

Process variables (coded terms)		Responses					
wheatgrass powder (g)	Baking time (minutes)	TPC (mg/100g)	TFC (mg/100g)	DPPH (%)	Vitamin C (mg/100g)	Iron (mg/100g)	Overall acceptability
5.00	15.00	10.65	0.46	64.58	9.58	8.56	8.24
10.00	25.00	13.25	0.58	80.25	10.37	11.25	7.46
10.00	25.00	14.45	0.61	81.56	11.35	10.65	7.46

10.00	25.00	14.56	0.62	74.58	12.15	11.65	6.49
10.00	25.00	15.45	0.65	80.56	11.65	10.54	7.86
10.00	39.14	16.38	0.64	81.68	10.26	12.56	6.62
10.00	10.86	17.59	0.62	78.68	11.35	11.68	7.46
5.00	35.00	11.56	0.59	71.68	8.56	6.26	6.86
15.00	15.00	13.56	0.64	80.58	11.59	10.47	4.28
15.00	35.00	14.56	0.67	78.68	12.54	16.48	6.46
2.93	25.00	7.54	0.48	58.56	6.48	4.46	6.57
17.07	25.00	14.65	0.78	78.59	13.48	16.46	4.36
10.00	25.00	13.54	0.66	74.59	11.64	15.84	6.65

Model fitting from RSM

The effects of wheatgrass powder and baking time on total phenolic content (TPC), total flavonoid content (TFC), DPPH, iron, and overall acceptability of baked wheatgrass cup cake are shown in Table 2.

The independent and dependent variables were fitted to the second-order model equation and examined for the goodness of fit. The analyses of variance were performed to determine the lack of fit and the significance of the linear, quadratic and interaction effects of the independent variables on the dependent variables (Table 3).

The lack of fit test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression Varnalis et al, 2004.

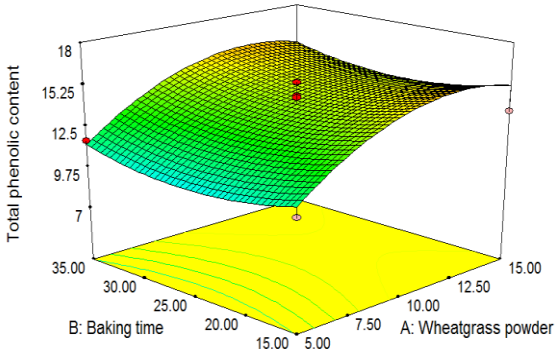

Figure 1. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total phenolic content (TPC) of wheatgrass cupcake.

Table 3. Estimated coefficient for the different response variables

Variables	D.f	Estimated Coefficients					
		TPC	TFC	DPPH	Vitamin C	Iron	O.A.
Model	5	14.25	0.62	78.31	11.43	11.99	7.18
A	1	2.00	0.086	6.42	1.99	3.64	-0.94
B	1	0.025	0.024	1.18	-0.20	0.62	-0.048
AB	1	0.023	-0.025	-2.25	0.49	2.08	0.89
A^2	1	-1.94	-0.0070	-4.99	-0.68	-0.97	-0.81
B^2	1	1.00	-0.0070	0.81	-0.27	-0.14	-0.020
R^2	0.8617	0.8370	0.9146	0.9012	0.9012	0.8442	0.8732
Adj R^2	0.7630	0.7206	0.8535	0.8306	0.8306	0.7329	0.7826
CV%	9.22	6.97	3.57	6.96	6.96	16.5	8.30

TPC=Total phenolic content; TFC= Total flavonoid content; O.A=Overall acceptability; R^2=Coefficient of multiple determinations; CV= coefficient of variance.

Coefficient of determination or R^2 is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for good fit model, R^2 should be at least 80%.

The results showed that the models for all the response variables were highly adequate because they have satisfactory levels of R^2 of more than 80% and that there is no significant lack of fit in all the response variables indicating a high proportion of variability as explained by the data. Therefore, the response surface models developed were adequate.

Effect of amount of wheatgrass powder and baking time

The effect of different amount of wheatgrass powder and baking time on the instrumental data (TPC, TFC, DPPH, ascorbic acid and Iron content) and the sensory attributes (overall acceptability) of baked wheatgrass cupcake are reported (Table 3) by the coefficient of the second-order polynomials (Rifna et al., 2019) . To aid visualization, the response surfaces for these response variables are shown in Figs. 1–6.

Effect on the Total phenolic content (TPC)

Total phenolic content (TPC) is one of the important antioxidant properties of the formulated product. In the present study, It can be observed (Fig.1) that the total phenolic content (TPC) of the baked wheatgrass cupcake depended on the amount of the wheatgrass powder added, as its linear, quadratic and interaction effects were positive at $p \leq 0.05$. Thus, an increase in the amount of wheatgrass powder might probably lead to an increase in total phenolic content of product. This may be due to the higher antioxidant content of wheatgrass powder. Similarly, the effect of baking time showed positive linear, interaction and quadratic effects $p \leq 0.05$) on the phenolic content of baked wheatgrass cupcake. Because in some conditions, heat-processing treatments (baking, roasting) may also be helpful for increasing antioxidant content. Heat treatments (baking and roasting) leads to chemical oxidation of phenol and non-enzymatic browning reaction associated with strong antioxidant potential (Manzocco et al.,2000).

Effect on total flavonoid content (TFC)

Total flavonoid content of backed wheatgrass cupcake was shown in Table 3, and Fig. 2, it is clear that the scores for total flavonoid content were affected by the backing time and amount of wheatgrass powder added. Table 3 showed that total flavonoid content was affected by the amount of wheatgrass powder used, with positive linear and negative quadratic and inaction effects at $p \leq 0.05$. The total phenolic content was high initially and it decreases as the amount of wheatgrass was increased gradually. However, the interaction and quadratic effects of baking time on total flavonoid content were negative at $p \leq 0.05$ and the effect was linear, owing to a positive a $p \leq 0.05$ (Table 3). As the baking time was increased, it had changed the product total flavonoid content.

Hence, a higher amount of wheatgrass powder and moderate level of baking time might increase the total flavonoid content of baked wheatgrass cupcake.

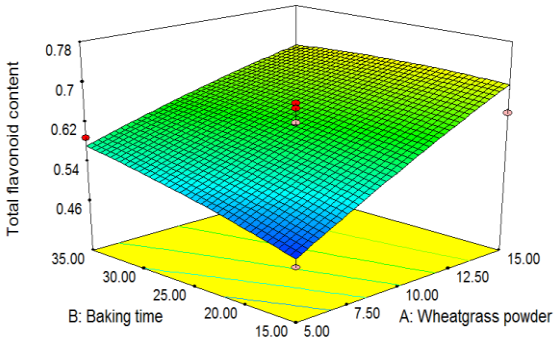

Figure 2. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total flavonoid content (TFC) of wheatgrass

Effect on DPPH radical scavenging activity

Replacement of wheatgrass powder had a positive effect on the DPPH radical scavenging activity indices at positive linear and negative quadratic terms, showing significant levels at $p<0.001$ and $p<0.001$, respectively (Table 3). However It can be observed that the positive effect of baking time on the DPPH radical scavenging activity at linear ($p<0.001$) and quadratic ($p<0.05$) term (Table 3). Thus increasing the replacement level of wheatgrass powder and baking time would increase the DPPH radical scavenging activity indices to positive values. Result also showed that the interaction effect on DPPH ($p \leq 0.05$) was negative meaning that the DPPH was dependent on both of these variables. DPPH content was increased when increase in the amount of wheatgrass powder added and with prolonged baking time (Fig. 3).

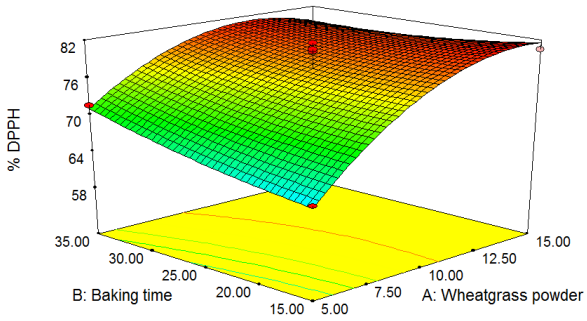

Figure 3. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total % DPPH (TPC) of wheatgrass cupcake.

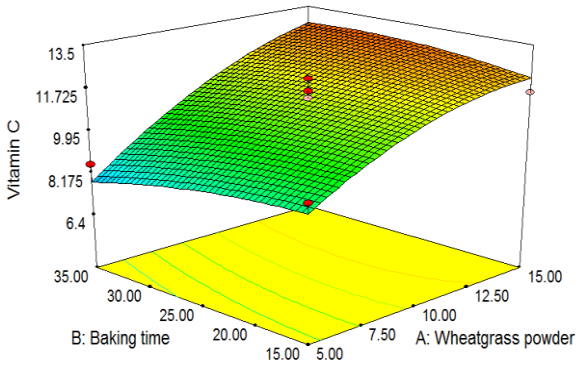

Figure 4. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on ascorbic acid of wheatgrass cupcake.

Effect on ascorbic acid content

Baking time had a negative effect on the Ascorbic acid content at linear and quadratic terms, showing significant levels of $p \leq 0.05$ (Table 4).because ascorbic acid is not heat sable and its destroy when temperature is high, a positive linear effect $p \leq 0.05$ of the amount of wheatgrass powder on the ascorbic acid content was found. This indicates that the presence of wheatgrass powder could enhance the ascorbic acid content of the baked wheatgrass cup cake (Fig. 4). The highest amount of ascorbic acid content for baked wheatgrass cupcake obtained when the amount of wheatgrass powder added was increased and baking time deceased.

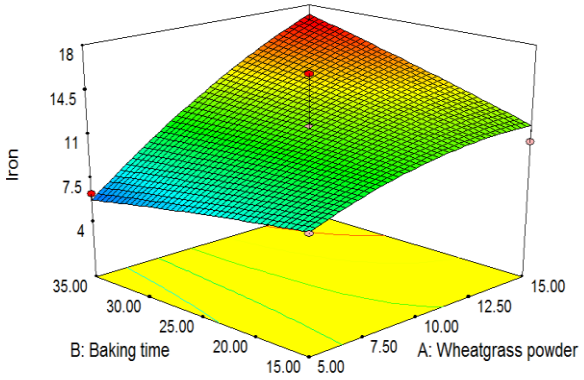

Figure 5. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on iron content of wheatgrass cupcake.

Table 4. Analysis of variance for the response variables

Variables	D.f	F-Values					
		TPC	TFC	DPPH	Vitamin C	Iron	O.A.
Model	5	8.72	7.19	14.99	12.77	7.59	9.64
A	1	20.06*	31.85*	44.94*	55.46*	30.23*	22.84*
B	1	0.00310	2.41	1.52	0.57	0.88	0.061
AB	1	0.00127	1.36	2.76	1.71	4.93	10.33*
A^2	1	16.52*	0.19	23.65*	5.69*	1.89	14.80*
B^2	1	4.41	0.19	0.63	0.89	0.042	0.0198
Lack of fit		3.48^{ns}	2.83^{ns}	0.12^{ns}	1.72^{ns}	0.35^{ns}	0.75^{ns}

TPC=Total phenolic content; TFC= Total flavonoid content; O. A.=Overall acceptability; *=Significant at P<0.05; ^{ns}= not significant; Df=Degree of freedom; F= ratio of variance estimates.

Effect on iron content

Figure 5 shows the response surface plot at different replacement level of wheatgrass powder and baking time on iron content. Table 3 indicated that iron content was affected by wheatgrass powder, with positive linear ($p<0.05$) and negative quadratic effects at $p<0.05$. However, the same pattern also can be observed on the positive linear and negative quadratic effects of baking time on wheatgrass cupcake (Table 3). As the wheatgrass powder replacement level and baking time increased the iron content of wheatgrass cupcake also was increased.

Effect on overall acceptability

For the evaluation of sensory attribute of formulated product, overall acceptability was considered as response variable. In this study the hedonic ratings of sensory attribute i.e. overall acceptability was observed 6.86 (like moderately) by the panelists (Table 2). Overall acceptability of the optimized product was found increase with increase in the amount of wheatgrass powder, and baking time. Figure 1 shows the response surface for the effect of independent variables on the overall acceptability of wheatgrass cupcake. As shown in Table 3, overall acceptability was negatively related to the linear and quadratic effects of wheatgrass powder ($p<0.05$) and baking time ($p<0.05$). The overall acceptability was significantly decreased with the increase level of wheatgrass powder and baking time (Figure 6). However he interaction effects of wheatgrass powder and baking time were positive at $p<0.05$ respectively shows that the moderate amount of wheatgrass powder and optimum time period seemed to be more acceptable by the panelists, and could increased the overall acceptability of wheatgrass cupcake.

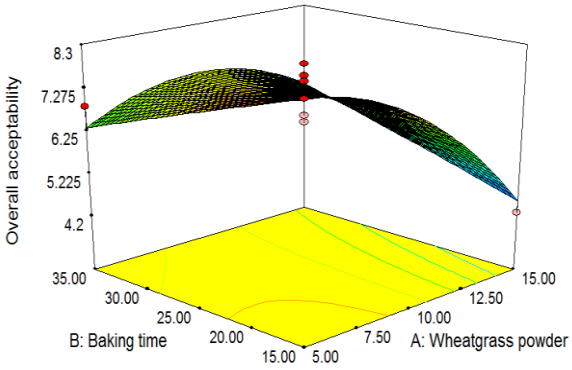

Figure 6. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on overall acceptability of wheatgrass cupcake.

The representative antioxidant materials in wheatgrass are phenolic compounds, including flavonoids and ascorbic acid content. Significantly, these compounds have been reported to have antioxidant activity and are thought to be responsible for the antioxidant activity of backed wheatgrass cupcake samples by inhibiting free radicals (Borek 2001). However, the increased amount of wheatgrass powder and the baking time resulting in the highest antioxidant activity, including total phenolic content, total flavonoid content and DPPH radical scavenging activity and highest ion content was found when increased amount of wheatgrass powder and moderate period of baking time; however, ascorbic acid content largely increased in baked wheatgrass cupcake backed for less time period.

Other active constituents may contribute to the antioxidant activity of the backed wheatgrass cupcake. Many studies have indicated that the presence of browning products is related to increases in antioxidant activity, and browning products have been shown to exert antioxidant action by breaking the free radical chain through the donation of hydrogen atoms (Eichner. 1981;Manzocco et al.,2000). A positive and highly significant relationship between total phenolics and antioxidant activity in plant products has also been previously demonstrated (Stratil, Klejdus, and Kubán. 2006). In this study, the browning intensity gradually increased during the wheatgrass cupcake manufacturing process, and it exhibited a trend

similar to that of total phenolic content, total flavonoid content and DPPH radical scavenging activity of wheatgrass cupcake backed at various time periods temperatures. Moreover, all these properties were enhanced in backed wheatgrass cupcake backed for long period of time and at high temperatures (Nencini et al., 2011).

Optimization and characterization

In this study, Response surface methodology was used for the optimization of independent variables i.e. amount of wheatgrass powder, and baking time and their effect on responses i.e. (TPC, TFC, DPPH, ascorbic acid and Ion content) and the sensory attributes (overall acceptability). It reveals that the terms in each model had a significant effect on the responses- TPC, TFC, DPPH, ascorbic acid, Ion content and overall acceptability, suggesting a good fit of each model. The response optimization was achieved as per the desired criteria based on the acceptance of the product. The solutions could be achieved from the software with the maximum desirability as well as the acceptance and the optimum variable levels by being at random starting points and proceeding on the path of the steepest slope to a maximum. The best among them was taken as the optimum. Wheatgrass powder 5.00 g, with 35:00 minutes baking time achieving the desirability of 1 and OAA of 6.86 on nine point hedonic scale was the optimized ingredient composition with the best fit. The predicted response value of acceptability, TPC, TFC, DPPH, ascorbic acid and Ion content scores were 7.18, 14.25, 0.62, 78.30, 11.43, 11.98 as against actual values 6.86, 11.56, 0.59, 71.86, 8.56, 6.26 respectively, which were in concurrence with each other.

Proximate Composition

Nutritional composition of wheatgrass cupcake is presented in Table 5. Wheatgrass cupcake possessed good quantities of protein 12.65%, fiber 8.8%, along with minerals such as calcium 160mg/100 g, iron 12.46mg/100 g and phosphorous 86.45 mg/100 g, ascorbic acid 8.46 as compared to control. Increasing addition of wheatgrass powder (5–15%) has shown good enhancement in protein, minerals and fiber in cupcake when compared to control. Rahman and Hiregoudaret (2014) produced muffins using 2.5-7.5% of dry wheatgrass powder, and that muffin formulated with replacement of wheat flour with up to 5.0 per cent wheatgrass had higher protein and fiber content as compared to muffin prepared with 100 per cent wheat flour. This study demonstrated that wheat grass powder offers a great potential to be used in a variety of food products to enhance their nutritional quality.

Table 5.Proximate Analysis

Parameters	Control	Optimized wheatgrass cupcake
Moisture g/100g	15 ± 0.81	13.00±0.65
Protein g/100g	8.5 ± 0.84	12.65 ± 1.23
Fat g/100g	7.46 ± 0.65	5.50±1.12
Ash g/100g	2.83 ±1.24	3.50±0.65
Fiber g/100g	1.2± 0.40	8.8 ± 0.73
Carbohydrate g/100g	48.2±2.56	49.5±1.15
Phosphorus mg/100g	56.00±1.36	78.33±2.21
Calcium mg/100g	78.00±2.14	160.34±2.45
Iron mg/100g	6.70±0.20	12.46±1.36

The nutritive value of wheatgrass powder supplemented formulation was found higher than that of control product. It is clear that supplementation of the basic formula with the wheatgrass powder resulted in higher dietary fiber, and mineral matter content. This fulfills approximately one third nutritional requirement of school going children (Table 5). The fiber and minerals content was relatively high in this product, which indicates that incorporation of natural plant fibers, and their minerals in food products thus increasing the mineral and fiber consumption in daily diet.

Antioxidant analysis

Antioxidant potential of optimized formulation wheatgrass cupcake was shown in (Table 6). Wheatgrass powder supplemented optimized formulation contained higher antioxidant potential including 0.71 mmolFe(II)Eq/g FRAP value, 0.68 % Reducing capacity, and 65.65umolAAE/g Metal chelating activity than control 0.25 mmolFe(II)Eq/g FRAP value, 0.32 % Reducing capacity, 38 µmolAAE/g Metal chelating activity respectively. Incorporation of wheatgrass powder, gave an excellent antioxidant effect on the wheatgrass cupcake as compared with control. Addition of wheat grass enhanced the antioxidant effect of the optimized formulated product. The higher efficiency of the wheatgrass powder could be due to the persistence of this natural antioxidant during processing. In addition, natural antioxidants are safe and impart health benefits to the consumer.

Table 6. Antioxidant analysis

Treatments	FRAP (mmolFe(II)Eq/g)	Metal chelating (µmol/AAE/g)	Reducing powder (%)
Control	0.25±0.23	38.12±0.86	0.32±0.23
Optimised wheatgrass cupcake	0.71±0.15	65.67±0.75	0.68±0.06

Anti-nutritional analysis

The anti-nutritional factors of optimized product are summarized in (Table 7). Highest tannin, trypsin inhibitor and phytate content was found in optimized wheatgrass cupcake (0.56%, 20%, 38.67%) respectively, and lowest was in case of control (0.43%, 18%, 34.33%) respectively.

It must be noted that anti-nutritional factors (tannin, trypsin inhibitor and phytate content) of wheatgrass cupcake was found higher than control product. Studies suggest that anti-nutritional factors can be reduced by various food processing techniques.

Table 7. Anti- nutritional analysis

Treatments	**Tannin (mg/100g)**	**Phytate (%)**	**Trypsin inhibitor (%)**
Control	0.43±0.11	34.33±1.52	18.00±1.25
Optimized wheatgrass cupcake	0.56±0.21	38.67±1.52	20.00±1.41

4. Conclusions

The wheatgrass cupcake formulation can serve as a good source of dietary fiber, minerals and is a novel approach for increasing the mineral and fiber consumption in daily diet. Wheatgrass can be considered as a good source of natural antioxidants and has the potential to enhance the health benefits to the consumer.

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EFFECTS OF SMOKING ON THE NUTRITIONAL COMPOSITION OF FLESH AND OIL CHEMISTRY OF ATLANTIC MACKEREL (*SCOMBER SCOMBRUS*) OIL

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ABSTRACT

Fish constitute a major part of human diet. It a good source of proteins, lipids and minerals. Fishes are processed before they are consumed, to offer palatability and preservation. The study investigated the effects of processing on the nutritional properties, *in vitro* antioxidant capacity and fatty acid profile of oil. Processing methods used were sun drying and smoking methods. On milled fish samples, nutritional analysis such as minerals and proximate analysis were performed using standard protocols. After processing, oil from *Scomber scombrus* as extracted using soxhlet extractor and n-hexane as solvent. *In vitro* antioxidant assay, fatty acid profile and physiochemical parameters of the oil performed. Result showed protein, fat, sodium and fibre were significantly ($P<0.05$) higher in sundried sample compared to smoked. Saponification value, peroxide value and iodine value were significantly ($P<0.05$) higher in oil obtained from smoked fish when compared to sundried. Fatty acid profile showed the presence of four fatty acids. From the result of this study, it can be concluded that smoking affected the nutritional properties of the fishes, especially the oil chemistry.

1. Introduction

The roles played by fish in human nutrition has been fully established (Tufan *et al.,* 2016). They are good sources of minerals, amino acids (Oluwaniyi *et al.,* 2010), vitamins and lipids (Dobreva *et al.,* 2011). The oils are rich in omega-3 fatty acids (Venugopal, 2009), and have been have been implicated to be useful in managing many disease conditions such as obesity, diabetes, cancer (Gogus and Smith, 2010).

The fish Atlantic mackerel known scientifically as *Scomber scrombus* is readily available in the Atlantic. It is highly consumed in Nigeria, considering that it is cheaper and offers good source of protein and amino acids

(Kim and Lall, 2000), but first undergoes processing to be fit for consumption. Processing methods used in Nigeria's traditional system includes sun drying, boiling, frying and roasting (Oluwaniyi *et al.,* 2010).

Generally, it is known that method of processing food items has impact on the nutritional property of the food materials. Some processing method leads to loss of vital components of the food, while others may improve the nutritional quality of the food item. Studies by Oluwaniyi *et al.,* (2010), reported that processing (boiling and roasting) had a desirable effect on the amino acids constituents of fishes.

In Nigeria, fish smoking is a very common practice, as it is seen to preserve the fish for a longer time than any other traditional method. The study therefore investigates the effect of smoking on the fatty acids profile, antioxidant properties of oil obtained from Horse Mackerel, and looks at how this processing method affects it nutritional properties.

2. Materials and methods

2.1. Procurement of the raw material

Fish sampling and handling

The fish samples (*Scomber scrombus*) locally called titus were purchased from a commercial market in Umuahia, Abia State, Nigeria. They were bought iced and transported to the Department of Biochemistry, Michael Okpara University, Umudike, Abia State in an ice pack. The fish samples were washed in running water, and cut into parts with a knife. The head region was discarded and the remaining parts were properly washed again to remove the presence of blood.

Sample treatment

The fish (wet) was divided into two sections. Part A served as control and was dried under the sun for three days in a locally made iron fish basket covered with net to prevent the presence of flies, while part B which served as the test group was smoked. Smoking was achieved using fire wood and wood shavings. Briefly, the firewood and wood shavings were burnt to generate smoke through a channel (iron drum). The fishes placed on an iron mesh was kept on top of the drum. Smoking was achieved for seven hours. The samples were milled after processing and stored in air tight container.

Proximate analysis of milled sample

Proximate analysis was determined by the method described by AOAC (1990)

Mineral estimation of milled sample

Minerals were estimated by the method described by James, 1995.

Oil extraction

Dried fish samples were milled into fine powder and oil extraction was achieved using n-hexane as solvent in a soxhlet extractor.

Physicochemical property of oil

Physiochemical analysis was determined by the method described by AOAC (1990). Colour of the obtained oils were determined by physical eye observation. Five persons allowed to sight the sample and make colour observation.

***In vitro* antioxidant potentials of oil**

DPPH scavenging potentials of the oil was determined by the method described by Manzocco *et al.,* 1998. Reducing power of the oil was determined by the method described by Oyaizu, 1986.

Fatty acid characterization

Fatty acid characterization of the oil was determined by the method described by Ezeagu *et al.,* 2005.

Statistical analysis

Data obtained was statistically analysed. For data containing two variables, student T- test was employed, while data with three variables was analysed using analysis of variance ANOVA). Significant difference was set at 95% confidence level. Result was reported as mean±S.D.

3.Results and discussions

3.1.Results

Table 1. Proximate composition of fish samples

Parameter	Sundried fish	Smoked fish
Protein (%)	54.08±0.22*	52.09±0.07
Fat (%)	22.30±0.21*	20.20±0.03
Fibre (%)	7.76±0.03*	7.18±0.02
Moisture (%)	6.52±0.31	7.33±0.10*
Ash (%)	6.79±0.03	7.12±0.11*
Carbohydrate (%)	5.45±0.02	5.41±0.01

Values reported as mean±S.D of triplicate determinations. (*) indicates significant difference at 95% confidence level.

Table 2. Mineral estimation of fish samples

Parameter	Sundried fish	Smoked fish
Sodium (mg/100g)	9.03±0.02	9.20±0.01*
Potassium (mg/100g)	5.35±0.16	5.38±0.01
Calcium (mg/100g)	2.40±0.00	2.44±0.01
Phosphorous (mg/100g)	6.41±0.01	6.44±0.01
Magnesium (mg/100g)	6.10±0.00	6.04±0.00

Values reported as mean±S.D of triplicate determinations. (*) indicates significant difference at 95% confidence level.

Table 3. Physicochemical properties of fish oil

Parameter	Sundried fish	Smoked fish
Colour	Opaque	Dark
Saponification value	108.16±0.03	135.19±0.00*
Peroxide value	2.01±0.01	2.42±0.01*
Iodine value	118.72±0.01	128.63±0.01*

Values reported as mean±S.D of triplicate determinations. (*) indicates significant difference at 95% confidence level.

Table 4. DPPH scavenging activity of oil obtained from fish samples

Concentration (mg/ml)	Sundried fish (% inhibition)	Smoked fish (% inhibition)	Vitamin C (% inhibition)
10	14.13 ± 0.02^{b}	10.10±0.07	50.12 ± 0.01^{a}
20	16.92 ± 0.02^{b}	12.01±0.00	55.74 ± 0.02^{a}
40	19.33 ± 0.01^{b}	14.02±0.00	78.10 ± 0.00^{a}
80	24.53 ± 0.00^{b}	18.24±0.00	80.13 ± 0.04^{a}
100	28.55 ± 0.00^{b}	20.21±0.27	90.54 ± 0.13^{a}

Values reported as mean±S.D. (a) indicates significantly higher *(P<0.05)* than the sundried and smoked fish group. (b) indicates significantly higher *(P<0.05)* than the smoked fish group.

Table 5. Reducing power activity of oil obtained from fish samples

Concentration (mg/ml)	Sundried fish (OD at 700nm)	Smoked fish (OD at 700nm)	Vitamin C (OD at 700nm)
10	0.71 ± 0.00^{b}	0.51 ± 0.00	1.42 ± 0.00^{a}
20	0.78 ± 0.00^{b}	0.59 ± 0.00	1.56 ± 0.00^{a}
40	0.90 ± 0.00^{b}	0.68 ± 0.00	1.72 ± 0.00^{a}
80	1.10 ± 0.00^{b}	0.73 ± 0.00	1.82 ± 0.00^{a}
100	1.29 ± 0.00^{b}	0.81 ± 0.00	1.88 ± 0.01^{a}

Values reported as mean±S.D. $^{(a)}$ indicates significantly higher *(P<0.05)* than the sundried and smoked fish group. $^{(b)}$ indicates significantly higher *(P<0.05)* than the smoked fish group.

Table 6. Fatty acid profiles of fish oil samples

Fatty acid (% composition)	Sundried fish	Smoked fish
Myristic (C14:0)	12.84	16.98
Palmitic (C16:0)	39.83	41.29
Oleic (C18:1)	34.89	34.61
Linoleic (C18:2)	8.28	11.24

3.2. Discussion

Generally, fish processing has been shown to alter nutritional values of fishes, and this effect is dependent on the method of processing (Oluwaniyi *et al.,* 2010). The proximate composition of fish samples is presented in Tab. 1. Protein, fat and fibre composition was significantly *(P<0.05)* higher in the sundried samples compared to smoked sample. Moisture and ash content was significantly *(P<0.05)* higher in the smoked samples compared to the sundried samples. From the result obtained from this study, it becomes clear that smoking lowered the composition of protein, fat and fibre in the fish samples. Mathew *et al.,* (2014), reported that smoking affects nutritional component of fishes.

Result for sodium concentration was significantly *(P<0.05)* higher in the smoked sample when compared to the sundried sample. However, all other minerals were not affected by smoking as shown in Tab. 2. The value reported for sodium in this study is like the value reported by Mathew *et al.,* (2014).

The colour of the oil obtained from the smoked fish showed great deviation. Colour report from scoring individuals showed that the fish oil was black in colour as against the sun-dried fish oil wish was opaque and clear in colour. This could be because of large amount of carbon emitted from the smoke that was deposited on the fish samples before oil extraction. Saponification, peroxide and iodine value was also significantly *(P<0.05)* higher in the oil obtained from smoked samples when compared to sundried samples. The increase in saponification, peroxide and iodine value can be to the damage and oxidation caused by hydrocarbon compounds present in the smoke. Emmanuel *et al.,* (2018), reported that hydrocarbons from fossil fuels processing can generate oxidants that damage nutritional oils.

Result for *in vitro* antioxidant capacity of the oil showed that oil from sundried samples was more potent than smoked fish oil. DPPH scavenging activity and reducing power activity was significantly (P<0.05) higher in sundried fish samples when compared to smoked fish samples, although Vitamin C used as standard had the highest activity. This indicates that sun drying method preserved more antioxidant in the oil than smoking. Also, smoking can generate free radicals in the oil which is likely to reduce

the number of antioxidants present in the oil sample.

Result for fatty acids showed the same type of fatty acids in both oil samples, however few deviations were observed in the concentration of myrsitic and linoleic fatty acids. This indicates that smoking does not affect fatty acid profile of oil.

4.Conclusions

From the result of this study, it can be concluded that smoking affects the nutritional composition of fishes, and affects majorly the oils obtained from the fishes negatively, which may in turn affect the application of such oil in medicine.

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BLUE HONEYSUCKLE BERRY (*LONICERA CAERULEA* L.), AS RAW MATERIAL, IS PARTICULARLY PREDISPOSED TO THE PRODUCTION OF FUNCTIONAL FOODS

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ABSTRACT

The aim of this work was to present the characteristics of the blue honeysuckle berry and its practical application in the food industry. Blue honeysuckle berries are a source of valuable and essential nutrients. They are becoming more and more popular also because of their valuable medicinal properties. Blue honeysuckle berry, due to the presence of compounds with strong antioxidant and anti-inflammatory properties, reduces the harmful effects of free radicals. It is a rich source of vitamin C, contains polyphenols, and is popularly used as an ingredient of dietary supplements and medicinal preparations. Due to its high nutritional value, its cultivation is of utmost importance. Its inherent strength at low temperatures and early maturation render it valuable as a raw material. The growing interest of producers in new products rich in health-promoting properties makes them more attractive to the potential consumer. In addition, consumers are constantly seeking better alternatives, healthier products of plant origin, in a bid to rule out the negative aspects, and this will be an alternative to the widely existing food products.

1. Introduction

Blue honeysuckle (*Lonicera caerulea* L.) is a plant belonging to the Caprifoliaceae family. Other names of this plant include, but are not limited to, "haskap," "sweet berry honeysuckle," "kamchatka honeysuckle," "edible honeysuckle," and "honeyberry" (Jurgoński et al., 2013; Jurikova, et al., 2012a; Becker and Szakiel, 2019; Rupasinghe et al., 2018). In Japan and Canada, it is most commonly known as "haskap," and in areas of Siberia and Russia it is known as "zhimolost" (Becker and Szakiel, 2019; Celli et al., 2014). Currently, this plant is cultivated in Japan, Russia, Canada, Poland, Czech Republic, Slovakia, Austria, and other countries (Oszmiański and Kucharska, 2018; Auzanneau et al., 2018).

It is a long-lived, fruit-bearing shrub that originated from distant Siberia and northeastern Asia. Its natural habitat includes wet areas along rivers and bogs as well as high mountains (Celli et al., 2014). The first mention of this plant dates back to the 17th century, and the first attempts at

domestication took place in Russia at the beginning of the 20th century. Around 1950, work in Russia intensified to develop cultivars with the highest possible yield, larger and sweeter fruits, and mechanical harvesting characteristics (i.e. balanced ripening of fruits). Similar work began only at the end of the 20th century in Canada and in several European countries (i.e. Poland, Czech Republic, Lithuania, Finland, and Slovakia) (Celli et al., 2014; EFSA, 2018; Becker and Szakiel, 2019).

Currently, the widely grown Canadian varieties are obtained from the cross-pollination of *L. caerulea* var. *kamtschatica* with the Canadian variety *Lonicera kamtschatica* var. *villosa* and the Japanese (Hokkaido) variety *L. caerulea* var. *emphyllocalyx* (Thompson and Barney, 2007). In turn, Polish varieties are derived from the cross-pollination of *L. caerulea* var. *kamtschatica* with *L. caerulea* var. *edulis* (Becker and Szakiel, 2019). Only the above mentioned species result in tasty, aromatic, sweet–sour fruits of the highbush blueberry or bilberry type. On the other hand, fruits from shrubs of varieties with *Lonicera altaica* and *Lonicera pallasii* in their pedigree are characterized by a marked tartness and bitterness resulting mainly from the high content of iridoid glycosides and esters of malic and citric acid (Jurikova, et al., 2012a; Celli et al., 2014).

The varieties in Poland include “Wojtek,” “Jolanta,” “Atut,” “Duet,” “Brazowa,” “Czarna,” and “Warszawa” (Becker and Szakiel, 2019; Kaczmarska et al., 2015; Ochmian et al., 2012; Ochmian et al., 2008); while the most popular Canadian varieties are “Blue Belle,” “Blue Bird,” “Blue Moon,” “Blue Velvet,” “Tundra,” “Aurora,” “Borealis,” “Indigo Gem,” and “Honeybee” (Becker and Szakiel, 2019; Rupasinghe et al., 2018; Rupasinghe et al., 2012).

The fully mature shrub is dense and upright (Figure 1).

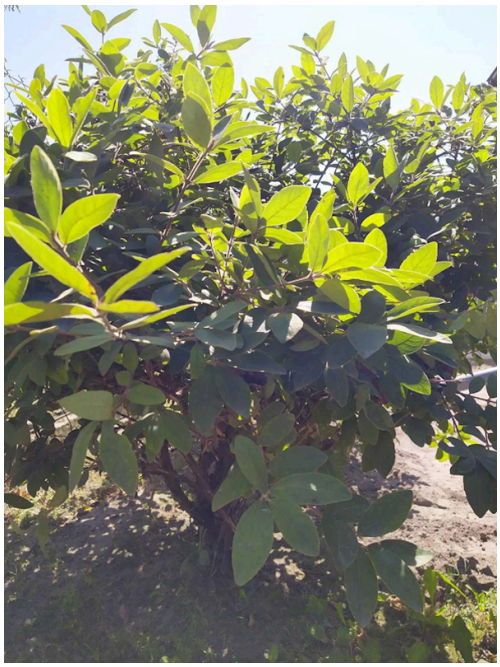

Figure 1. Blue honeysuckle bush.

The shrubs can reach a height of 2 m and a width of 1.5–2 m. The flowers are pale yellow, melliferous, and have a delicate, pleasant aroma (Figure 2).

The bush blooms with the development of leaves. In Canada (Saskatoon), Blue Honeysuckle begins to flower at the beginning of May, while in Poland it usually blooms at the end of April (Gawroński et al., 2014). However, the time of flowering is highly influenced by climatic conditions and, above all, temperature. It has been proven that there can be large differences in flowering time (even more than 2 weeks) of the same varieties in different years. The other important factor is the variety-depending on the variety, flowering time can last from 7 to 15 days (Dawson, 2017). As this is not a self-pollinating plant, it requires a different variety-which flowers at the same time-to be present close by, for cross-pollination will occur. A solitary blue honeysuckle shrub can also bear fruit, but less abundantly (Frier et al., 2016).

Figure 2. Blue honeysuckle flowers.

Blue honeysuckle starts bearing fruit in the second year after planting, and the full yield (3-5 kg) can be harvested 8–15 years after planting (Dawson, 2017). The berries are fleshy, elongated, navy blue in color, and covered with a waxy, blue coating (Figure 3).

In addition, the fruit contains about 20 small seeds, which are undetectable during eating. The weight of the fruit varies from 0.3 to 3.8 g while the length varies from 2 to 3 cm, depending on the variety and climatic conditions. The flavor of the berries is sweet and sour, slightly tart or bitter (Becker and Szakiel, 2019; Celli et al., 2014; Jurikova et al., 2012a; Ochmian et al., 2008). Blue honeysuckle is long-lived and can bear fruit for up to 30 years. Shrubs that are 20- to 25-year-old can die out or yield less, but treatments such as pruning and removing older stems and branches can help the plant to grow afresh (Becker and Szakiel, 2019; Dawson, 2017). This is a plant that tolerates shaded areas, but for maximum yield, it is recommended to have full sun exposure. The soil requirements are relatively minimal and acidification is not necessary unlike high blueberries. It tolerates a wide range of soil pH and the most favorable pH range is 5.5-8.0. Blue honeysuckle can grow on sandy and clay soils as well as peaty and slightly acidic soils (Dawson, 2017). It is very rarely pest-attacked and therefore does not require special protection against fungal diseases and other pathogens (Celli et al., 2014). Blue honeysuckle demonstrates very high frost resistance-shrubs can withstand temperatures down to -40°C and flowers down to -8°C (Ochmian et al., 2008). As a result, the climate in Central Europe, Northern Europe, Canada, and USA is favorable for blue honeysuckle (Becker and Szakiel, 2019).

Figure 3. Blue honeysuckle fruits.

2. The content of bioactive compounds and pro-health properties of blue honeysuckle berries

Blue honeysuckle berries are a valuable source of vitamins, minerals, and secondary metabolites with bioactive properties that are important for maintaining proper human health. Properties of *L. caerulea* L. have been appreciated for centuries by the folk medicine of China, Japan, and northern Russia (Kaczmarska

et al., 2015). This plant was even called the "elixir of life" by the indigenous Ainu family living on the island of Hokkaido (Celli et al., 2014). The raw material was used to treat fever, headaches, and urinary tract diseases (Kaczmarska et al., 2015), and the fruits were used in coronary heart disease, respiratory infections, and liver and gallbladder disorders. An infusion of leaves and flowers of this plant was used as a medicine against bacterial and viral infections of the oral cavity and throat, in cold and flu and as a diuretic. Additionally, the fruits were used to treat gastrointestinal disorders and eye diseases (Ochmian et al., 2012; Caprioli et al., 2016; Becker and Szakiel, 2019). Contemporary scientific research has confirmed the therapeutic properties of blue honeysuckle, which result from the health-promoting chemical composition of this plant. It was found that extracts from blue honeysuckle berries are effective both in chemoprevention and in chemotherapy. Their anticancer property is related to the induction of antioxidant defense enzymes, inhibition of cancer cell proliferation, and factors causing metastases (Rupasinghe et al., 2018). Its cardiovascular benefit has also been proven. A recent study on the oral intake of blue honeysuckle extracts by elderly people showed a significant decrease in diastolic blood pressure and relative heart rate (Bell & Williams, 2018). It was also found that consumption of these berries inhibited postprandial serum triacylglycerol and glucose levels in rats (Takahashi et al., 2014) and decreased plasma high-density lipoprotein cholesterol levels (Jurgoński et al., 2013). Blue honeysuckle berries also have strong antidiabetic properties. In one study, blue honeysuckle berries showed the strongest α-glucosidase inhibitory activity among fruits such as highbush blueberry, bilberry, blackcurrant, sweet cherry, and red gooseberry (Podsędek et al., 2014). Inhibition of α-glucosidase and β-fructosidase allows delaying disaccharide digestion, which is important for postprandial hyperglycemia control in patients with diabetes (Johnson et al., 2011). In addition, in several studies antibacterial properties of blue honeysuckle berries were observed. Palíková et al. showed that freeze-dried berries and its extracts rich in polyphenolic compounds decreased artificial adhesion and biofilm formation of pathogenic microorganism strains, i.e. *Candida parapsilosis*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus mutans*. Adhesion to tissues and biofilm formation are the culminant stages of microbial colonization, and then infection (Palíková et al., 2008; Rupasinghe et al., 2018). Another important observation showed that blue honeysuckle berries effectively inhibited the development of bacteria often transmitted through food such as *Escherichia coli*, *Campylobacter jejuni*, and *Listeria monocytogenes*, but did not inhibit the growth of bacteria such as *Bifidobacterium bifidum* (Raudsepp et al., 2013). Moreover, polyphenols present in blue honeysuckle berries improved the functioning of the intestinal microflora (Taira et al., 2015). The beneficial effect of blue honeysuckle berries on thyroid diseases is also shown. A mouse with hyperthyroidism, which was given an oral extract of blue honeysuckle berries, was shown to have decreased thyroid hormones secretion in a study (Rupasinghe et al., 2018).

It should be noted that the chemical composition of blue honeysuckle may differ depending on the genetic characteristics of the different varieties, the climatic conditions, the geographical location of the crop, the agrotechnical treatments used (irrigation and fertilization), and the harvest period (Kaczmarska et al., 2015). Fully ripened fruits contain between 12.4% and 20.3% of dry matter, with a predominance of fructose and glucose (Dawson, 2017; Rupasinghe et al., 2018). In some cultivars grown in Poland, the presence of sorbitol was also detected, while sucrose was found in cultivars originating from Canada (Wojdyło et al., 2013; Rupasinghe et al., 2015; Senica et al., 2018). In general, compared to

other popular berries, blue honeysuckle berries contain much lower sugar content (Senica et al., 2018). They are characterized by a high content of organic acids, among which citric acid is the most dominant. Wojdyło et al. found that citric acid constituted 47% of all organic acids, among which malic, phytic, oxalic, quinic, and shikimic acids were also present. At the same time, oxalic, quinic, and shikimic acids were present in the lowest amounts and constituted, respectively, 5%, 4%, and 1% (Wojdyło et al., 2013). Blue honeysuckle berries are characterized by a high content of vitamin C, which is comparable to or higher than the content in fruits considered to be the best sources of this vitamin (oranges, strawberries, raspberries, blackberries) (Rupasinghe et al., 2018). The vitamin C content can reach up to 187 mg/100 g fresh weight (FW) (Jurikova et al., 2012b). Among the mineral components, potassium is dominant, followed by phosphorus and calcium, magnesium and iron in smaller amounts, and trace amounts of manganese, copper, and zinc (Dawson, 2017; Rupasinghe et al., 2018). Blue honeysuckle berries are also a good source of pectin, comparable to raspberries (Wojdyło et al., 2013). Fiber may constitute on average 8.3% (Caprioli et al., 2016), carbohydrates from 10.2% to 15.6% (Rupasinghe et al., 2012), and proteins from 2.1% to 8.4% (Rupasinghe et al., 2018). Fat content may vary between 0.01% and 4.8% depending on the location and variety (Rupasinghe et al., 2012).

Blue honeysuckle is considered particularly important for its high content of antioxidant compounds from the polyphenol group, which have the ability to neutralize free radical activity (Jurikova et al., 2012b). Polyphenols are a very large group of compounds with different structure and physical, chemical, and biological properties. Depending on the position of the phenolic ring and the degree of oxidation of the pyranone ring, the following are distinguished: phenolic acids, flavones, flavanones, flavonols, flavan-3-ols, anthocyanins, and chalcones (Jurikova et al., 2012a; Wojdyło et al., 2013; Panche et al., 2016).

Blue honeysuckle berries are known for their remarkably high content of anthocyanins (from 400 to 1500 mg/100 g) (Jurikova et al., 2012a). Anthocyanins are the compounds responsible for the dark blue color of these fruits. The most abundant anthocyanin is cyanidin-3-glucoside (79%–92%), whereas cyanidin-3,5-diglucoside, peonidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-rutinoside, and pelargonidin-3-glucoside occur in smaller amounts (Wang et al., 2015; Rupasinghe et al., 2018). Furthermore, Wang et al. also reported the presence of cyanidin-3-acetylhexoside and peonidin-3-acetylhexoside in cultivars grown in China (Wang et al., 2015). Research has also shown that a higher amount of anthocyanins is contained in the skin than in the flesh of these fruits. The total content of anthocyanins in blue honeysuckle berries is comparable to the content in chokeberries and elderberries—which are two of the richest sources of these ingredients (Becker and Szakiel, 2019). Numerous studies have confirmed a wide range of pro-health and therapeutic properties of anthocyanins, i.e. antioxidant, anticancer, antidiabetic, and antibacterial properties, besides improving the functioning of the visual system (Khoo et al., 2017).

The next group of polyphenolic compounds present in blue honeysuckle berries is phenolic acids. In terms of chemical structure, phenolic acids are divided into hydroxybenzoic acids with carbon skeleton C_6–C_1 and hydroxycinnamic acids built on skeleton C_6–C_3 (Becker and Szakiel, 2019). Among the hydroxybenzoic acids identified in blue honeysuckle fruits are salycil acid, gentistic acid, protocatechic acid, gallic acid, and vanilic acid. In turn, among hydroxycinnamic acids chlorogenic acid, caffeic acid, m-cumaric acid, and p-cumaric acid were isolated. The main phenolic acid in these fruits is chlorogenic acid, the proportion being significantly determined by the conditions and location of cultivation

(Jurikova et al., 2012a). In varieties grown in Canada, the content of this acid ranged from 35.0 to 44.0 mg/100 g FW (Khattab et al., 2015), in Poland 17.24–60.37 mg/100 g FW (Kucharska et al., 2017), in Slovenia 22.45–46.06 mg/100 g FW (Senica et al., 2018), and in the Czech Republic 86.62–267.14 mg/100 g FW (Jurikova et al., 2012b). Recent studies have shown that yearly climatic conditions have a significant influence on the content of this acid. Among the cultivars cultivated in Switzerland in the years 2014–2016, a higher content of phenolic compounds, including chlorogenic acid, was noted in 2014 which was characterized by longer sunshine time and lower precipitation sums (Auzanneau et al., 2018). It is worth noting that chlorogenic acid is a compound with pro-health properties. In animal studies, the consumption of chlorogenic acid resulted in pharmacological properties against insulin resistance, obesity, and hepatic steatosis caused by a high-fat diet (Ma et al., 2015). Furthermore, Onakpoya et al. have proven in their clinical trials that chlorogenic acid shows antihypertensive properties (Onakpoya et al., 2015).

The content of flavan-3-ols, present in the form of monomers (catechins) and polymers (procyanidins), was also identified in blue honeysuckle berry. However, procyanidins occur in higher amounts (228.6–512.0 mg/100 g DM), compared to catechins (22.2–136.1 mg/100 g DM) (Wojdyło et al., 2013). Procyanidins are compounds with strong antioxidant properties that protect against the development of many diseases caused by excessive oxidative stress in the body. They also affect the taste of raw materials—they can cause astringency or bitterness (Rauf et al., 2019). Flavonoles are the next group of polyphenols present in blue honeysuckle berries. Wojdyło et al. showed that the average content of flavonoles in Polish varieties ranges from 55.7 to 170.0 mg/100 g DM (Wojdyło et al., 2013). Flavonoles are present in blue honeysuckle berries in lower quantities than in fruits such as bilberries, blackcurrants, and blueberries (Jurikova et al., 2012a). The identified flavonoles include quercetin, quercetin derivatives (quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside), and luteolin derivatives (luteolin 7-*O*-α-glucoside) (Rupasinghe et al., 2018). According to a recent report, saponin compounds are also present in blue honeysuckle berries. The saponin content, depending on the variety, ranged from 235.78 to 640.79 mg/100 g FW (Senica et al., 2018).

Iridoids are a very interesting group of compounds that have been recently identified in fruits of blue honeysuckle. They are a large group of secondary metabolites, belonging to the group of cyclopentane monoterpenes. The basic structure of iridoids has a skeleton built of a ring of cyclopentane and pyrane (Oszmiański and Kucharska, 2018). In plants, they can defend against predators and are synthesized in response to the attack of pathogens (Whitehead and Bowers, 2013; Kucharska et al., 2017). Iridoids are compounds often found in medicinal plants—examples include morinda roots (*Morinda citrifolia* L.) and the bog rosemary (*Andromeda polifolia* L.). However, they rarely occur in fruits-with the exception of cornelian cherry, cranberry, lingonberry, and bilberry (Heffels et al., 2017; Kuchska et al., 2017). Some types of iridoids may be responsible for the bitterness of plant raw materials; an example could be secoiridoids that are also present in blue honeysuckle berries. Recent studies have shown that the most abundant iridoid in blue honeysuckle berries is loganic acid (Kucharska et al., 2017). However, iridoids such as loganin, sweroside, secologanin, secoxyloganin, pentosides of loganin, and pentosyl-sweroside have also been identified (Kucharska et al., 2017; Oszmiański and Kucharska, 2018; Kucharska and Fecka, 2016). Iridoids are biologically active compounds showing anti-inflammatory, neuroprotective, hepatoprotective, hypotensive, and antibiotic properties (Heffels et al., 2017; Oszmiański and

Kucharska, 2018; Kucharska and Fecka, 2016). Loganin has been shown to be effective in alleviating neurological diseases. In one study, it effectively inhibited the activity of β-secretase, which is a protease involved in the production of β-amyloid aggregates—which is one of the causes for Alzheimer's disease (Youn et al., 2013). In addition, loganin also helped alleviate diabetes mellitus by improving liver function and reducing nephropathy (Park et al., 2011; Tundis et al., 2008). On the other hand, secologanin derivatives showed analgesic, anti-inflammatory, and anti-allergic properties (Tundis et al., 2008).

3. The possibilities of application of blue honeysuckle berries in the food industry

Consumption of both fresh and processed blue honeysuckle berries is particularly popular in Japan and Canada. Until recently, the distribution of fresh blue honeysuckle berries in the European Union was not regulated by law, although there has been increasing interest in the cultivation of this fruit in many countries (Auzanneau et al., 2018). It was not until December 2018 that *L. caerulea* L. was included in the list of traditional foods. Regulation (EU) 2015/2283 of the European Parliament and of the Council permitted blue honeysuckle berries to be legally marketed in the European Union. In comparison, fresh fruit was introduced to the market in Japan as early as in 1950, and in 1970 production was intensified to meet the demands for bakery and confectionery products with these berries (EFSA, 2018).

Fresh fruits intended for consumption are juicy and, depending on the variety, more or less acidic with a bit of bitter taste (Jurikova et al., 2012a). The remaining fruit can be successfully used for processing. Blue honeysuckle berries are used, among others, in the production of jams, jellies, wines, juices, soft drinks, yogurts, candies, puffed snacks, cakes, and ice creams (Celli et al., 2014; EFSA, 2018).

The first very important step affecting the quality of this raw material is harvesting and then postharvest processing. Blue honeysuckle berries are usually ready for harvest at around 6 weeks after pollination, when they have a uniform dark blue color (Dawson, 2017). In smaller farms, manual harvesting is usually used, whereas on larger farms, machine harvesting is used. An unquestionable advantage of blue honeysuckle is the possibility of machine harvesting-especially in countries with high labor costs, i.e. Canada (Celli et al., 2014; Thompson and Barney, 2007). Depending on the intended use, an appropriate way of processing the fruit after harvesting is very important. Fruits which are meant for sale as fresh fruits should be kept at low temperatures as soon as possible and transported to the market because they have a shelf life of only a few days. However, fruits for export or for processing should be frozen immediately. A good way of using fruits is to extract their juice immediately after harvesting. Pasteurized juices can be stored in refrigerated conditions for a longer period of time (Dawson, 2017).

According to a literature review, blue honeysuckle fruits are considered raw materials with a wide spectrum of therapeutic and health-promoting properties. Therefore, they can be successfully used in the production of functional food and dietary supplements. However, there is relatively less research on the influence of technological processes on the chemical composition of the products from blue honeysuckle berries. It should be noted that in order to obtain products with the highest possible content of bioactive ingredients and consequently the best healthy value, it is necessary to adjust the process parameters during processing in an appropriate and optimal way.

Due to the short harvesting period as well as the short shelf life of the fresh fruit, freezing is a basic technological procedure used in the processing of blue honeysuckle berries. Khattab et al. studied the effect of freezing at −18 and −32°C and blanching as pretreatment conditions before freezing. A reduction in polyphenols

content and antioxidant activity was observed during the time of storage (especially in the first 3 months). Moreover, storage at −32°C did not significantly increase the content of polyphenolic compounds in the fruits in comparison to the raw materials stored at −18°C. Nevertheless, the use of steam blanching before freezing of berries improved the retention of polyphenolic compounds (Khattab et al., 2015). Another study reported that the freezing process had no effect on the content of anthocyanins; however, upon storing frozen products a significant decrease of the anthocyanin content was observed. Additionally, the effect of thawing on the content of anthocyanins in blue honeysuckle berries was investigated. Microwave thawing (1000 W, 17 min) resulted in lower anthocyanin losses compared to room temperature thawing (25±2°C/12 h) and refrigerated thawing (4°C/22 h). Moreover, despite longer thawing time, higher content of anthocyanins was observed in blue honeysuckle berries thawed in refrigerators than those thawed at room temperature (Khattab et al., 2016).

Blue honeysuckle berries after thawing can be utilized primarily for the production of juices. The processing of blue honeysuckle berries into not from concentrate (NFC) juices may be more beneficial, as NFC juices are getting more popular among consumers and have a higher content of nutrients and health-promoting substances than from concentrate (FC) juices (Włodarska et al., 2016). A characteristic of blue honeysuckle berries is their high level of acidity, which is often a feature that is not sensory acceptable to consumers (Wojdyło et al., 2013; Włodarska et al., 2016). For this reason, blue honeysuckle berry juice would be particularly suitable for the production of mixed juices based on juices from fruit containing more sugars, i.e. apples, pears, and peaches. Mixing juices from different fruits is also a way to create an innovative product with functional properties (Grobelna et al., 2019; Lachowicz and Oszmiański, 2018). An important stage in guaranteeing the safety of juices is their preservation process. However, the conventional process of pasteurization at high temperature (above 85°C) degrades valuable nutrients and bioactive compounds from the group of polyphenols, which are often labile and sensitive to technological processes, especially at high temperature. Piasek et al. (2011) compared the preservation of blue honeysuckle berry juices by the conventional method with the method using an EnbioJet microwave pasteurizer. The microwave method of preservation resulted in juices which had no significant degradation of phenolic acids and flavonoids, and lower loss of anthocyanin content that did not exceed 25%.

The next step in the processing of blue honeysuckle berries is the drying process. The drying process can be used to produce powders that can be encapsulated and then marketed as dietary supplements (Celli et al., 2014). However, in the case of blue honeysuckle berries, the drying process of the whole fruit may be complicated because of the thick layer of wax covering the peel which, on the one hand, plays a physiological role in protecting against external factors and pests, but on the other hand it reduces water transport besides slowing the drying process (Oszmiański et al., 2016; Chu et al., 2017). Oszmiański et al. investigated the possibility of using pomace for the production of health-promoting powders from blue honeysuckle berries. It was found that peel-based pomace powders obtained from peel-based pomace contained 4.3 times more bioactive compounds than powders obtained from fresh fruit. In turn, whole fruit-based pomace powders contained twice as much less bioactive compounds than peel-based pomace powders (Oszmiański et al., 2016). Moreover, the utilization of pomace made it possible to streamline the freeze-drying and crushing process because of lower water content in relation to fresh fruit. Interestingly, it has been shown that iridoids evenly accumulate in whole fruits (Oszmiański and Kucharska, 2018), while polyphenols predominate in the skin

(Oszmiański et al., 2016). Therefore, it was concluded that in order to produce powders richer in iridoids, it is more beneficial to produce them from pomace obtained from whole or crushed berries, rather than only from skins (Oszmiański and Kucharska, 2018). Moreover, the content of iridoid in juices after pressing was also examined. The juices showed a higher content of secologanin and a lower loganin content in comparison to fruits. The technological process of juice production could have caused the breakdown of the bond between C-7 and C-8 of the cyclopentane ring and the formation of a particle of secologanin which is a very bitter substance. As a consequence, the authors concluded that the blue honeysuckle juice might be bitter as compared to berries (Oszmiański and Kucharska, 2018).

It is worth recalling that blue honeysuckle berry is a raw material with proven antibacterial properties (Palíková et al., 2008; Raudsepp et al., 2013; Taira et al., 2015) and would, therefore, find potential use as an antimicrobial agent in food processing. As previously pointed out, blue honeysuckle berries are rich in anthocyanins (Rupasinghe et al., 2018); hence, it would be feasible to obtain a natural red coloring agent from these. This is particularly valuable in the era of growing consumer awareness and the quest for natural food additives.

4. Conclusions

The blue honeysuckle berry is a unique horticultural plant, which has been steadily growing in popularity in recent years. Its main advantage is its high content of bioactive compounds and therefore it can be used as a very good component of functional food, dietary supplements, and even medicinal products. However, in the design of high quality functional products based on blue honeysuckle berries it is extremely important to choose appropriate processing methods for this raw material. Future research should especially focus on the influence of different technological processes on the content of bioactive compounds in products from these berries. It is particularly important to maximize the potential of this magnificent plant in the era of more and more common lifestyle diseases.

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FIRST REPORT OF NUTRITIONAL VALUE AND CONSUMER ACCEPTABILITY OF 'KATI' PRODUCED FROM SORGHUM USING LACTIC ACID BACTERIA AS STARTER CULTURES

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ABSTRACT

Most fermented cereal-based foods are source of nutrients and energy for human being. Hence, a large number of fermented cereal products are consumed daily in Africa. 'Kati', an indigenous food to Akoko in Ondo State, Nigeria was produced using different Lactic acid bacteria (LAB) as starter cultures. Nutrient contents and sensory evaluation of 'Kati' produced with different LAB as starter culture were assessed. *Saccharomyces cerevisiae* have the highest occurrence (20.8%) during the steeping of sorghum. *Lactobacillus plantarum* was most predominant bacterium in the fermented slurry with the value of 19.5%. 'Kati' produced with *Lactobacillus* spp. have moisture (64.0 to 67.23%), ash (0.39 to 0.47%), crude fibre (1.05 to 2.31%), protein (2.02 to 5.15%) and carbohydrates (24.12 to 27.35%) contents. The fermented food has minimal value of phytates (0.64-0.77 mg/100g), phenols (11.47-14.75 mg/100g), tannins (0.40-0.51 mg/100g), and oxalates (0.11-0.18 mg/100g). 'Kati' produced with each *Lactobacillus* spp. were preferred to panellists in terms of general acceptability. LAB generally regarded as safe (GRAS), can be used as starter culture to improve nutritional contents and organoleptic property of traditional foods in order to gain wide acceptance by consumers.

1.Introduction

Sorghum, millet, maize, wheat, rice, barley, rye and oat are grains (cereals), mostly considered as one of the important food sources. They are widely cultivated and available in greater quantities since they are the major nutrients for human (Lafiandra *et al*., 2014). Although, bioactive compounds in cereals played significant roles but cereals are still found deficient in some basic components such as essential amino acids and vitamins (Sandhu *et al.*, 2017). Despite the nutritional deficiency in cereals, its components remain better substrate of fermented foods (Achi and Asamudo, 2019). Several indigenous fermented foods and drinks are produced from cereals by simple biotechnological techniques to alleviate food insecurity (Blandino *et al.*, 2003).

Often time, the desirable biochemical changes and significant modification of cereals were achieved by the presence of microorganisms and appropriate enzymes involved during fermentation (Campbell-Platt, 1994), which make the final product more nutritious, digestible, tastier and safer for consumers. Fermented products have a longer shelf life than their original substrate, hence, fermentation is advantageous in food preservation (Egwim *et al*., 2013). Besides prolonged shelf life and digestibility of fermented foods, fermentation improves nutrient level in food by enhancing bioavailability of minerals, eliminating the risk

of antinutrients, improving the food safety by inhibiting microbial pathogens (Assohoun *et al*., 2013). Fermented foods are widely accepted as a result of expanding scientific evidences pointing to their beneficial effects on human health.

Africa are known to have an age-old history of traditionally fermented foods rich in probiotics (Egwim *et al*., 2013). Unfortunately, some of these fermented foods are not widely known or accepted due to different methods of production with chance inoculation, use of rudimentary equipment, and consumption within the rural community. 'Kati' is one of the understudied indigenous foods that is consumed in Akoko community, Ondo State, Southwestern Nigeria. Research documentations have been made on many fermented cereal products from sorghum such as: 'Gowè', 'Kunun-zaki' and 'Ogi-baba' (Oguntoyinbo and Narbad, 2012), maize products: 'Mawè', 'Ogi' and 'Koko' (Adimpong *et al*. 2012), rice products: 'Sake', 'Dosa', 'Idli', 'Miso' and 'Dhokla' (Kumari *et al.,* 2015). The fermented foods are produced through traditional fermentation with mixed cultures of Lactic Acid Bacteria (LAB). Some LAB have been used as starter cultures in laboratory trials due to their higher lactic acid production, rapid acidification, superior shelf life quality attributed to foods as well as improving organoleptic properties of the final products. Hence, a greater degree of controlled fermentation processes has been achieved with the use of starter cultures to produce some traditional foods (Adesulu and Awojobi, 2014). There is a need to research on local foods that are consumed since ages by identifying and revealing the best starter cultures associated with the food production. Therefore, this study aimed to produce 'Kati' from sorghum (white or and red) using different LAB as starter cultures. The nutrient contents and sensory evaluation of produced 'Kati' were assessed.

2. Materials and methods

collection of samples

White and red sorghum used for this study were purchased from King's market, Akure, Nigeria. The samples were collected in a locked bag and transported to the laboratory for further analysis.

2.1.Preparation of 'Kati'

Each grain (500 g) of white, red and mixture (1:1) was weighed into different sterile plastic bowl containing water. The grains were thoroughly washed for two consecutive times. Thereafter, each group of grains was steeped into different sterile bowls containing 2,500 ml of water for 72 h and well covered. Thereafter, samples were washed with sterile water and wet milled using a clean grinder. The milled samples of white, red or mixture was fermented for 24 h and thereafter, molded in wrapped leaves: *Ficus carica* and *Thaumatococcus daniellii*. The samples were cooked in aluminum pot under smoldering fire for 45-50 min.

2.2.Enumeration and isolation of microorganisms

Microbial evaluation of steep water and fermented milled sorghum (red, white and mixture) were carried out using the method of Cappuccino and Sherman (1999). Briefly, 10 ml from steeping of sorghum or 10 g of milled sorghum after fermentation was transferred into 90 ml of sterile peptone water. Thereafter, 10-fold dilutions were prepared and 1.0 ml was dispensed from the dilution onto Petri dish using pour plate method. Cool nutrient agar, de Man Rogosa and Sharpe agar (MRS) and Sabouraud Dextrose Agar (SDA) were introduced for the cultivation of bacteria and fungi. The plates were incubated at 30^{o}C for 24 h and 25^{o}C for 48 h for the growth of bacteria and fungi. The plates containing MRS was incubated under anaerobic condition. Gram's staining and some biochemical tests such as catalase, oxidase, coagulase, motility, methyl red, Voges-proskauer, starch hydrolysis and sugars fermentation were carried out. The biochemical results were compared to Bergey's Manual of Systematic Bacteriology (Krieg *et al*., 2010). The fungi isolates were identified using method of Samson *et al*. (2010).

2.3.Determination of temperature, pH and total titratable acidity of fermented slurry

The temperature of the sample was determined with thermometer (HANNA HI 9828). pH was determined at intervals of 48 h using Jenway pH meter. The total titratable acid (TTA) was determined using the method of AOAC (1990), briefly, 20 ml of milled sorghum was diluted with distilled water (20 ml) and titrated with 0.1 M NaOH into an end point of permanent pink colour using phenolphthalein as indicator.

2.4.Sensory evaluation of produced Kati

The sensory evaluation of 'Kati' produced from white, red and mixed sorghum was initially determined. Sensory evaluation was conducted as described by Meilgaard *et al*. (2007) for taste, colour, texture, aroma and overall acceptability by 10-member panelists selected from public and academic environment based on familiarity and interest on 'Kati'. The parameters were rated on a 9 points hedonic scale. Kati from mixture of white and red sorghum was highly accepted after sensory evaluation and was selected for further studies.

Having known the best substrate for 'Kati' production, it was re-produced using different LAB isolated from fermented slurry of mixed sorghum (white and red). This is to reveal the best starter culture for the production of 'Kati'. The ready-to-eat 'Kati' was purchased from Arigidi Akoko and used as control in this experiment.

2.5.Proximate and mineral analysis of 'Kati' produced with different starter cultures

The proximate analysis was carried out using method of AOAC (1990). The moisture content was estimated by drying method. Ash content was determined putting 5 g in crucible and then placed in muffle furnace at 550 ^{o}C for 4 h. The fat content was determined using the Soxhlet type of direct solvent extraction method. The thimble was removed, placed in a hot-air oven and dried at 105^{o}C for 1 h. The thimble was placed in a desiccator and allowed to cool. Crude fibre was determined by defatting 2 g of sample. Briefly, sulphuric acid (200 ml of 1.25%) was added and the content was boiled for 30 min. The sample was filtered under vacuum followed by repeated washing with distilled water. The sample was later returned to the flask with the addition of 200 ml of 1.25% NaOH. This was boiled for 30 min and filtered. The sample was thoroughly washed with distilled water, followed by 10% HCl and further washing with distilled water to free the sample of any adhering acid. The sample was further treated with 10 ml of petroleum ether and 10 ml of ethanol. The sample was scooped into an empty crucible and placed in a hot-air oven at 105^{o}C for 1 h. Protein content of sample was determined by micro-Kjeldahl method. The percentage nitrogen content in each sample was calculated and multiplied by 6.25 to get the percentage protein content. The total carbohydrate content of each sample was estimated by difference.

% carbohydrates = 100 - (% moisture + % ash + % fat + % protein + % crude fibre).

The mineral; potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) contents in 'Kati' were analyzed from ash samples using atomic absorption spectrometer. Phenol and tannin content in 'Kati' were determined using methods stated by Makkar and Goodchild (1996). The quantity of oxalates and phytate in 'Kati' was determined using the methods of Krishna and Ranahan (1980).

3.Results and discussions

The bacterial and fungal count during steeping of sorghum and its fermented slurry was recorded in Table 1. The mixed samples have the highest count in all the days except the fungal count at 0 hour (h), in which the white and mixed sorghum have the same count. It was observed that both bacterial and fungal count increased steadily from 0 to 72 h but higher microbial count was observed in mixed sorghum than red or white sorghum. This could be as a result of time needed for the organisms to adapt to the new environment. Findings of Ogodo *et al*. (2019) attributed stabilization of microorganisms to utilization of available nutrient in medium during fermentation. The

steady increase after initial hour could be as a result of microbial build up due to non-disturbance of the water during steeping. It was reported by Van-Nierop *et al.* (2006) that population of microorganisms increased during steeping and conditions (temperatures, moisture and airflow) enable grain germination as well as microbial growth. The decrease in bacterial and fungal count after 48 h could be as a result of depletion of nutrient and increase in acid content of the medium which may affect non-lactic bacteria (Ogodo *et al*., 2019).

Table 2 shows percentage occurrence of microorganisms isolated during steeping of sorghum. *Saccharomyces cerevisiae* possessed the highest occurrence (20.8%), while *Candida albicans* and *Staphylococcus aureus* have the same least value of 3.0%. *Saccharomyces cerevisiae*, *Corynebacterium* spp. and *Lactobacillus* spp. were mainly present at 24- 72 h, which may due to limited oxygen availability during steeping. Aeration during steeping enhances proliferation, resulting in a coat of bacteria, yeasts and fungal spores on steeped grains (Justé *et al.*, 2011). Increase in acidity of the steeping water favours LAB and contributed to continuous decreasing of other bacteria and fungi (Okeke *et al*., 2015).

Saccharomyces cerevisiae (20.8%) was the most dominant microbe amongst other microbes isolated during the stepping process. Gobbetti *et al.* (1994) and Steinkraus (1996) proposed that LAB create an acidic environment (lower pH) conducive to yeast proliferation, while the yeasts provide vitamins and other growth factors such as amino acids for the lactic acid bacteria. Ali and Mustafa (2009) reported that, the simultaneous increase in numbers of both LAB and yeasts could be attributed to their symbiotic association in fermented sorghum dough. The isolation of other microorganisms, which did not have definite role could either occur as contaminants form stepping water or body contact, Holzapfel (1997) revealed that all microbial genera are not of equal importance in fermentation therefore, candidate isolates for starter culture development have to be evaluated for their contribution during fermentation.

Table 3 shows percentage occurrence of LAB isolated from fermented slurry of sorghum. *Lactobacillus plantarum* was most predominant in the fermented slurry of sorghum with the value of 19.5%, while *Lactobacillus jensenii* had the least occurrence of 9.8%. In this study, LAB predominantly isolated from traditionally fermented 'Kati' were *Lactobacillus casei*, *Lactobacillus salivarius, Lactobacillus jensenii*, *Lactobacillus cellobiosus, Lactobacillus plantarum, Lactobacillus delbrueckii,* and *Lactobacillus fermentum*. Most of LAB isolated from the fermented sorghum are similar to what isolated from other fermented foods (Abegaz, 2007; Chelule *et al.*, 2010 and Mukisa *et al*., 2016). Similarly, members of lactobacilli can be detected in a variety of habitat include fermented foods and dairy products (Admassie, 2018). LAB survive in acidic environment during fermentation. Its prevalence could also be as a result of its fast and predominant growth under fermentation conditions (Soro-Yao *et al.*, 2014). LAB have found in many traditional foods to improve organoleptic properties and shelf life. The functional properties displayed by LAB can be attributed to their ability to produce heat-stable antimicrobial compounds or ribosomally synthesized antimicrobial peptides called bacteriocins, which prevent the growth of other microbes (De Vuyst and Vandamme, 1994). *L. plantarum* was the predominant among the isolated LAB. This could be as a result of its higher acid tolerance. LAB obtain energy through substrate-level phosphorylation following two metabolic pathways for hexose fermentation (homofermentative and heterofermentative) and thus, characterized by production of lactic acid as major end metabolic product (Mora-Villalobos *et al*., 2020).

The elimination of some microorganisms, which present during steeping could be as a bio-functionality displayed by the LAB. The bacteria produce lactic acid, diacetyl, acetaldehyde and hydrogen peroxide as fermentation end-products. These products possess eliminate or retard the growth of many spoilage microorganisms, which enables them to

be used as bio-preservatives in foods, feeds and beverages (Justé *et al.*, 2011).

Table 4 shows the physicochemical properties of milled sorghum during fermentation. The pH of white sorghum (5.9-4.5); red sorghum (5.0-4.2) and mixed sorghum (5.3-4.4) decreased as the fermentation progressed from 0-48 h, while TTA increased from 2.2 to 3.5. The decrease in pH is suitable for lactic acid bacteria to grow and remain viable within a medium containing higher amount of lactic acid. Obadina *et al*. (2013) and Omemu *et al*. (2018) reported the decreased in pH and increase in TTA during fermentation process of traditionally fermented food products. Table 5 reveals the consumer acceptability of 'Kati' from white, red and mixed sorghum. 'Kati' from mixed white and red sorghum was most preferred with overall acceptance of 6.88. Fermented foods and beverages are widely accepted by consumers due to their enhanced nutritional content, digestibility, microbial stability and detoxification (Anal, 2019).

The proximate composition of produced 'Kati' using different starter cultures was recorded in Table 6. The moisture, ash, crude fibre, protein and carbohydrates contents (%) ranged from 64.0 to 67.23, 0.39 to 0.47, 1.05 to 2.31, 2.02 to 5.15 and 24.12 to 27.35, respectively. The higher moisture content could be attributed to the steeping of sorghum in water for period of time and addition of water during cooking. The low content of ash could be due to complete utilization of minerals by microorganisms involved during fermentation for their metabolism. The result of the anti-nutrient composition of 'Kati' produced using different starter cultures was recorded in Table 8. Sorghum has significant amounts of phytate. Phytate has been recognized as anti-nutrient factor that reduces bioavailability some macro- and micro-elements (Soro-Yao *et al.,* 2014). 'Kati' produced with *Lactobacillus* spp. have minimal value of phytates (0.64-0.77 mg/100g), phenols (11.47-14.75 mg/100g), tannins (0.40-051 mg/100g), oxalates (0.11-0.18 mg/100g). This suggests that *Lactobacillus* spp. could produce enzymes, which help to degrade the anti-nutrients during fermentation (Adeyemo and Onilude, 2013). LAB remove some non-nutrients component and synthesize vitamins, bioactive peptides, conjugated linoleic, exopolysaccharides, bacteriocins, sphingolipids that are known for health benefits (Şanlier *et al*., 2019).

The sensory evaluation of quality and acceptability of 'Kati' (Table 7) indicated that, samples produced with different LAB were well accepted for consumption. Sensory evaluation remains a mechanism to reported acceptance and consumption of foods (Yang and Lee, 2019). It has been realized that, sensory evaluation could contribute pertinent, valuable information related to marketing consequences and simultaneously provide direct actionable information (Delwiche, 2009). Fermentation makes food more palatable by enhancing its aroma and flavour with better taste. Fermented foods are more accepted by consumers than unfermented one due to their organoleptic properties (Blandino *et al.,* 2003).

Findings of Hasan *et al.* (2014) and Dimidi *et al*. (2019) suggested that viable LAB such as *Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium bifidum* interfere with gut colonization to prevent proliferation of food borne pathogens, thereby preventing manifestation various gastrointestinal infections.

Table 1. Bacterial and fungal count from steeped water and fermented slurry of sorghum

Bacteria (cfu mL^{-1})				**Fungi (sfu mL^{-1})**		
Steeped water						
	White	Red	Mixed	White	Red	Mixed
0	1.8×10^6	1.7×10^6	2.0×10^6	4.0×10^3	0.3×10^4	4.0×10^3
24	3.2×10^6	2.8×10^6	3.8×10^6	2.5×10^4	2.3×10^4	2.8×10^4
48	4.0×10^5	2.0×10^5	6.0×10^5	3.5×10^4	3.0×10^4	4.0×10^3
72	6.0×10^5	4.0×10^5	8.0×10^5	4.0×10^3	3.0×10^3	4.5×10^3
Fermented slurry of sorghum						
0	1.0×10^3	1.0×10^3	2.0×10^3	1.0×10^3	1.0×10^3	2.0×10^3
24	1.2×10^3	3.0×10^3	3.5×10^3	1.5×10^3	2.0×10^3	2.5×10^3
48	2.0×10^3	3.5×10^3	3.5×10^3	3.0×10^3	3.0×10^3	3.5×10^3

Table 2. Occurrence of microorganisms during steeping of sorghum for 'Kati' production

Isolates	**White**				**Red**				**White and red**				**Number of isolates**	**% Occurrence**
	0	24	48	72	0	24	48	72	0	24	48	72		
Saccharomyces cerevisiae	-	+	+	+	-	+	+	+	-	+	+	+	14	20.8
Corynebacterium spp	-	+	+	+	-	-	-	+	-	+	+	+	12	18.0
Lactobacillus spp.	-	+	+	+	-	+	+	+	-	+	+	+	9	13.4
Clostridium bifermentans	-	-	-	-	+	+	-	-	+	+	-	-	9	13.4
Aspergillus niger	+	-	-	-	+	+	-	-	+	-	-	-	6	9.0
Aspergillus flavus	+	-	-	-	+	-	-	-	+	-	-	-	6	9.0
Fusarium oxysporium	+	+	-	-	-	-	-	-	+	-	-	-	4	6.0
Mucor mucedo	+	-	-	-	+	-	-	-	-	-	-	-	3	4.4
Candida albicans	-	-	-	-	-	-	-	-	+	-	-	-	2	3.0
Staphylococcus aureus	+	-	-	-	-	-	-	-	-	-	-	-	2	3.0

-: absent, +: present

Table 3. Occurrence of LAB in milled sorghum at different hour(s) of fermentation

***Isolates**	**White**			**Red**			**White and red**			**Number of isolates**	**% Occurrence**
	0	24	48	0	24	48	0	24	48		
Lactobacillus plantarum	+	+	+	+	+	+	+	+	+	16	19.5
Lactobacillus fermentum	-	+	+	+	+	+	+	+	+	14	17.1
Lactobacillus delbrueckii	-	+	+	-	+	+	-	+	+	12	14.6
Lactobacillus casei	-	+	+	-	+	+	-	+	+	12	14.6
Lactobacillus cellobiosus	+	+	+	-	+	+	+	+	+	11	13.4
Lactobacillus salivarius	-	-	+	-	+	+	-	+	+	9	11.0
Lactobacillus jensenii	-	+	+	-	+	+	-	+	+	8	9.8

-: absent, +: present, *each of LAB was used as starter culture to produce 'Kati' with mixed sorghum

Table 4. Physicochemical parameters of milled sorghum during fermentation at different hour(s)

White sorghum				**Red sorghum**			**Mixed sorghum**		
Time (h)	Temp (^oC)	pH	TTA (%)	Temp (^oC)	pH	TTA (%)	Temp (^oC)	pH	TTA (%)
0	26	5.9	2.2±0.0	25	5.1	2.0±0.0	25	5.3	1.7±0.0
24	29	4.8	3.0±0.3	30	4.7	2.4±0.3	30	4.6	1.9±0.0
48	30	4.5	3.5±0.5	31	4.2	2.5±0.4	32	4.4	2.0±0.0

Table 5. Sensory evaluation of 'Kati' produced from white, red and mixed sorghum

Sensory properties	**White**	**Red**	**White and red**
Taste	5.70^b±0.85	5.60^b±0.90	6.65^a±2.00
Colour	6.00^b±0.76	5.60^c±0.66	6.98^a±0.76
Texture	6.60^b±2.00	5.31^c±2.00	7.00^a±0.89
Aroma	5.00^c±0.76	5.50^b±0.90	6.90^a±1.20
Overall acceptance	5.83^b±0.68	5.50^b±0.71	6.88^a±2.00

Values with the same superscript in a row are not significantly different at $P \geq 0.05$

Table 6. Proximate (%), mineral (µg/g) and anti-nutrient (mg/100g) of 'Kati' produced from mixture of white and red sorghum with LAB starter cultures

Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Moisture	66.59±0.07	64.00±0.77	66.37±0.06	67.23±0.13	67.16±0.14	66.58±0.06	67.14±0.16	65.02±0.06
Ash	0.47±0.01	0.41±0.06	0.45±0.03	0.43±0.05	0.42±0.01	0.40±0.03	0.39±0.01	0.41±0.02
Fat	1.95±0.05	2.73±0.02	1.73±0.03	2.53±0.05	1.72±0.06	1.34±0.04	2.72±0.07	4.10±0.77
Crude fibre	1.72±0.05	1.86±0.08	2.31±0.05	1.72±0.03	1.39±0.07	1.05±0.06	1.47±0.07	1.05±0.01
Protein	5.15±0.02	5.05±0.04	2.09±0.03	3.48±0.02	2.02±0.06	3.28±0.06	2.07±0.06	2.68±0.84
Carbohydrates	24.12±0.25	25.95±0.03	27.05±0.73	24.61±0.06	27.29±0.05	27.35±0.01	26.21±0.08	26.74±0.02
Na	15.40±0.01	14.80±0.01	15.00±0.02	14.20±0.04	14.60±0.02	19.00±0.02	17.10±0.01	21.03±0.01
K	85.50±0.02	93.00±0.02	92.06±0.02	94.20±0.02	89.40±0.02	94.90±0.01	83.00±0.02	97.02±0.22
Ca	30.00±0.02	21.05±0.01	22.20±0.00	18.04±0.02	26.05±0.01	20.02±0.00	17.50±0.02	36.04±0.01
Mg	30.03±0.02	45.00±0.02	38.04±0.02	47.03±0.00	42.02±0.01	32.00±0.00	48.50±0.00	52.22±0.02
Phytates	0.73±0.00	0.74±0.08	0.64±0.04	0.67±0.08	0.78±0.04	0.94±0.04	0.77±0.04	0.73 ±0.01
Phenols	11.47±0.05	12.15±0.01	12.47±0.02	12.28±0.06	11.89±0.08	12.35±0.06	12.70±0.01	14.75+0.02
Tannins	0.46±0.01	0.49±0.01	0.48±0.02	0.50±0.03	0.48±0.02	0.50±0.01	0.51±0.02	0.40±0.01
Oxalates	0.12±0.00	0.13±0.00	0.16±0.01	0.14±0.00	0.18±0.00	0.17±0.00	0.16 ± 0.02^{0}	0.11± 0.00

1: 'Kati' produced with *Lactobacillus casei,* 2: 'Kati' produced with *Lactobacillus salivarius*,
3: 'Kati' produced with *Lactobacillus jensenii,* 4: Kati' produced with *Lactobacillus cellobiosus,*
5: 'Kati' produced with *Lactobacillus plantarum,* 6: 'Kati' produced with *Lactobacillus delbrueckii*,
7: 'Kati' produced with *Lactobacillus fermentum* and 8: 'Kati' purchased as control

Table 7. Sensory evaluation of 'Kati' produced form mixture of white and red sorghum

Sensory properties	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Taste	$6.97^{c}\pm0.85$	$7.50^{b}\pm0.90$	$5.65^{d}\pm2.00$	$6.87^{c}\pm0.57$	$8.69^{a}\pm2.0$	$6.80^{c}\pm0.76$	$7.69^{b}\pm2.00$	$8.37^{a}\pm0.72$
Colour	$6.80^{c}\pm0.76$	$7.20^{b}\pm0.66$	$6.97^{c}\pm0.76$	$7.62^{b}\pm2.00$	$8.59^{a}\pm2.00$	$7.10^{bc}\pm1.10$	$8.10^{a}\pm0.70$	$7.03^{bc}\pm0.60$
Texture	$6.62^{b}\pm2.00$	$8.31^{a}\pm2.00$	$6.97^{b}\pm0.89$	$6.90^{b}\pm1.20$	$8.00^{a}\pm0.70$	$7.13^{b}\pm0.97$	$7.19^{b}\pm2.00$	$7.20^{b}\pm0.76$
Aroma	$7.10^{b}\pm0.76$	$7.50^{b}\pm0.90$	$6.90^{bc}\pm1.20$	$7.10^{b}\pm1.10$	$8.10^{a}\pm071$	$7.23^{b}\pm0.69$	$7.53^{b}\pm0.94$	$8.73^{a}\pm2.00$
Overall acceptance	$6.87^{c}\pm0.68$	$7.63^{ab}\pm0.71$	$6.63^{c}\pm2.00$	$7.13^{b}\pm0.57$	$8.35^{a}\pm0.50$	$7.07^{b}\pm0.78$	$7.58^{ab}\pm2.00$	$7.83^{a}\pm2.00$

Values followed by the same superscript in a row is not significantly different at $P\geq0.05$

1: 'Kati' produced with *Lactobacillus casei,* 2: 'Kati' produced with *Lactobacillus salivarius*,
3: 'Kati' produced with *Lactobacillus jensenii,* 4: 'Kati' produced with *Lactobacillus cellobiosus,*
5: 'Kati' produced with *Lactobacillus plantarum,* 6: 'Kati' produced with *Lactobacillus delbrueckii*,
7: 'Kati' produced with *Lactobacillus fermentum* and 8: 'Kati' purchased as control.

4. Conclusions

Starter cultures in 'Kati' will serve as probiotics, which are live microorganisms and when consumed in adequate amounts could confer health benefits on the host. Fermented foods containing LAB can be attributed to the presence of some essential nutrients and bioactive compounds that have potential to improve human health.

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ANISAKIASIS OF FISH PRODUCTS AND ITS SANITARY CHARACTERISTICS

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ABSTRACT

The article is devoted to the study of the defeat of fish products (Atlantic herring (Clupea harenqus) with fresh-frozen, salted, pickled and smoked) parasitic nematodes Anisakis simplex. It was noted that for the period from 2015 to 2018, the rate of invasion by this parasite increased from 9.2 to 54.4%. It was experimentally established that fish of various culinary processing had different degrees of helminth damage. The most anisizoid larvae were in pickled herring, and least of all - in smoked. It is obvious that the established differences in digital data are associated not only with the type of culinary processing, but with the observance of sanitary standards for the storage of fish raw materials and finished products. Removing the viscera from the fish carcass and observing the thermal regime significantly reduce the level of invasion.

1.Introduction

Fish and fish products are valuable food products - a source of high-quality proteins, unsaturated fatty acids, fat-soluble vitamins and minerals. Fishing is one of the main sources of providing residents of Ukraine with full-fledged food. In the structure of the Ukrainian fish market, about 80% is imported products. An increase in the supply of fish from abroad to the domestic market of Ukraine has increased the frequency of detection of helminthological pathogens previously diagnosed sporadically, in particular, opisthorchiasis, clonorhosis, metagoniosis, nanofeetosis, diophilobothiosis, coriandrosis, anisakidosis, and others.

According to the literature, virtually all sea fish can be infected with different types of worms, up to 30 species of which constitute a potential danger to humans or cause unwanted changes in fish, as in technological raw materials. Of the helminthiasis dangerous to humans in sea fish most often occur nematodes of the family of *Anisakids* (Mikulich, 2013; Shibata, Ueda, Akaike & Saida, 2014).

Anisakiasis is a zoonotic gelmina characterized by a defeat of the gastrointestinal tract as a result of parasitism in the human body at the stages of the larvae of the worm family *Anisakidae*.Anisakis – pathogenic worms, the representatives of nematodes of the family *Anisakidae* (*Anisakis simplex, Pseudoterranova decipiens, Hysterothylacium aduncum, Contracaecum osculatum*). According to studies by various scientists, fish invasiveness with anisakiasis reaches high rates (20-50%). Localized in the body, on the surface or in the tissues of the internal organs, rarely in the muscles (often below the middle line of the body of fish), sea and passage Pacific fish (cod, mackerel, hake, flounder, knot, herring, pink salmon etc.) (Gaevskaya, 2005; Baird et al., 2014).

It is dangerous that the parasites are stored in the fish after freezing, harvesting and heat treatment. Larvae of *A. simplex* can survive and

retain allergenic properties even during long-term storage in frozen form (-20 ± 2°C for 11 months). Is can tolerate a temperature rise of up to 45°- 60°C and above they die within 10 minutes. Thus, the thermal processing of fish does not guarantee its decontamination from the anisotropic larva (Sondak, Gritsik & Rud, 2006; Buchmann & Mehrdana, 2016).

However, the relationship between the degree of invasion of Anisakis and the various types of culinary processing of fish has not yet been studied. Obviously, a detailed study of this issue can improve sanitary safety and reduce the number of low-quality products.

According to monitoring of the Ukrainian trading network, currently frozen fish products dominate the market, accounting for about 90% of all imports. In addition, there are different types of fish processing: marinades, cheeses, pickled, smoked and others. The most popular and affordable object is Atlantic herring (about 20% of all imports). It was repeatedly noted earlier that this species is characterized by the highest prevalence of Anisakidae invasion (up to 100%), respectively, such products can be potentially dangerous for the consumer (Gaevskaya, 2005; Baird et al., 2014). In this regard, the aim of our study was to study the interannual dynamics of the number of Anisakidae parasites in the Atlantic herring (Clupea harenqus) after various culinary processing.

2. Materials and methods

The main goal of the study is to determine the extent of herring damage during various culinary treatments by the larvae of *Anisakis simplex* in the markets of Vinnitsa (by 2015-2018 years). The **object** of the study was herring frosting, salting, smoked, and marinated herring, the **subject** - the dynamics of lesion with anisakis.

The selection fish products samples was carried out 2015 – 2018 year by the work of the expansion network in accordance with the established rules and regulations. In the course of research are used same methods such as organoptic and parasitological. The parasitological examination of fish reveals visible parasites, as well as parasites, muscles, under the skin or shines. At the same time, we paid attention to the inclusions, which differ in color or consistency from normal tissues and form regions of meat of sparse consistency. To detect parasites in meat use a method of parallel cuts (Bogatko, Vlasenko & Golub, 2011; Fotina, Berezovskiy, Petrov & Gorchanok, 2013).

Detection of parasites or inclusion, like living parasites, is initially considered under magnifying glass or binocular. Then, they are considered under the small and middle levels of the microscope. The vital activity of parasites is determined by the method of irritation.

The following parameters were determined to the dynamics of anisakis in different types of culinary processing: the number of damaged specimens, the severity of the invasion, the intensity of the invasion and the index of invasion. The number of specimens affected was determined by simple counting when the fish were opened and examined. Extensiveness of the invasion was determined by dividing the number of damaged specimens by the number of specimens (25 pieces) and multiplying by 100. The intensity of the invasion was determined by counting the parasites of one fish. The invasion index was determined by dividing the number of parasite larvae into the total number of parasites found in the sample (Bogatko et al., 2011). A sanitary-microbiological assessment of the affected fish was carried out using a reaction to reductase and peroxidase (benzyidine test) (Fotina et al., 2013).

The digital material was processed statistically. The resulting digital data was processed using the MS EXEL 98 and Windows program, statistically processed by Student. The results were considered statistically significant at $p < 0.1$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

3.Results and discusions

3.1.Results

It has been experimentally established that different types of fish products have different levels of infection with *Anisakis simplex* larvae. Smoked fish suffered the least. However, in the

period from 2015 to 2018, the rate of invasion increased by this parasite increased from 9.2 to 54.4%. Samples of salty fish at the beginning of our study had no parasites (2015). Since 2016, it has grown by 60.4%. The largest infection of anisakis salty fish has in 2017 (Fig. 1).

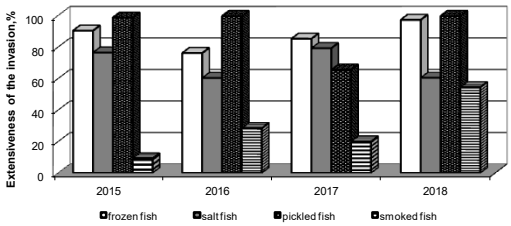

Figure 1. Dynamics of invasion of herring Anisakis simplex of different culinary processing

The graphic material shows that marinated fish products are the most affected. The degree of damage has reached almost 100%. This means that virtually every carcass of fish during the analysis was infected with parasites. Only in 2017, the level of invasion decreased by 33% compared with the previous one. Frozen fish also had a high incidence of defeat by Anisakis simplex larvae. In the baseline (2015) and reporting (2018) years, the severity of the invasion was close to 100%. The least was contaminated with frozen fish products in 2016 (by 21.2% compared to the reported year).

Parasitological studies have shown that in the base year (2015) all marinated fish were damaged by Anisakis simplex larvae. In it we counted up to 57 larvae per 1 carcass of fish. In addition, the marinated herring had the highest rate of invasion (33.3 units / carcass) compared to other types of fish products (Table 1). In the reported year (2018) The number of carcasses of fish with larvae of the parasitic nematode in the group of frozen and pickled products was the same. However, in the marinated fish, the invasion index was 10 units higher compared to frozen fish. In this group, we found the highest intensity of invasion (Table 1).

Table 1.The dynamics of infection to *A. simplex* of fish products over the past 5 years *(M ± m, n=25)*

Indicator	**Type of fish products (Atlantic herring (*Clupea harenqus*)**			
	frozen	**salt**	**pickled**	**smoked**
2015 year (base)				
The number of specimens affected, units	23,6 ± 0,52	19,3 ± 0,06***	25,0 ± 0,01*	2,3 ± 0,91***
The intensity of the invasion, units	5-19	7-20	9-57	1-17
The invasion index, units	12,6 ± 1,78	13,5 ± 0,77	33,3 ± 0,85***	9,03 ± 0,45*
2016 year				
The number of specimens affected, units	19,0 ± 1,03	15,0 ± 0,07***	24,9 ± 0,03**	7,1 ± 0,09***
The intensity of the invasion, units	3-15	1-23	7-43	0-7
The invasion index, units	9,3 ± 0,03*	12,3 ± 0,36	23,5 ± 3,31**	3,5 ± 0,09***

2017 year				
The number of specimens affected, units	21,3 ±0,71**	19,8 ± 1,69	23,9 ± 2,03	5,3 ± 2,73
The intensity of the invasion, units	3-25	5-19	7-28	0-3
The invasion index, units	14,3 ± 0,06	12,0 ± 0,31*	17,5 ± 4,35**	1,5 ± 0,01***
2018 year (reporting)				
The number of specimens affected, units	24,3 ± 2,75	15,1 ± 3,71	24,9 ± 1,33	13,6 ± 2,75*
The intensity of the invasion, units	5-23	1-18	11-37	3-15
The invasion index, units	14,3 ± 2,75	9,5 ± 0,33***	24,8 ± 7,24	9,3 ± 0,33

In the dynamics of the intensity of the invasion over the past five years, this indicator was the highest in the group of frozen fish and salty fish products in 2017, in the group of pickled and smoked fish - in 2018. A group of frozen fish, this figure has increased by 1.7 units. In salted herring, for the last five years, the parasite larvae were less than 4.0 units per 1 carcass of fish ($p < 0.001$). In the group of pickled fish products, the invasion index decreased by 8.5 units ($p < 0.001$), while smoked fish - on the contrary, increased by 0.27 units ($p < 0.01$).

During our previous studies, the infection of the marinated herring Anisakis simplex in the markets of Vinnitsa and villages of urban type Lityn was studied. In 2019, we repeated the experiment to study the viability of the larvae of this parasite. The results of the comparative analysis are presented in table 2.

Table 2. The dynamics of *A. simplex* infected marinated herring and the presence of live larvae *(M ± m, n = 5)*

Index	Year:	
	2015	2018
The intensity of infection with parasites, units	7,6 ± 1,88	7,2 ± 5,22
Intensity of infection with parasites of the abdominal cavity, units/kg	43,0	35,6
including live parasites, units	0,20 ± 0,19	1,7 ± 1,40
% of total	2,63	23,19

The material in the table indicates an increase in the number of live larvae of the *A. simplex* in fish in the reporting year 2019. At the same time, the index of invasion in 2015 and 2018 was almost the same. By the number of parasites per kilogram of edible part of the fish,

the invasion index decreased by 7.4 units, but remains fairly large.

The connection between the defeat of fishery products by *A. simplex* larvae and the sanitary condition was investigated. During the study of the reaction to peroxidase, a positive effect was found in gills of all types of culinary processing. In muscle extracts, the reaction to reductase was positive only in the group of pickled products (table 3).

Table 3. Peroxidase and Reductase tests for fish products *(M ± m, n=5)*

Indicator	Kind of fish processing			
	frozen	salt	pickled	smoked
Peroxidase test: - gills	positive (+)	+	+	+
- muscles	negative (-)	-	+	-
Reductase test, time of counting change of color, minutes: - up to 40 minutes	negative, the indicator did not change the color (-)	-	-	-
- from 40 min up to 2.5 hours	-	-	indicator changed the color (+)	-
- more than 2.5 hours	-	-	+	+

The reaction to the reductase was positive in pickled and smoked fish. In addition, in the marinated herring, the indicator changed the color for almost 1 hour. All other samples of fish products had a negative reaction to reductase.

3.2.Discussions

Anisakiasis was first detected in the Netherlands in 1955 after eating slightly salted herring. Every year in many countries new cases are registered. According to studies of various scientists, the invasiveness of fish with anisakiasis reaches high rates. The greatest incidence is observed in Atlantic herring - (59-100%), the frequency of invasion is 1–38 individuals, the invasion index is 12 larvae per carcass of fish (Gaevskaya, 2005; Baird et al., 2014). Our studies have confirmed this trend of Atlantic herring pollution. The total volume of herring samples of different types of culinary processing in Vinnitsa was somewhat higher than the data on the loss of imported fish, as shown in the article. F. Baird et al. aaccording to their data, the degree of damage by parasites of fish was within 59%, the intensity of the invasion - up to 38, and the index of invasion - up to 12 (Baird et al., 2014). However, according to experimental data R.V. Puzyr, S.A. Tkachuk, (2013) the extensiveness of the

invasion - the herring was 89%, the intensity of the invasion - 7-22 units. Apparently, discrepancies in the data are related not only to the type of culinary processing, but also to the observance of sanitary norms for the storage of fish raw materials and finished products.

In studying the localization of larvae of anisakis, it was established that the determining factor is not a kind of culinary processing, but a kind of processing of raw materials. Most of the invasions were found on the internal organs of fish. According to literature data, the abdominal cavity and internal organs (82.2%), less often the muscles of the abdominal wall (13.3%) and back muscles (4.5%) are most often affected (Grigoryeva, 2009). There are cases of invasion not only in frozen fish, but even in fish canned fish from whole fish (Pravettoni, Primavesi & Piantanida, 2012; Caballero et al., 2013; Baird et al., 2014). Therefore, in our opinion, removal of

insects from the carcass of fish can reduce the level of invasion.

According to the results of our research, the largest number of larvae was found in the intestines of pickled fish, and the least - in cartridges of smoked fish. We found that in the period from 2015, the degree of invasion of smoked fish has almost doubled, and salty - by 60.4%. Perhaps in the production of such fish products, producers did not sufficiently control the quality of raw materials or used inappropriate fish.

An analysis of experimental data showed that for one pickled herring, according to calculations, more than 5 larvae. In accordance with sanitary norms (Bogatko et al., 2011; Fotina et al., 2013) such products are not allowed for sale In our opinion, this is due to the selection of low-quality raw materials that have not been properly treated with cold. Seafood disinfection from anisakis larvae can be carried out by freezing and heating (Mikulich, 2013; Buchmann & Mehrdana, 2016, Rodríguez et al., 2016). Typically, pickled fish is made from previously frozen foods. In Europe and the USA, sanitary rules regulate the freezing of fish, which cannot be subjected to further heat treatment at -20 ° C for 5 days. In ordinary physiological and acetic acid solutions used to prepare fish, Anisakias larvae can remain viable for many days or even months (Mikulich, 2013; Buchmann & Mehrdana, 2016, Rodríguez et al., 2016). Also, the cause may be improperly prepared marinade with a low salt content (less than 14%), favorable for the life of larvae.

The smallest contamination was observed in smoked fish products. The removal of the intestines from the fish before smoking caused a decrease in the degree of invasion of the body by 78%. Anisakias larvae can tolerate fever up to 45 ° C, but at a temperature of 60 ° C and above they die within 10 minutes. However, thermal treatment of fish in the temperature range 45-60 ° C does not guarantee its disinfection from larvae (Baird et al., 2014; Prester, 2015), as evidenced by their presence in smoked samples.

We also established a connection between the sanitary state of fish products and damage to A. simplex larvae. It is proved that the more parasites were detected in fish (pickled herrings), the better the reaction was to peroxidase and reductase. This, in turn, indicates the damage to fish products by microorganisms and their inability to consume people. For example, according to the reaction to the reductase, marinated fish products were classified as conditionally fresh. Similar data were obtained in other studies by other researchers (Puzyr & Tkachuk, 2013; Shibata et al., 2014).

4.Conclusions

The practical significance of this study is to increase the effectiveness of sanitary and epidemiological control of fish products and public awareness of the risk of anisakiosis. Studies have shown that in recent years there has been a tendency to increase the invasion of herring on anisaki of all types of cooking. The highest intensity, intensity and index of invasion were found in pickled fish. The analysis of carcasses showed that up to 89% of all larvae are localized in the abdominal cavity. Smoked fish had the slightest damage with anisakis.

Anisakis simplex larva invasion negatively affects the sanitary state of fishery products. It was found that herring, marinated with a large number of parasites, had a positive reaction to peroxidase and reductase. In the future, we will investigate the biochemical composition and properties of fish products depending on the degree of defeat by Anisakis simplex larvae. We will also develop biologically active additives that quickly disinfect live larvae of this species.

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STABILITY AND RHEOLOGICAL PROPERTIES OF ICE CREAMS PRODUCED WITH DAIRY BY-PRODUCTS

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ABSTRACT

The majority of dairy industries (cheese, butter, ricotta, etc.) discard their by-products in the environment causing intense pollution due to the high concentration of organic matter in these products. An interesting solution would be the reuse of these by-products in foods, such as ice cream. However, unpleasant changes in this emulsion may occur, such as undesirable phase separation. In this context, the aim of this work was to observe the effects of the application of dairy wheys on ice cream's rheological properties and stability. Ice cream formulations differed by flavor (cream and chocolate), by type (milk, cheese whey, ricotta whey and butter whey), and by proportions of wheys (0, 25, 50, 75 and 100%). A commercial sample was also evaluated as a comparison. The evaluated parameters were zeta potential (ZP), particle size (PS), rheological behavior, desorption, and concentration of Ca+Mg. The results showed that the addition of whey, regardless of flavor and origin, reduced the viscosity and increased PS and desorption, but did not compromise the ZP of most of the samples (78.57%). This behavior was concentration-dependent. The Ca+Mg content of the wheys and the flavorings had no influence on the desorption index. Thus, the analyses revealed that different dairy by-products in ice creams could be used without significantly compromising important quality parameters and, at the same time, help to preserve the environment. However, further experiments should be conducted (e.g. sensory analysis) in order to better understand the technological potential of dairy by-products application in ice creams.

1.Introduction

Dairy producers are one of the food industry's examples that produce large quantities of effluent with high organic load, i.e. high Chemical (COD) and Biochemical Oxygen Demands (BOD). These by-products can impose serious challenges in local sewage treatment systems, representing a significant environmental impact when discarded without proper treatment (Janes et al., 2008; Silva, 2011). Among the alternatives that minimize environmental aggression, the partial or total replacement of powdered milk, eggs, fats, sugar, and even protein in the formulations of several other processed food has high added value,

considering the nutritional e technological potential of these dairy effluents. In addition, these by-products are inexpensive and could help the economy of many companies while reducing raw material costs, and therefore lowering the cost of production (Singh et al., 2012; Božanić et al., 2014).

One of the most popular desserts in the world is the ice cream and its consumption has been constantly increasing over the years, mainly due to its sweet taste and soft texture. It is a product appreciated by people of all ages and at any time of the year, being considered a healthy and nutritious food, not only because of its high energy value, but also because of its high digestibility (Aboulfazli et al., 2015; Kumar et al., 2016, Makares et al., 2014, Vadiveloo et al., 2014).

The macromolecules present in ice creams (fats, proteins and complex carbohydrates) significantly contribute to the perception of texture and taste (Akesowan, 2009). Among the ice cream quality parameters, two of the most important are the viscosity and stability of its visual characteristics, which should not show any phase separation during and right after the melting (Tharp et al., 1998).

The formation and stability of dispersed colloidal systems as ice cream, depend on many factors such as protein content, salt concentration and ionic strength (Corredig et al., 2011, Tharp et al., 1998, Bodyfelt et al., 1988). Little is known regarding phase separation of ice cream blends, considering that investigations on their stability behavior focus mainly on the physicochemical characteristics and sensory qualities when new ingredients are added, thus leaving a gap in terms of understanding this mechanism (BahramParvar et al., 2008; Cheng et al., 2015).

Taking into consideration the pollutant potential of dairy by-products, the reuse of these wastes in human foods, as well as the possible modifications that they can cause in the composition and structure of food (desirably or not), the aim of this study was to evaluate the behavior of the addition of ricotta whey, cheese whey, and butter whey in rheological properties and ice cream stability.

2. Materials and methods

All procedures were carried out in three experimental replicates.

2.1. Formulation and processing of ice creams

The residues were obtained from the draining step of the production of rennet cheese, butter, and ricotta as liquid whey and then immediately cooled/frozen in cold chamber at -18±2°C. They were kindly provided from an agro-industry located at Federal Institute of Alagoas (IFAL), Campus Satuba, being transported frozen to the Laboratory of Biochemical Engineering of the School of Chemistry at Federal University of Rio de Janeiro (UFRJ) in a time not exceeding 12 hours.

Cream and Chocolate flavors were used in the production of ice creams with different ingredient proportions (% w/w) (Table 1), where the ingredients were purchased in a local market of Rio de Janeiro-RJ. Milk was replaced by different proportions of liquid/thawed residues and a control sample (0% of whey/100% of milk) was also made. A sample of a commercial brand with a high market share was acquired for comparison purposes with the developed ice cream.

The procedure to obtain the ice creams was done using a specific equipment to make the samples (Cuisinart® ice cream model, Ice 100 model), in which the basic unit operations of its processing were provided: homogenization of the ingredients (less emulsifier), pasteurization of the mixture, maturation, beating/freezing (emulsifier addition). The ice creams were transferred to plastic polypropylene recipients with a capacity of 250 mL, then stored in a freezer with the temperature set at -18±2ºC.

Table 1. Ice cream formulations with different whey percentages (% w/w).

Ingredients (%)	**Cream flavor**						**Chocolate flavor**				
	0%	25%	50%	75%	100%		0%	25%	50%	75%	100%
Whole Milk	66.86	16.72	33.43	50.14	---		58.14	43.61	29.07	14.53	---
Ricotta whey or Cheese whey or Butter whey	---	50.14	33.43	16.72	66.86		---	14.53	29.07	43.61	58.14
Refined sugar	17.44	17.44	17.44	17.44	17.44		17.44	17.44	17.44	17.44	17.44
Cream milk	11.63	11.63	11.63	11.63	11.63		11.63	11.63	11.63	11.63	11.63
Thickener and Stabilizer	0.58	0.58	0.58	0.58	0.58		0.58	0.58	0.58	0.58	0.58
Emulsifier	0.58	0.58	0.58	0.58	0.58		0.58	0.58	0.58	0.58	0.58
100% cocoa powder	---	---	---	---	---		11.63	11.63	11.63	11.63	11.63
Cream flavoring	2.91	2.91	2.91	2.91	2.91		---	---	---	---	---

2.2. Particle size and Zeta Potential

The mean particle size (PS) and zeta potential (ZP) of the ice cream mixes samples were determined using the Malvern Zetasizer Nano Series (Malvern Instruments, UK) at a constant temperature of 25 °C. Measurements were performed with an approximately 1:10.000 sample dilution in deionized water (Aboulfazli et al., 2014; Aboulfazli et al., 2015).

2.3. Rheological behavior

Rheological tests were carried out in melted ice cream samples (Aboulfazli et al., 2014; Aboulfazli et al., 2015) with a rotational viscometer OFITE 900 (OFI Testing Equipment, EUA) equipped with a Couette coaxial cylinder geometry. The viscometer was coupled to a Julabo F25 (GmbH, Germany) refrigerated circulator and all measurements were performed at a constant temperature of 4.0°C ± 0.1°C. Apparent viscosity and shear stress were assessed by linearly increasing the shear rate from 1.7 to 1,021 s^{-1}. Thus, the representative viscosity value of 50 s^{-1} (i.e., the shear rate equivalent to that performed by the mouth during chewing according to Bourne, 2002) was captured and compared to values found in the literature.

Rheological data were used to calculate the consistency index (K) and flow behavior index (n) through power-law model, represented by Eq. 1 (Rossa et al., 2012). Viscosity curves were also obtained and discussed throughout the text.

$$\sigma = K(\gamma)^n \quad \cdots\cdots \text{Eq. 1}$$

σ = shear stress (mPa);
K = consistency index (mPa s^n);
γ = shear rate (s^{-1});
n = flow behavior index.

2.4. Stability Analysis

Stability analysis of ice creams was carried out according to their desorption as described by the methodology of Cheng et al. (2015) with some modifications. The melted samples were poured into 100 mL glass beakers, slightly sealed and resting for one week. In each day, it was observed the presence of phase separation and the volume of the serum fraction formed in each sample was registered. The desorption index (DI) was determined by plotting the volumes recorded versus time, and the percentage of desorption was determined by the following equation:

$$\% DI = (H_{serum\ fraction}/H_{ice\ cream}) * 100$$

Where:
$H_{serum\ fraction}$ = height of the whey layer formed (mL);
$H_{ice\ cream}$ = total height of the ice cream (mL).

2.5. Determination of Calcium (Ca) and Magnesium (Mg)

The determination of Calcium (Ca) and Magnesium (Mg) in the main ingredients, both liquid and solid, was performed by titration with EDTA (ethylenediamine tetraacetic acid) solution as described in the methodology by Bird et al. (1961).

2.6. Statistical analysis

All parameters were performed in triplicate and the results of zeta potential, particle size, rheological behavior, desorption, and Ca + Mg concentration were expressed as the mean value ± standard deviation (SD). One-way ANOVA with post hoc was used to compare data obtained from the application of different dairy wheys and their proportions in the ice creams. The Tukey's HSD test was used when the difference was detected with 95% confidence ($p < 0.05$). Statistical analyses were performed using Statistica 7.0 software (StatSoft - USA).

3.Results and discussions

3.1. Particle size and Zeta Potential

The zeta potential of emulsions determines whether the particles tend to coalesce or not, that is, it evaluates constant stability thereof. Along with particle size, zeta potential measurements can be used to predict the stability of ice cream emulsions. According to Achouri et al. (2012)

and Malvern (2004), a zeta potential with a value (in magnitude) of more than 30 mV indicates the impedance of the droplet aggregation of an emulsion, which in turn, provides the increase of its stability by electrostatic repulsion.

According to table 2, it is observed that the zeta potential increased (it becomes less negative) along with the particle size in a concentration-dependent manner as wheys were added. This behavior indicates that the dairy by-products do not help to prevent the aggregation of droplets present in the ice cream mixes, meaning that the ice creams mixes containing the smallest quantity of residues were more stable, even though only 21.43% (6) of the total samples (28) are considered unstable since the threshold between stable and unstable suspensions is usually at ± 30mV (particles with zeta potential greater than + 30mV or less than -30mV are normally considered stable) (Malvern, 2004; et al., 2006; Tangsuphoom et al., 2009). In this way, chocolate flavor samples, except for the ice cream mix with 100% butter whey, were considered to be stable and those with cream flavor formulation of up to 75% of ricotta whey, 25% of cheese whey, and 75% of butter whey, also presented values of zeta potential below -30mV.

The zeta potential of ice creams mixes developed with 100% milk (without any residues) was similar to the vanilla-flavored ice cream mix also developed with 100% milk by Aboulfazli et al. (2014) (-36.56 mV) and Aboulfazli et al. (2015) (-36.56 and -35.10 mV). Regarding other formulations, the same study by Aboulfazli et al. (2014) when evaluating the effect of vegetable milks on the physical and rheological properties of ice cream, found that the formulation with coconut milk was more unstable (-30.70 mV) than soy milk (-35.50 mV) and the majority of samples developed in the present study (66.67% of samples). The zeta potential variation between the samples was also found in the research by Cheng et al. (2015) (-25 to -50 mV) when analyzing the effects of milk protein-polysaccharides interactions on the stability of ice cream mix model systems.

Compared to the cow's milk and vegetable milk-based ice creams mixes by Aboulfazli et al. (2014) (810-2541 nm) and Aboulfazli et al. (2015) (810-8.628 nm), the particle size values of ice cream mixes of the present study were lower (308-755 nm). Furthermore, Whelan et al. (2008) when studying the physicochemical and sensorial optimization of a low glycemic index in ice cream made with cow's milk, also presented high values (≈ 4,850 nm). For the particle sizes found by Cheng et al. (2015) (610-990 nm) in coconut oil and cow's milk ice creams mixes, only three samples presented sizes greater than 610 nm (100% cream flavored cheese whey, and 100% cream and chocolate-flavored ricotta whey).

The digestive process is affected by a wide variety of factors, including particle size, which is usually related to the surface area available for enzymatic action (Al-Rabadi et al., 2009). Based on this, Blasel et al. (2006) found that the starch access by α-amylase significantly decreases for every 100 μm increase in grain particle size of milled corn. Regarding the ingestion of breads, de la Hera et al. (2014) when evaluating the effects of flour particle size on gluten-free bread at an in vitro digestibility model, found difficulties in the digestibility of the starch as particle size increased, since the larger the particle size is, the larger is the quantity of low digestible starch and resistant starch.

Therefore, since the smaller the particle size the better the digestibility of a food, the ice creams mixes of the present study were shown to be more digestible, although the particle size increased with the addition of different wheys (those made with ricotta whey had higher sizes than the cheese whey and butter whey ice creams, respectively).

Table 2. Data of zeta potential, particle size, and desorption index (DI) of ice cream samples.

Sample	Zeta potential (mV)		Particles size (nm)		Desorption Index (DI) (%)	
	Cream flavor	Chocolate flavor	Cream flavor	Chocolate flavor	Cream flavor	Chocolate flavor
Ricotta whey						
Commercial	-40.70 ± 1.01^{a}	- 42.20 ± 1.47^{a}	334.57 ± 3.5^{c}	307.90 ± 10.69^{e}	15.00 ± 1.00^{d}	3.00 ± 1.00^{ab}
0%	-35.57 ± 2.05^{b}	-38.53 ± 1.96^{ab}	343.27 ± 10.92^{c}	308.97 ± 7.05^{e}	10.00 ± 2.00^{e}	2.00 ± 1.00^{ab}
25%	-33.28 ± 1.33^{bc}	-36.15 ± 2.00^{bc}	346.23 ± 2.82^{c}	353.37 ± 4.04^{d}	15.00 ± 1.00^{d}	1.00 ± 1.00^{b}
50%	-31.00 ± 0.60^{cd}	-33.77 ± 2.22^{c}	349.20 ± 7.45^{c}	397.77 ± 5.90^{c}	20.00 ± 0.00^{c}	1.00 ± 0.00^{b}
75%	-30.22 ± 0.90^{cd}	-33.35 ± 0.76^{c}	551.88 ± 15.09^{b}	541.10 ± 10.99^{b}	30.00 ± 1.00^{b}	3.00 ± 1.00^{ab}
100%	-29.43 ± 1.33^{d}	-32.93 ± 0.81^{c}	754.57 ± 29.05^{a}	684.43 ± 16.65^{a}	39.00 ± 3.00^{a}	4.00 ± 1.00^{a}
Cheese whey						
Commercial	-40.70 ± 1.01^{a}	- 42.20 ± 1.47^{a}	334.57 ± 3.65^{d}	307.90 ± 10.69^{d}	15.00 ± 1.00^{c}	3.00 ± 1.00^{a}
0%	-35.57 ± 2.05^{b}	-38.53 ± 1.96^{b}	343.27 ± 10.92^{d}	308.97 ± 7.05^{d}	10.00 ± 2.00^{d}	2.00 ± 1.00^{a}
25%	-32.73 ± 1.11^{bc}	-34.58 ± 0.98^{c}	376.02 ± 9.91^{cd}	340.05 ± 8.74^{cd}	13.00 ± 1.00^{c}	2.00 ± 0.00^{a}
50%	-29.90 ± 1.64^{c}	-30.63 ± 0.67^{d}	408.77 ± 9.00^{c}	371.13 ± 12.60^{c}	15.00 ± 3.00^{c}	1.00 ± 0.00^{a}
75%	-25.97 ± 0.78^{d}	-30.38 ± 0.40^{d}	566.83 ± 23.81^{b}	447.12 ± 13.97^{b}	20.00 ± 2.00^{b}	1.00 ± 1.00^{a}
100%	-22.03 ± 0.49^{e}	-30.13 ± 0.45^{d}	724.90 ± 39.01^{a}	523.10 ± 18.42^{a}	25.00 ± 2.00^{a}	1.00 ± 0.00^{a}
Butter whey						
Commercial	-40.70 ± 1.01^{a}	- 42.20 ± 1.47^{a}	334.57 ± 3.65^{e}	307.90 ± 10.69^{c}	15.00 ± 1.00^{a}	3.00 ± 1.00^{a}
0%	-35.57 ± 2.05^{b}	-38.53 ± 1.96^{b}	343.27 ± 10.92^{e}	308.97 ± 7.05^{c}	10.00 ± 2.00^{b}	2.00 ± 1.00^{ab}
25%	-34.52 ± 1.26^{b}	-35.15 ± 0.95^{c}	384.08 ± 8.76^{d}	375.05 ± 11.39^{b}	1.00 ± 1.00^{d}	1.00 ± 0.00^{ab}
50%	-33.47 ± 0.55^{b}	-31.77 ± 0.25^{d}	424.90 ± 9.26^{c}	441.13 ± 26.17^{a}	2.00 ± 0.00^{cd}	0.00 ± 0.00^{b}
75%	-30.05 ± 0.45^{c}	-30.73 ± 0.20^{d}	491.53 ± 0.96^{b}	463.90 ± 19.75^{a}	3.00 ± 0.00^{cd}	0.00 ± 0.00^{b}
100%	-29.82 ± 0.46^{c}	-29.70 ± 0.53^{d}	558.17 ± 11.10^{a}	453.39 ± 19.48^{a}	4.00 ± 1.00^{c}	0.00 ± 0.00^{b}

Data are presented as mean values ± Standard deviation (SD). Mean values followed by the same lowercase letter in the column do not differ between themselves by ANOVA with post hoc Tukey test and 95% of confidence ($p < 0.05$).

Table 3. Rheological parameters of the ice cream obtained by Power Law model (or Ostwald de Waale model).

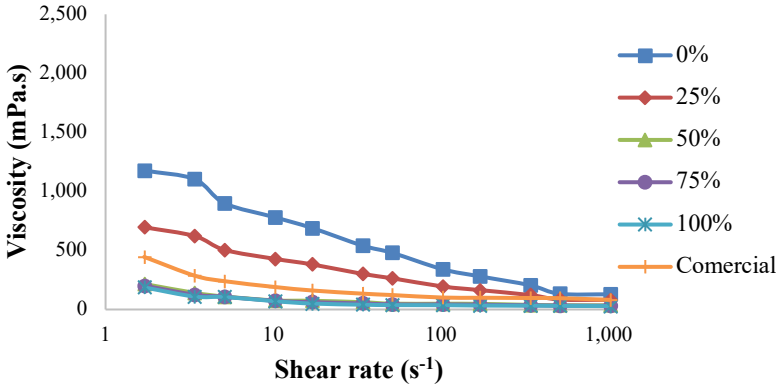
Sample	Apparent Viscosity (mPa.s) 50 s^{-1}		Consistency Index K (mPa.s^n)		Flow Behavior Index (n)		R^2	
	Cream	Chocolate	Cream	Chocolate	Cream	Chocolate	Cream	Chocolate
Ricotta whey								
Commercial	122.20 ± 0.91^{c}	289.40 ± 1.54^{c}	364.99 ± 2.11^{c}	913.66 ± 1.23^{e}	0.76 ± 0.007^{a}	0.72 ± 0.012^{a}	0.909 ± 0.003^{a}	0.981 ± 0.002^{a}
0%	480.20 ± 1.05^{a}	462.70 ± 0.87^{a}	1757.80 ± 3.32^{a}	2305.60 ± 2.21^{a}	0.63 ± 0.005^{a}	0.60 ± 0.009^{a}	0.972 ± 0.002^{a}	0.996 ± 0.001^{a}
25%	274.65 ± 1.03^{b}	455.85 ± 1.20^{a}	957.30 ± 1.02^{b}	2117.30 ± 1.01^{ab}	0.65 ± 0.003^{a}	0.60 ± 0.007^{a}	0.983 ± 0.003^{a}	0.996 ± 0.003^{a}
50%	49.10 ± 0.80^{d}	349.00 ± 1.01^{b}	175.65 ± 2.02^{d}	1926.50 ± 1.14^{bc}	0.74 ± 0.009^{a}	0.60 ± 0.007^{a}	0.912 ± 0.003^{a}	0.989 ± 0.007^{a}
75%	43.10 ± 1.01^{d}	289.00 ± 1.33^{c}	160.65 ± 2.42^{d}	1656.30 ± 3.01^{cd}	0.73 ± 0.005^{a}	0.61 ± 0.003^{a}	0.898 ± 0.005^{a}	0.991 ± 0.003^{a}
100%	37.10 ± 0.93^{d}	229.00 ± 1.87^{d}	144.84 ± 3.07^{d}	1389.30 ± 1.20^{d}	0.73 ± 0.010^{a}	0.63 ± 0.002^{a}	0.862 ± 0.001^{a}	0.979 ± 0.005^{a}
Cheese whey								
Commercial	122.20 ± 0.91^{c}	289.40 ± 1.54^{ed}	364.99 ± 2.11^{c}	913.66 ± 1.23^{b}	0.76 ± 0.007^{a}	0.72 ± 0.012^{a}	0.909 ± 0.003^{a}	0.981 ± 0.002^{a}
0%	480.20 ± 1.05^{a}	462.70 ± 0.87^{a}	1757.80 ± 3.32^{a}	2305.60 ± 2.21^{a}	0.63 ± 0.005^{a}	0.60 ± 0.009^{a}	0.972 ± 0.002^{a}	0.996 ± 0.001^{a}
25%	267.75 ± 0.86^{b}	398.10 ± 1.01^{b}	973.31 ± 1.27^{b}	2120.30 ± 0.92^{a}	0.65 ± 0.006^{a}	0.61 ± 0.005^{a}	0.988 ± 0.001^{a}	0.993 ± 0.004^{a}
50%	55.30 ± 0.97^{d}	393.50 ± 1.32^{b}	204.77 ± 1.01^{d}	1950.70 ± 1.34^{a}	0.73 ± 0.002^{a}	0.61 ± 0.008^{a}	0.840 ± 0.002^{a}	0.987 ± 0.003^{a}
75%	53.55 ± 1.23^{d}	324.55 ± 0.98^{c}	183.63 ± 2.33^{d}	1908.00 ± 1.09^{a}	0.75 ± 0.003^{a}	0.60 ± 0.004^{a}	0.847 ± 0.003^{a}	0.986 ± 0.002^{a}
100%	51.80 ± 0.79^{d}	305.60 ± 0.89^{cd}	162.29 ± 2.02^{d}	1882.40 ± 1.11^{a}	0.77 ± 0.009^{a}	0.60 ± 0.005^{a}	0.855 ± 0.002^{a}	0.987 ± 0.003^{a}
Butter whey								
Commercial	122.20 ± 0.91^{c}	289.40 ± 1.54^{e}	364.99 ± 2.11^{c}	913.66 ± 1.23^{c}	0.76 ± 0.007^{a}	0.72 ± 0.012^{a}	0.909 ± 0.003^{a}	0.981 ± 0.002^{a}
0%	480.20 ± 1.05^{a}	462.70 ± 0.87^{a}	1757.80 ± 3.32^{a}	2305.60 ± 2.21^{a}	0.63 ± 0.005^{a}	0.60 ± 0.009^{a}	0.972 ± 0.002^{a}	0.996 ± 0.001^{a}
25%	272.30 ± 0.97^{b}	433.55 ± 0.91^{b}	1008.80 ± 3.03^{b}	2267.00 ± 2.12^{ab}	0.65 ± 0.009^{a}	0.60 ± 0.002^{a}	0.992 ± 0.004^{a}	0.994 ± 0.006^{a}
50%	74.40 ± 0.85^{d}	404.40 ± 1.27^{c}	275.95 ± 2.01^{cd}	2228.20 ± 1.01^{b}	0.72 ± 0.007^{a}	0.60 ± 0.007^{a}	0.819 ± 0.007^{a}	0.990 ± 0.003^{a}
75%	69.30 ± 1.17^{d}	386.65 ± 1.85^{cd}	239.43 ± 2.01^{d}	2131.00 ± 1.23^{b}	0.74 ± 0.004^{a}	0.60 ± 0.005^{a}	0.837 ± 0.001^{a}	0.989 ± 0.003^{a}
100%	64.20 ± 1.69^{d}	368.90 ± 1.03^{d}	201.83 ± 3.23^{d}	2029.10 ± 2.01^{b}	0.76 ± 0.010^{a}	0.60 ± 0.009^{a}	0.862 ± 0.005^{a}	0.985 ± 0.001^{a}

Data are presented as mean values ± standard deviation (SD). Mean values followed by the same lowercase letter in the column do not differ between themselves by ANOVA with post hoc Tukey test and 95% confidence ($p < 0.05$).

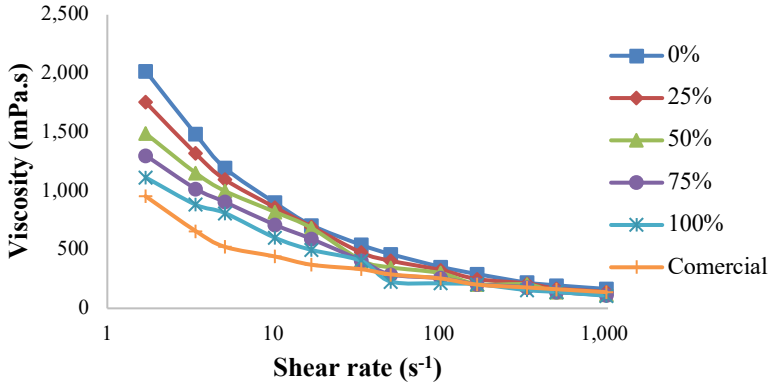
3.2. Rheological behavior

The effect of melted ice cream type (chocolate or cream) and the concentration of dairy whey products (ricotta whey, cheese whey, and butter whey) in the viscosity is shown in Table 3 and Figures 1, 2, and 3. As one can observe, all samples demonstrated pseudoplastic behavior, meaning that apparent viscosity is a function of the imposed shear rate and it decreases as the shear rate increases. The viscosity reduction may be attributed to structural changes occurring during the shearing process: the emulsified particles tend to decrease in size and to orient themselves towards the flow, which has an impact on the viscosity values (Rossa *et al*., 2012).

By the information exposed in Table 3, there is a reduction on apparent viscosity as the whey content increases. This fact is attributed to the lower stability for melted ice creams with high whey content, which reflects in the increase of desorption index (Table 4 and Figure 4) and the reduction of zeta potential (Chiewchan *et al*., 2006).

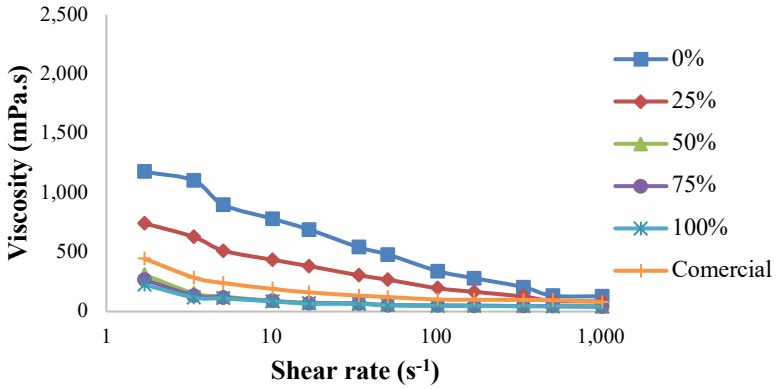


(a)

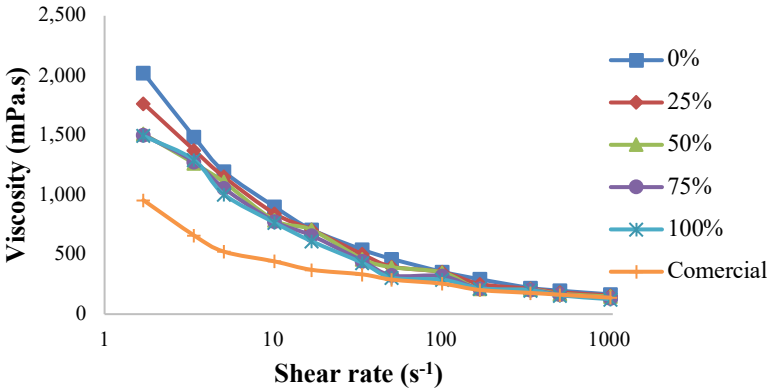


(b)

Figure 1. Effect of shear rate on apparent viscosity of cream (a) and chocolate (b) ice creams made with ricotta whey.

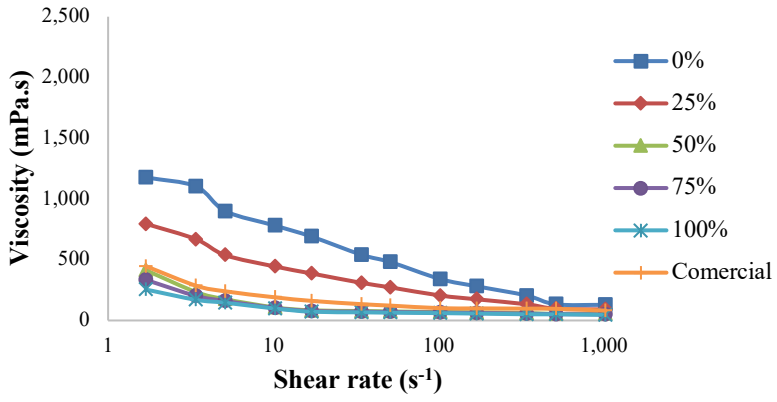


(a)

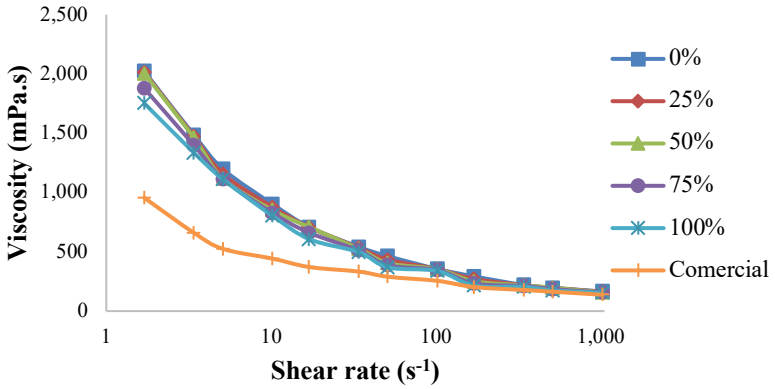


(b)

Figure 2. Effect of shear rate on apparent viscosity of cream (a) and chocolate (b) ice creams made with cheese whey.



(a)



(b)

Figure 3. Effect of shear rate on apparent viscosity of cream (a) and chocolate (b) ice creams made with butter whey.

The highest apparent viscosity values were obtained from butter whey, followed by cheese whey and ricotta whey, irrespective to the melted ice cream type. The qualitative behavior between the commercial melted ice cream brand assessed (which has great acceptance in the Brazilian market) and the formulated melted ice creams is evident as whey products are added to the samples.

For the same whey product, chocolate samples exhibited higher apparent viscosity values compared to the cream samples, which is reflected by the higher parameter *K* (consistency index, Table 3). This constant was calculated through viscosity data fit from power-law model (also known as Ostwald de Waale model). Besides, the pseudoplastic behavior is more pronounced for chocolate melted ice creams, reflected in the low values of parameter *n* (flow behavior index) when compared to the cream flavor. It is likely that this result was influenced by the presence of cocoa powder that is obtained from the cocoa paste prepared with seeds that underwent fermentation, drying, roasting, grinding and pressing for the separation of cocoa butter (Medeiros *et al*., 2009). Precisely because it is devoid of cocoa butter (which according to Gao *et al*., 2015 causes viscosity reduction), cocoa powder can cause the inverse effect.

At 20 s^{-1} all samples (except 0 and 25% ricotta whey) presented lower apparent viscosity than those developed with cow's milk (294 mPa.s), coconut milk (287 mPa.s), and soy milk (1,012 mPa.s) as seen in the study of Aboulfazli *et al.* (2015). In the present investigation, the values varied in a relatively large range for both flavors: chocolate (371.4 mPa.s, commercial sample to 708.8 mPa.s, 100% milk) and cream (50.2 mPa.s, 100% ricotta whey to 690.1 mPa.s, 100% milk).

Choi *et al*. (2014) obtained apparent viscosity values at 340.5 s-1 for the vanilla melted ice cream investigated, ranging from 26.3 to 198.9 mPa.s. In this study, this property ranged from 155.4 mPa.s (100% ricotta whey) to 219.7 mPa.s (100% milk) for chocolate melted ice cream and 32.2 mPa.s (100% ricotta whey) to 135.2 mPa.s (100% milk) for the cream melted ice cream. At 50 s^{-1}, Li *et al.* (2015) had samples varying from 100 to 430 mPa.s. At this same shear rate our results ranged from 289.4 mPa.s (commercial sample) to 462.7 mPa.s (100% milk) for chocolate type and 37.1 mPa.s (100% ricotta whey) to 480.2 mPa.s (100% milk) for the cream type.

The average apparent viscosity for the melted ice creams assessed by Whelan *et al.* (2008) at 30 s^{-1} was 51.5 mPa.s. On the other hand, in this investigation the apparent viscosity of chocolate melted ice cream ranged from 333.2 mPa.s (commercial sample) to 540.8 mPa.s (100% milk), whereas the cream samples

ranged from 40.5 mPa.s (100% ricotta whey) to 540.9 mPa.s, i.e., a much broader range.

In Figures 1, 2 and 3, it is clear the viscosity convergence of all samples to relatively low values (~ 100 mPa.s) as the shear rate is increased to ~ 1,000 s^{-1}. This fact is likely to be connected to the structural breakage of the melted ice cream proteins network due to shear imposed, as emphasized by Rossa *et al*. (2012).

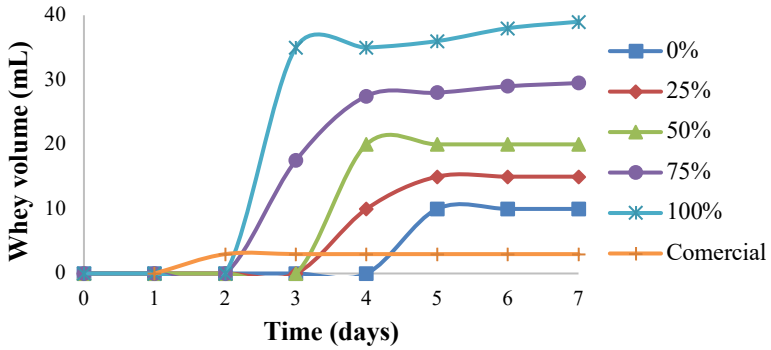
Considering the high values for the linear correlation coefficient (R^2) presented in Table 3, it is possible to attest that power-law model provided good adjustment parameters, since 72% of the samples exhibited $R^2 > 0.90$. Thus, the rheological behavior of the samples can be satisfactorily described by this model.

3.3. Stability analysis

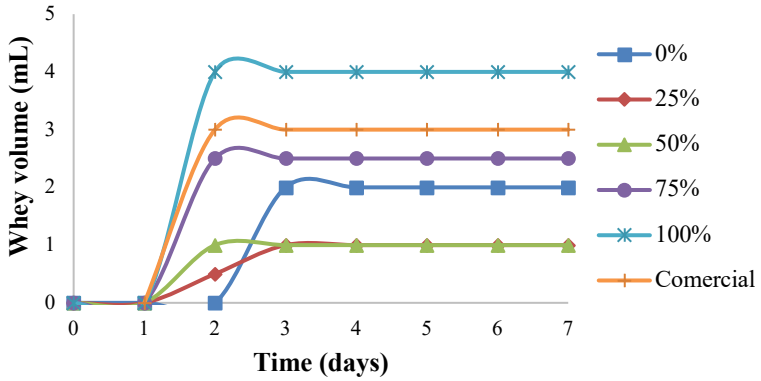
Besides a palatable taste, it is expected from a good ice cream to have moderate melt resistance in the form of uniform homogeneous liquid and the appearance of the original blend (without phase separation), exhibiting a natural color and particles regularly distributed as long as a certain consistency is also maintained when the ice crystals melt. The heterogeneity can be identified by the presence of clots, slag, large, varied size air bubbles, and/or phase separation (Bodyfelt et al., 1988, Marshall, 2003).

Due to the fact that emulsions are thermodynamically unstable, they are susceptible to coalescing interactions immediately after production or during storage. For this reason, this effect was investigated in the present study by measuring the total whey content (Table 2) at different time intervals, from one to seven days after preparation (Figures 4, 5 and 6).

Generally, the samples with ricotta whey desorbed more than the samples with cheese whey and butter whey, respectively.

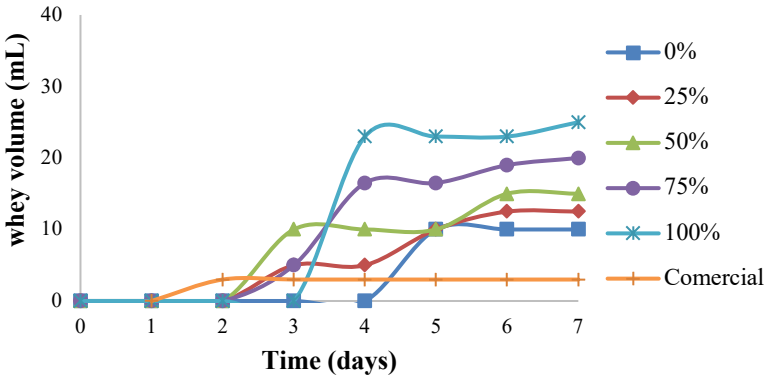


(a)

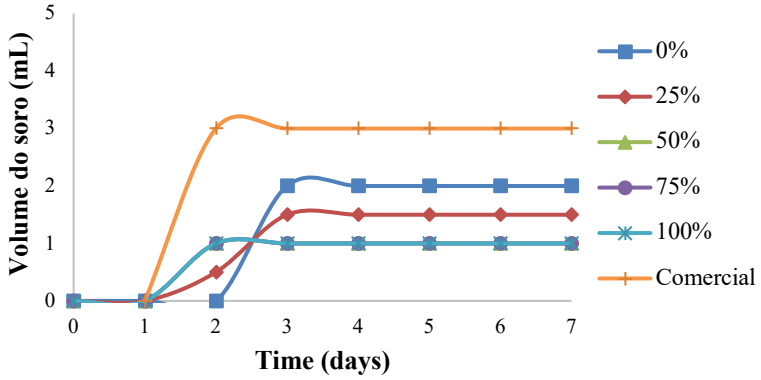


(b)

Figure 4. Effect of ricotta whey concentration in the desorption of cream (a) and chocolate (b) flavor ice cream.

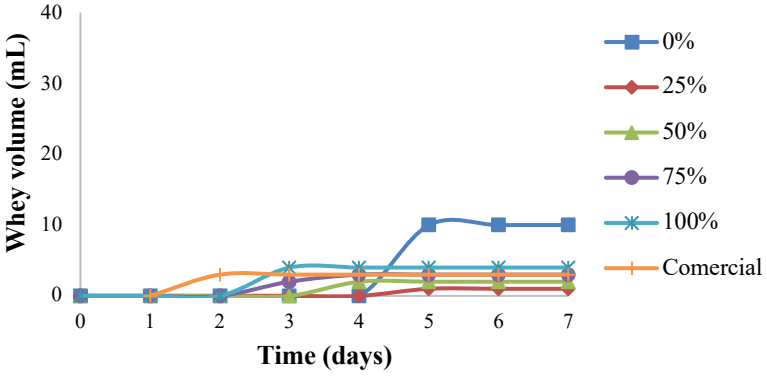


(a)

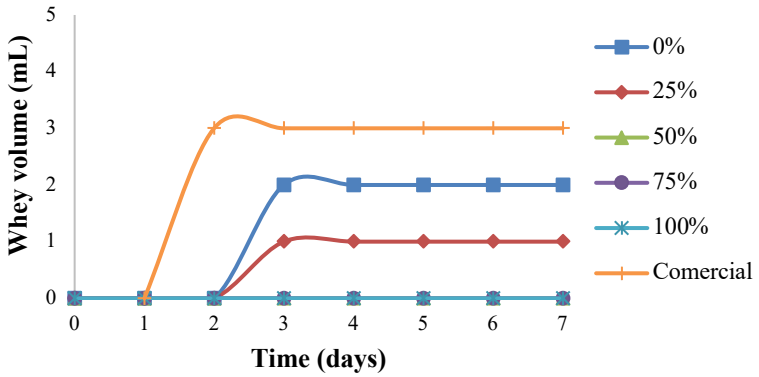


(b)

Figure 5. Effect of cheese whey concentration in the desorption of cream (a) and chocolate (b) flavor ice cream.



(a)



(b)

Figure 6. Effect of butter whey concentration in the desorption of cream (a) and chocolate (b) flavor ice cream.

Taking into consideration the chocolate flavored ice creams, only the 100% ricotta whey sample desorbed more than the 0% commercial samples. Besides the type and amount of flavorings, the greater presence of liquid ingredients in the cream flavored ice cream formulations (66.86% versus 55.14% in the chocolate flavor - Table 1) promoted a different behavior, which was the large whey formation right in the early days for most of these ice creams. Samples with butter whey had the lowest D and only the samples with 25% ricotta whey, and 25 and 50% cheese whey behaved similarly to the 0% commercial samples.

Thus, according to the results above, chocolate ice creams show a remarkably superior macroscopic stability than the cream flavor ice cream, regardless of the type of whey added.

3.4. Determination of Calcium (Ca) and Magnesium (Mg)

According to Chen et al. (2011), there are three factors that regulate the stability of an emulsion: particle size, particle flocculation and particle distribution between the different phases. In this context, calcium and magnesium ions can destabilize an emulsion, causing precipitation and compromising foaming (Ramkumar et al., 2000).

Figure 7 shows the Ca + Mg concentration in the main liquid and solid ingredients used in the formulations. Among the liquid ingredients, milk had the highest concentration of Ca and Mg, followed by butter whey, cheese whey and ricotta whey, respectively. In contrast, presenting an unexpected behavior, the samples developed with butter whey desorbed less, showed smaller particle sizes and were more stable (Table 2) compared to the samples with cheese whey and ricotta whey. In addition, even the milk-based sample with higher zeta potential and smaller particle size, presented low DI among the samples. This demonstrates the great complexity of the characterization of these materials in terms of their stability, because the parameters are often conflicting between different samples (either favoring or disfavoring the stability).

Regarding the solid ingredients, powdered cocoa was 14.28 times higher in Ca + Mg content when compared to the cream flavoring powder, however, it did not negatively influence the stability of the ice creams since they were more stable (higher zeta potential, lower particle size, and lower desorption). This fact may be associated with the small percentage of these flavorings in the ice cream formulations (2.91% cream flavor versus 11.63% cocoa powder - Table 1) when compared to the liquid ingredients (66.86% for cream flavor versus 55.14% for the chocolate flavor - Table 1).

Other authors found similar results with that of the present study for Ca + Mg in cow's milk: Visentin et al. (2018) (150.52 mg / 100 mL), Franzoi et al. (2018) (123.35 mg / 100 mL), Martino et al. (2011) (102.00 mg / 100 mL), and Yildiz et al. (2005) (116.05 mg / 100 mL). Taking into consideration the different influences on food composition, Jensen et al. (2012) when analyzing the composition of cow's milk from different breeds, showed that Ca + Mg content varied between Jersey (158.59 mg / 100 mL) and Holstein-Friesian breed (122.55 mg / 100 mL). Haug et al. (2007) pointed out that the milk composition of cows varies in different regions of Norway: north (137.00 mg / 100 ml), south (133.90 mg / 100 ml), east (131.90 mg / 100 ml), and west 132.70 mg / 100 mL).

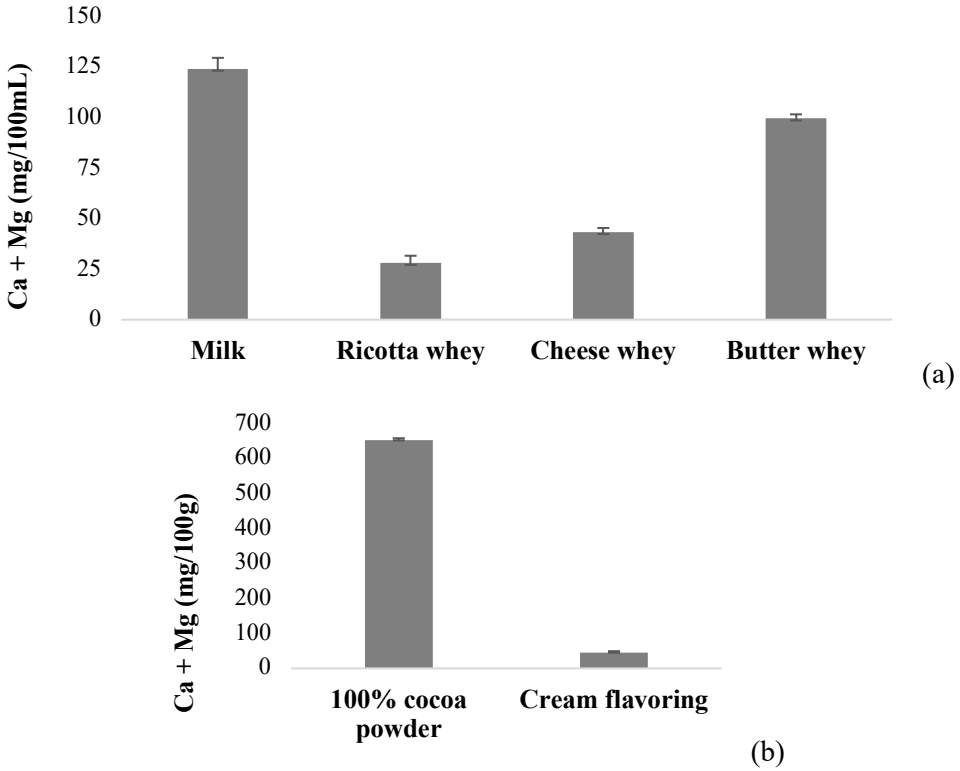


Figure 7. Content of Calcium and Magnesium in the main liquid (a) and solid (b) ingredients of ice cream. Data are presented as mean values ± standard deviation.

The content of Ca + Mg for cheese whey of the present research was lower, but similar to the one found by Martino et al. (54.80 mg / 100 mL), despite Franzoi et al. (2018) founding a lower content (28.87 mg / 100 mL). It should be noted that this variation occurs naturally and is mainly associated with the type of cheese from which the whey originates (Kandarakis, 1986).

4. Conclusions

The results showed that the high contents of ricotta whey (100% cream flavor), cheese whey (50% cream flavor), and butter whey (100% cream and chocolate flavor) had zeta potential values between +30 and -30mV, which compromises the stability of ice creams.

Regarding the rheological behavior, all samples showed predominant pseudoplastic character, with prevalence of the samples of chocolate over those of cream flavor. However, under high shear rates (~ 1000 s^{-1}), the apparent viscosity values converge to a Newtonian plateau of approximately 100 mPa.s

In the chocolate ice creams, practically no phase separation was observed, except in those developed with 75 and 100% of ricotta whey, that is, significant amounts of whey. In contrast, cream flavor samples, as indicated by their zeta

potential values, were more unstable and the phase separation occurred more quickly and intensely, evidencing the influence of the type of ice cream flavor on its stability, particle size, rheology, and desorption.

Ca + Mg content of the main ingredients used was not a strong and decisive factor in the destabilization of the samples. Although the wheys had lower concentrations of these minerals in comparison to milk, as well as the flavoring of cream in relation to the cocoa powder, they did not prevent or delay the phases separation of the ice cream for up to a week.

Thus, ricotta whey (up to 25%), cheese whey (up to 50%) and butter whey (up to 75%) were promising candidates for the production of ice cream. In addition, taking into consideration the difficulty of analyzing the stability and performance factors evaluated here, the present study indicates the importance thereof and suggests that other quality parameters should be also analyzed in order to clarify the influence of these wheys on the characteristics of ice cream.

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DIFFERENCES OF THE PHYSICOCHEMICAL INDICATORS OF BEVERAGES WILD ELDERFLOWER (*SAMBUCUS NIGRA*) FROM TELEAJEN VALLEY, ROMANIA, ACCORDING TO THE USED TECHNOLOGY

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ABSTRACT

Elderflowers beverages have a hydrating effect and a chemical composition that influences the sensory characteristics and the values of the main quality indicators. This paper focused on evaluating the modifications of 15 physicochemical quality indicators for 4 samples, elderflower beverages, which differ from one another by the applied technology, the parameters of operations and processes and the recipe. Fresh elderflowers, water, sugar and lemon were used for preparing the samples which is the classic technology of making "*socata*" and an original combination of obtaining a new product, namely "*the elderflower compote*". The analysis methods used were quantitative methods for determining the reducing sugar, the volatile components which give the volatile acidity, the fixed acidity, the physical methods based on different principles and modern equipment. Changes in all the quality indicators were reported as follows: the production yield of the liquid fraction 90-98 w/w %, density 1.0343-1.0590 g/cm^3, the kinematic viscosity 1.30087-1.8390 m^{2}s^{-1}, content values of the total soluble solids (TSS) 9.5-15.4 ^{o}Brix, with a correlation coefficient below 50 %, depending on the used method. Total sugar/acid ratio is 47.74-140.85 and total sugar 10.2-14.96 %. The new product, the compote, has values of the indicators that fall within the range of the elderflower beverages. Thus, it is characterized by the highest value of the production yield 98 w/w %, of the TS/TA ratio (140.85), of the density 1.0590 g/cm^3, of the kinematic viscosity 1.83902 m^{2}s^{-1}, of the TSS = 15.4 ^{o}Brix, of the TS 14.96 % and the lowest of the SDR- 4.5 % (direct reducing sugars).

1. Introduction

The wild elder shrubs (*Sambucus nigra*) are distinguished by their white and fragrant inflorescences occurring in May-June. The elderflowers are therefore a raw material available to both food and pharmaceutical manufacturers. Tea, syrup, tonic, ice cream, yoghurt and other beverages can be prepared as food (Vlachojannis *et al*., 2015; Mikulic Petkovsek *et al*., 2016; Olejnik *et al*., 2016).

There is a beverage made of elderflowers which is called "*cordial"*. It seems to originate in the Roman era, with powerful versions of the Victorian era, and now it is predominant in Central Europe, especially in England, Germany, Austria, Romania, Hungary and Slovakia. It is a syrup, flavored with fresh elderflowers and lemon as a source of citric acid. It is served diluted with tap water or carbonated water.

Spring flowers from the end of May until the beginning of June or later are used to this purpose. The hermaphrodite flowers have a diameter of 10-25 cm, the individual ones are white with five petals, 5-6 mm in diameter and are pollinated by insects. They are fragrant and somewhat succulent flowers with a particular aroma and flavor **(**Morton, 2004; Day, 2010**)**.

This juice, elderflower cordial, can be made relatively easily at home, but it is also a marketed product. There are currently soft drinks that use a combination of flavors that imitate the natural one. This drink, thus obtained, is cheaper and thus money and labor are saved (Morton, 2004). Elderflower beverages are prepared and consumed (Morton, 2004) as a refreshing and stimulating product designed for a medical purpose for various diseases (cardiac, diabetes).

There are technological studies on natural juices obtained from different raw materials such as fruits and vegetables, matured and consumed fresh or preserved. The reason why juices are recommended is that for medical reasons certain people cannot consume the vegetables as such (Mihalev *et a*l, 2018). In general, fruits are used for juices, and in human nourishment, flowers are used as spices. In the practice of their processing, flowers are used to make comfiture (roses, for example, or lilac) for their special sensory but also therapeutic characteristics (Walkowiak-Tomczak *et al.*, 2017).

Wild elderflowers *Sambucus nigra* belonging to the *Adoxaceae* family, *Sambucus* genus, are a morphological part of the shrub. They are extremely popular among consumers in Romania and throughout the world. In Romania, they are usually used fresh, and the manufactured beverages are based on other raw materials: water, sugar, lemon and high fermentation yeast belonging to the species *Saccharomyces cerevisiae*. According to FOASTAT, the production of wild elderflowers used in the beverage production is not specified (FOASTAT, 2017) but is a sustainable and, more importantly a free resource. These morphological parts are seasonal in nature as well as the juice production. In the way in which the technology is applied for the "*socata*", the nutrient principles and the volatile components are little affected by the operational parameters and the quality indicators have values that need to be determined.

The main objective of this study is to investigate by means of a comparative analysis the values of the quality indicators for a new product made of elderflowers, namely the fresh elderflower compote. As secondary objectives the following will be investigated: the production yield, the comparative analysis of the values of the physicochemical quality indicators (total acidity, volatility, relative density, pH, viscosity, reducing sugar, soluble dry matter, real dry matter, total sugar/total acidity ratio), the determination of the correlation coefficient for the soluble dry substance content values determined using apparatus operating according to different principles and the correlation between these values.

2. Materials and methods

2.1. Materials

2.1.1 Raw materials and technologies

The technology and the recipe were discussed in a prior study (Iancu, 2018). The studied samples are similar but have been coded differently because the analyses intended for this study differ from the sensory ones. The coding of the samples is different and is based on the differences in the applied technology. Thus, there were prepared: elderflower juice fermented with yeast (EjF); elderflower juice fermented with yeast preserved by pasteurization (EjFP), elderflower juice unfermented with yeast preserved by pasteurization (EjnFP), elderflowers compote with sugar (EC).

2.1.2 Determination of production yield

The Yield of juice For 100 g of the liquid fraction was calculated based on the formula:

$$JY = \frac{w_j}{w_{rw}} \cdot 100, [\%]$$

In which: JY - the yield in juice, w_{rw} - the amount of raw material [g] (solid part and liquid part), wj- the amount of juice obtained from this raw material (Kaack, 1997).

2.1.3. Physico-chemical characteriyzation of juice and compote

Appropriate choice of analysis package was done in order to highlight the quality indicators that change the most. The following analyzes were performed for the liquid fraction of the studied beverages: TA-titratable acidity (g/100g, expressed as citric acid)(AOAC 942.15, 2000); VA-acidity volatile (g/100g, expressed as degree of acidity)(AOAC 925.34, 2000)(steam distillation apparatus *Alcotest Raypa*, Spain); TSS-soluble solid content (^{o}Brix)(IS 13815:1993) (refractometer *Krüss*, Germany connected to a bath room ultrathermostated *Brookfield*, with the outer circulation); SDR-reducing directly sugar and total sugar (TS) content (%) (AOAC, Method 925.35, 2000); nSDR anreducing directly sugar (TS-SDR= nSDR)(%); the kinematic viscosity (m^2s^{-1}) (Ubbelhode 3 branches viscometer no.1) (Will *et al*., 2008); relative density (AOAC-988.06) (DMA35, Spain); pH (pH-meter Orion 2-STAR, England).

2.1.4. Statistical analysis of data

The results are presented as the mean ± standard deviation of 4 replicas. So, for the physico-chemical indicators for elderflower drinks, was been statistically analyzed. The calculation of the average value of the variables, the dispersion of the values and the correlation indices were done using the ANOVA statistical technique. Differences were considered significant at $P < 0,05$.

3.Results and discussions

3.1. Production yield

The elderflowers were picked up from May to June 2017 and they were used fresh. The harvest was made in a single geographical area (Teleajen Valley, Romania). Beverages made from water and inflorescences have been prepared that differ from one another by the applied technology. The yield in the extract ranges between 90 and 98 (w/w %) as shown in Table 1.

In other studies, the yield values in the aqueous sugar extract were reported to be between 69.6 and 82.7(w/w%). These values were influenced by the genotype and the geographic area where the elder grew, and the technology used was similar to that in this study. In wild elder samples, the yield value was reported at 69.8-80.8 (w/w %) (Kaack *et al*., 2006, 2008). Therefore, in the current study, the yield values in the juice are higher and are influenced by: the applied technology, the duration and the operational parameters of the fermentation process, the fermentation conditions, the fermentation degree, the recipe, the production losses.

3.2. Comparative analysis of the values of the physical-chemical quality indicators

The density of the analyzed sample, the relative density and the kinematic viscosity were investigated for the wild elder sweet beverages and listed in Table 1. These indicators were used to assess the quality of the fermented at 25^{o}C or unfermented vegetable juices. The following were used for the preparation of the juices: water, sugar, fresh inflorescences, lemon with rind and high fermentation yeast, the *Saccharomyces cerevisiae* species. All these influence the value of the sample density. It varies between 1.0343 and 1.0590 g/cm^3 with the highest value of 1.0590 g/cm^3 for the newly obtained product, the elderflower compote (EC). According to the applied technology, there aren't any fermentation, manipulation or thermal treatment losses for this type of product

preserved by abiosis. Density is the expression of the dry substance content in the unit of volume.

Consistent with the density, the fluid viscosity is their ability to resist flow. This is due to the mechanical interaction between the constituent particles. Thus, the more frequent these interactions, the higher the viscosity. In the studied samples, the kinematic viscosity value ranges between 1.30087 and 1.83902 m^2s^{-1}, with the highest value for the newly obtained product, the compote.

Table 1. Technological and physical characteristics of beverages from fresh elderflowers

Code sample	Yield of juice [w/w %]	Sample Density [g/cm^3]	Water Density at 20°C, [g/cm^3]	Relative Density	Kinematic viscosity at 20°C [m^2s^{-1}]
EjF[1]	90 ± 0.40	1.0461 ± 0.001	0.9987	1.0475 ± 0.0019	1.1451 ± 0.003
EjFP[2]	90 ± 0.48	1.0395 ± 0.006	0.9987	1.0408 ± 0.0012	1.3635 ± 0.02
EjnFP[3]	92 ± 0.75	1.0343 ± 0.001	0.9987	1.0356 ± 0.0013	1.30087 ± 0.004
EC[4]	98 ± 1.31	1.0590 ± 0.006	0.9987	1.06037 ±0.0030	1.83902 ± 0.005

The level of significance is $P < 0,05$ (n = 4). At the 0,05 level, the population mens are significantly different [1]elderflower juice fermented with yeast; [2]elderflower juice fermented with yeast preserved by pasteurization; [3]elderflower juice unfermented with yeast preserved by pasteurization; [4]c elderflower compote with sugar.

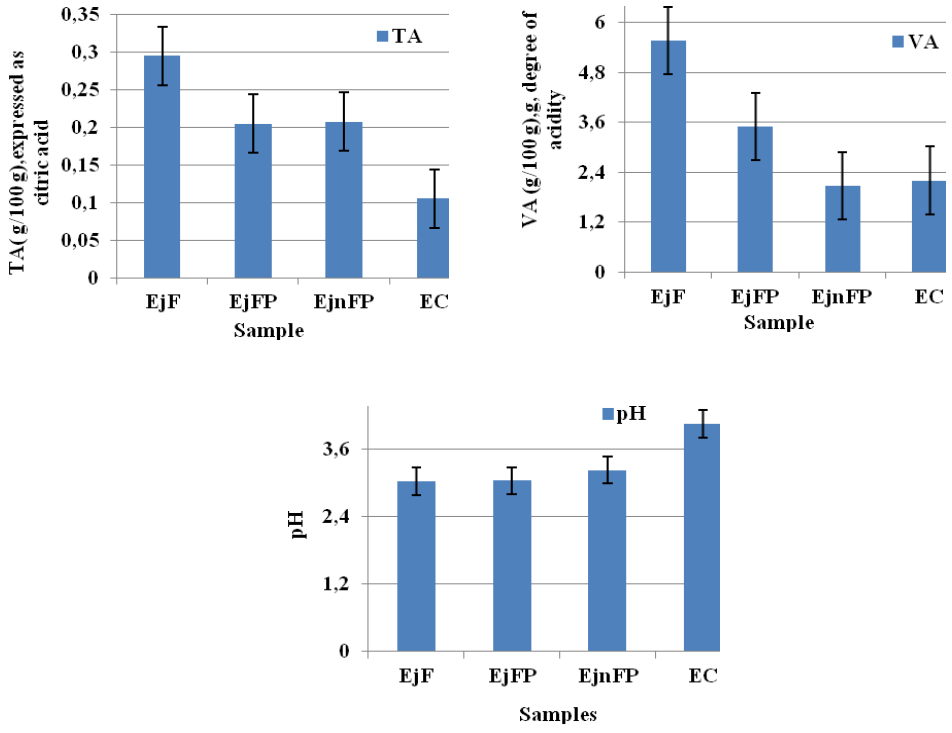

Figure 1. Total acidity (TA), volatile acidity (VA) and pH in the elderflowers juice and in the compote. The vertical bars correspond to the standard deviation (n = 4)

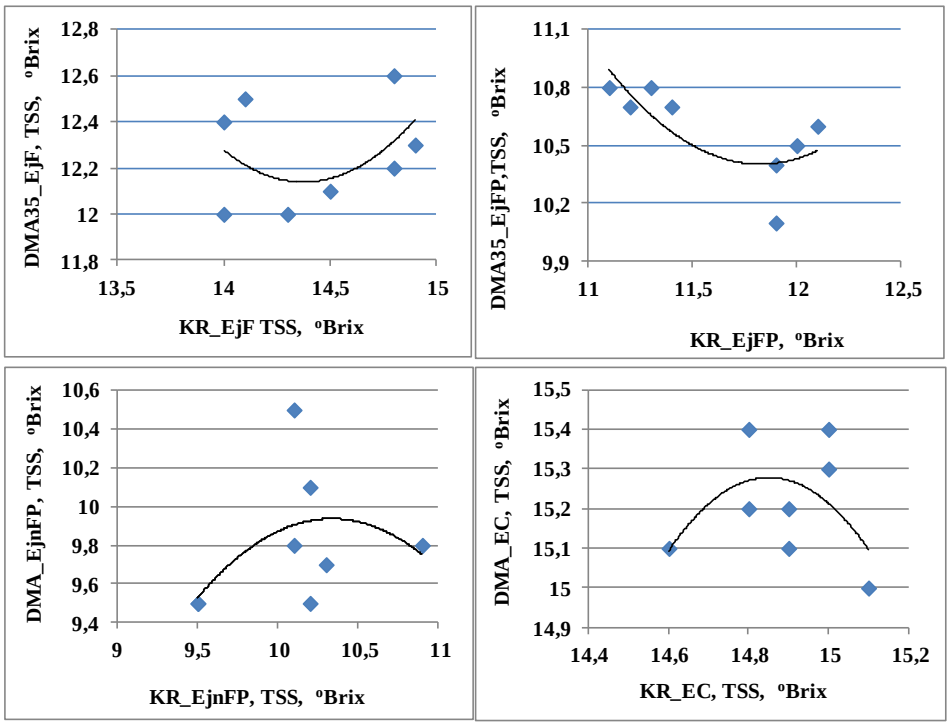

Figure 2. Soluble dry substance content (TSS) of the correlation values with the principle of the method used

Table 2. Variation of reducing sugar content determined chemically in elderflowes juice and total sugar/total acidity ratio

Sample	Variation in reducing sugars by the nature of the sample							
	[a]SDR(%)				[b]nSDR(%)			
	Mean	SE of Mean	α*	Sig.**	Mean	SE of mean	α*	Sig.**
EjF	10,525	0,18697	0,05	1	1,08	0,031	0,05	0-1
EjFP	9,625	0,04349			2,9475	0,0228		
EjnFP	8,0425	0,01436			2,7925	0,1525		
EC	4,565	0,04992			10,242	0,0476		
	Variation in total sugars and TS/TA ratio							
	[c]TS(%)				[d]TS/TA			
	Mean	SE of Mean	α*	Sig.**	Mean	SE of Mean	α*	Sig.**
EjF	11,62	0,19074	0,05	1	47,74	5,1898	0,05	0-1
EjFP	12,2725	0,16152			60,245	1,086		
EjnFP	10,2	0,09129			49,6225	0,847		
EC	14,96	0,02041			140,85	1,756		

* level of significance; ** the mean of mean differences; [a]-direct reducing sugar; [b]sugar which is not directly reducing; [c]-total sugar ; [d]-total sugar ratio / acids giving total acidity

One can observe the conformity between the values of the density, the relative density and the viscosity. That is, if one of them increases, the others increase as well because they are physical expressions of the chemical composition. The factors that influence these values are: the applied technology, the recipe used, the molecular structure of the constituent substances, the temperature at which the analysis was conducted.

The results of the modification of the chemical composition of the juices, expressed as TA (g/100g, expressed as citric acid), VA (g/100g, expressed in degrees of acidity) and pH are shown in the graphs in Figure 1. These quality indicators are an expression of volatile components and those with acidic chemical groups.

There are 58 volatile components identified in the extract of flowering flowers (alcohol, aldehydes, ketones, terpenes, esters), acids such as citric acid, ascorbic acid (Kaack *et al*., 2006; Pabi *et al*., 2014; Petruț *et al*., 2017), malic, tartaric, fumaric, shikimic in the total amount of 44.15 g/kg DW with the highest proportion of malic acid (30.19 g/kg DW) (Mikulic-Petkovsek *et al*., 2016), as well as those resulting from the fermentations that take place in the extract. In this study, the TA values are between 0.11 and 0.3 g/100g, expressed as citric acid, with the highest value for the EjF sample, which is fermented, and with the lowest value for the EC sample, the compote, which is only pasteurized. The same aspect applies to volatile acidity which ranges between 2 and 5.5 g/100g, expressed in degrees of acidity. The pH, another expression of the acidic components, has the same trend (inversely proportional, like value, to total acidity). It is noted that in the fermented samples the acidity is higher by about 43-64% compared to the unfermented ones. This aspect greatly influences the perception of the intensity of the sensory components: taste, aroma, flavor (Iancu, 2018). As influencing factors, from the variables of the procedures, it can be said that the production recipe, the fermentation degree, the thermal treatment and, according to other studies (Mikulic-Petkovsek *et al*., 2016) the genotype of the vegetal material bear a great influence.

The total soluble solid (TSS) content of the studied extracts and of the new product, the compote, are shown in Figure 2. It is a quality indicator which is present in all the analysis packages for the raw materials and the finished products from the vegetable canning industry (fruit and vegetable juices, sugar preserved vegetables and others) (Kaack *et al,* 2008; Marjan and Johari, 2010). That is why there are many items of equipment used for determination and which operate according to different physical principles. That is why the values of the TSS are not completely equal. Two devices (DMA 35 - portable electronic densimeter and Abbé refractometer) and two known physical principles were used for this study (the "*U*" -tube principle, respectively the principle of total light refraction). The variation of values is done by polynomial regression equations, and the variation coefficients range between 0 and 1.

At the sample EjF the variation is made by an equation of the form: $y_{EjF} = 0.9775x^2-28.105x+214.6$; $R^2_{EjF}=0.1528$, with a correlation of 15%. TSS values (refractometric method) are between (14-14.9) ^oBrix.

At the sample EjFP the variation is made by an equation of the form:

$y_{EjFP}=0.924x^2-21,871x+139.73$; $R^2_{EjFP}=$ 0.5821, with a correlation of 58%. TSS values (refractometric method) are between (11.1-12) ^oBrix.

At the sample EjnFP the variation is made by an equation of the form: $y_{EjnFP}=0.585x^2+12.095x–52.573$,$R^2_{EjnFP}=$ 0.259, with a correlation of 25%. TSS values (refractometric method) are between (9.5-10.3) ^oBrix.

At the sample EC the variation is made by an equation of the form:

$y_{EC} = - 2.916 x^2+86,632x–628,02$, $R^2_{EC}=0.278$, with a correlation of 27%. TSS

values (refractometric method) are between (14.6-15.1) ^{o}Brix.

As can be seen from the results, the correlations between Ox:(KR) and Oy:(DMA35) are described by polynomial equations, the points being spread throughout the surface of the graph (Figure 2). The values of the correlation coefficient are very low, below 50%, except for the EjFP sample. Therefore, it is recommended to refer to the standardized method, i.e. to the refractometric method.

The TSS content is given by the chemical composition of the extracts. Scientific literature reports that these *Sambucus nigra* concentrate elderflower extracts contain: sucrose

27.35 g/kg DW, glucose 19.71 g/kg DW, fructose 24.28 g/kg DW and a total sugar content of 71.34 g/Kg DW and an acid content of 44.15 g/kg DW (Mikulic-Petkovsek *et al*., 2016). Along with polyphenols which were found in the amount of 40137 mg GAE/kg (Mikulic-Petkovsek *et al*., 2016) the substances mentioned above forms TSS.

In the samples of this study, apart from these substances, the added sucrose and the components migrated from lemon in the liquid phase and from the autolysis of the yeast cells contribute to the value of the TSS content. Thus a value of 14.6 ^{o}Brix, for example, for the new product (the compote) (EC), is given by the added sugar, the sugars from the flowers, the acids in the elderflowers and the lemon slices. The TSS for the fresh or preserved vegetables and fruit juices ranges between 5^{o}Brix (for examples for the fresh juice of tomatoes) and 68 ^{o}Brix in syrup. Thus the values for the elderflower beverage are consistent with those for the beverages typical of the canned industry

The content of reducing sugars in the vegetables juice is an important quality indicator. It contributes to the TSS value and the TS/TA ratio with great importance on the sensory characteristics of the juices (taste balance). For the studied samples, the average value for the sugar content is shown in Table 2. By using the method recommended by ANOVA-One-Way (Fisher's LSD method), the confidence intervals created for all pairs differences between the level factors (very many values) are thus explained. The error rate was calculated and presented. According to the obtained results of the values for SDR and TS, at least one of the manufacturing means used to obtain the liquid fraction of the beverage varies significantly. For example, controlling the fermentation process is a key point in obtaining such different values.

At the Fischer test means comparison using equal 1 indicate that the difference of the mean is significant at the 0,05 level.

This method of statistical analysis was used because the variation of the results was great, which proves the influence of several factors such as: the variation of the

operational parameters of the technological schemes, the errors given by the analytical determinations. The only constant element was the variety of elderflowers and their condition (dried or fresh).

In conclusion, the highest direct reducing sugars (SDR) quantity is for the EjF sample (fresh fermented elderflower juice) with an average of 10.525 g/100g and the lowest of 4.565 g/100g in the EC sample (the elderflowers compote). These direct reducing sugars are given by the simple sugars: glucose and fructose that come from the elderflowers, from the sugars derived from the enzymatic hydrolysis of sucrose. If the fermentation of the mixture does not take place, the SDR is very law and is given only by the monosaccharides from the elderflowers.

In the fermented samples the TS content is much lower than the amount of sugar initially added to the sample (15%, on average, according to the recipe) as compared to that of the newly created product, the EC.

The TS/TA ratio, for example, is a quality indicator in choosing the fruit used in juice making. For balanced taste characteristics, it is recommended that this ratio should be around 15. The TS/TA ratio for the elderflower sucrose-free concentrated aqueous extract is

1.62 (Mikulic-Petkovsek *et al.,* 2016). Therefore the rest is given by the addition of sugar in the samples of this study. The values obtained for this ratio are 104.85 for the new product, the elderflower compote, the highest value, and 47.74 for the fermented and freshly consumed (EjF) variant. The higher this amount, the greater the quantity of sugars; meaning that the sweetness of the taste predominates. It is therefore noted that for the products presented here the recipe needs to be improved in terms of increasing the value of the acidic component of the taste. In a previous study it was shown that the EjF sample with the lowest value of the TS/TA ratio also obtained the highest score of the panelists in the sensory analysis (Iancu, 2018).

4. Conclusions

The correlated conclusions of the whole study on the fermented or not elderflower beverages are the following: the production yield is very high, with the highest value for the newly obtained product, the elderflowers compote, the values of the determined quality indicators are influenced by: the applied technology, the degree of fermentation (characterized by TSS and acidity), the duration of the fermentation, the nature of the used microorganisms (the use of the yeast species *Saccharomyces cerevisiae*), the method of preserving the juices, the shelf life, the chemical composition of the raw materials.

The closer to the neutral the pH value, the higher the TS/TA ratio. The value of the TS content in the studied samples, elderflower beverages, is close to the TSS value, demonstrating that the non-sugar in the liquid fraction is in a very small quantity, almost negligible, except for the EjF sample. In this sample, the difference in value, according to the data presented in the screenings, is due to the alcoholic fermentation, the lactic fermentation and other secondary fermentations and the lack of thermal treatment. The alcohol, the acids formed in fermentation may influence the TSS value, and therefore it is recommended to determine this value in the distilled liquid fraction rather than by direct reading.

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EXPERIMENTAL STUDY OF PRODUCTION AND CHARACTERIZATION OF DATE FRUIT POWDERS AND SYRUP

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ABSTRACT

In the present work, date pulp and pomace powders were produced under freeze drying conditions and syrup was extracted from Garn Ghzel date variety. Date products were characterized in terms of physicochemical properties (moisture, water activity, ash, soluble solids content, titrable acidity, pH and color) and functional properties (water holding capacity, wettability index, dispersibility and density). Freeze drying kinetic of date pulp and pomace was modeled using five empirical models (Newton, Page, Henderson and Papis, Logarithmic and Wang and Singh). Results showed that there is a slight difference between powders properties. Page and Logarithmic models best fitted the freeze drying kinetic of date pulp and pomace with the highest determination coefficient, R^2 (0.9635) and R^2 (0.9987) and the lowest chi-square χ^2 (0.000021) and χ^2 (0.000052) values respectively. Fick's law was used to determine the effective moisture diffusivity. Its values were 9.74×10^{-11} and 5.15×10^{-11} m^2/s for date pulp and pomace respectively. These results contribute added value to date technology.

1.Introduction

Date palm *(Phoenix dactylifera L.)* is extensively cultivated for its edible fruit belonging to the *Palmae (Arecaceae)* family (Yahaya *et al.*, 2015). The fruit of date palm is used due to its remarkable nutritional, health and economic value, in addition to its aesthetic and environmental benefits. Date composition is rich in carbohydrates, minerals, dietary fiber, vitamins, fatty acids, amino acids and protein diet (Al-Shahib and Marshall, 2003).

Algeria is one of the largest date producers in the world with a wide production diversity. Garn Ghzel variety is not widely consumed despite its high total and reducing sugar contents (Mrabet *et al.*, 2008). Date processing industries produce various date products such as paste, syrup, jam and vinegar. Date syrup is probably the most common derived date product (Ganbi, 2012). Production of date powder could improve handling, storage and blending ability of several products such as baked foods (Manickavasagan *et al.*, 2015).

In recent years, drying operations have made possible the production of various added value food products (Omolola *et al.*, 2015). Freeze drying is a drying method used to manufacture pharmaceutical and food thermolabile products (Nireesha *et al.*, 2013). It is considered a low processing drying method.

To our knowledge, no work has been reported on the production of powders from date pomace as well as the processing of Garn Ghzel variety.

Hence, the objective of this work is to produce pulp, pomace powders and syrup from Garn Ghzel date variety, to determine their

physicochemical and functional properties and to model their freeze drying kinetic.

2. Materials and methods

2.1. Vegetable materials

Date fruit (Garn Ghzel variety) was harvested at full maturity (Tamr) and stored at 4°C in a refrigerator, (SUMSUNG, South Korea). It was purchased from the region of Sidi Okba (Biskra, Algeria). The fruit was washed using water and divided into 2 parts; the first part used to characterize the pulp, the second part to produce date syrup and pomace.

Date syrup was prepared according to Al-farsi (2003) method with a slight modification: A chopper was used to cut dates into pieces of 1 cm, then dates were mixed with an equal amount of distilled water. The mixture was stirred for 20 min at 60°C in a water bath, (DK-420, China). Finally, the juice was extracted through a muslin cloth. Filtration was performed in two steps; coarse (50 µ filter paper) and fine (3 µ filter paper).

Concentration was performed on a hot plate, (IKA-COMBIMAG RCT, Germany) (90°C) (Daas Amiour *et al.*, 2014), until syrup reached 35°Brix.

Date pulp and pomace were freeze dried in a laboratory freeze dryer model (LD 2-8 CHRIST BETA PLUS, Germany) for 24 hours at a pressure and temperature of 0.12 mbar and -40°C respectively. The thickness of date pulp and pomace was measured using digital caliper, LCD screen 0.01mm-150mm (Electronic Vernier, UK) (Goula Athanasia and Adamopoulos Konstantinos, 2004). Samples were ground and sieved in a sieve of 1mm diameter (Figure 1).

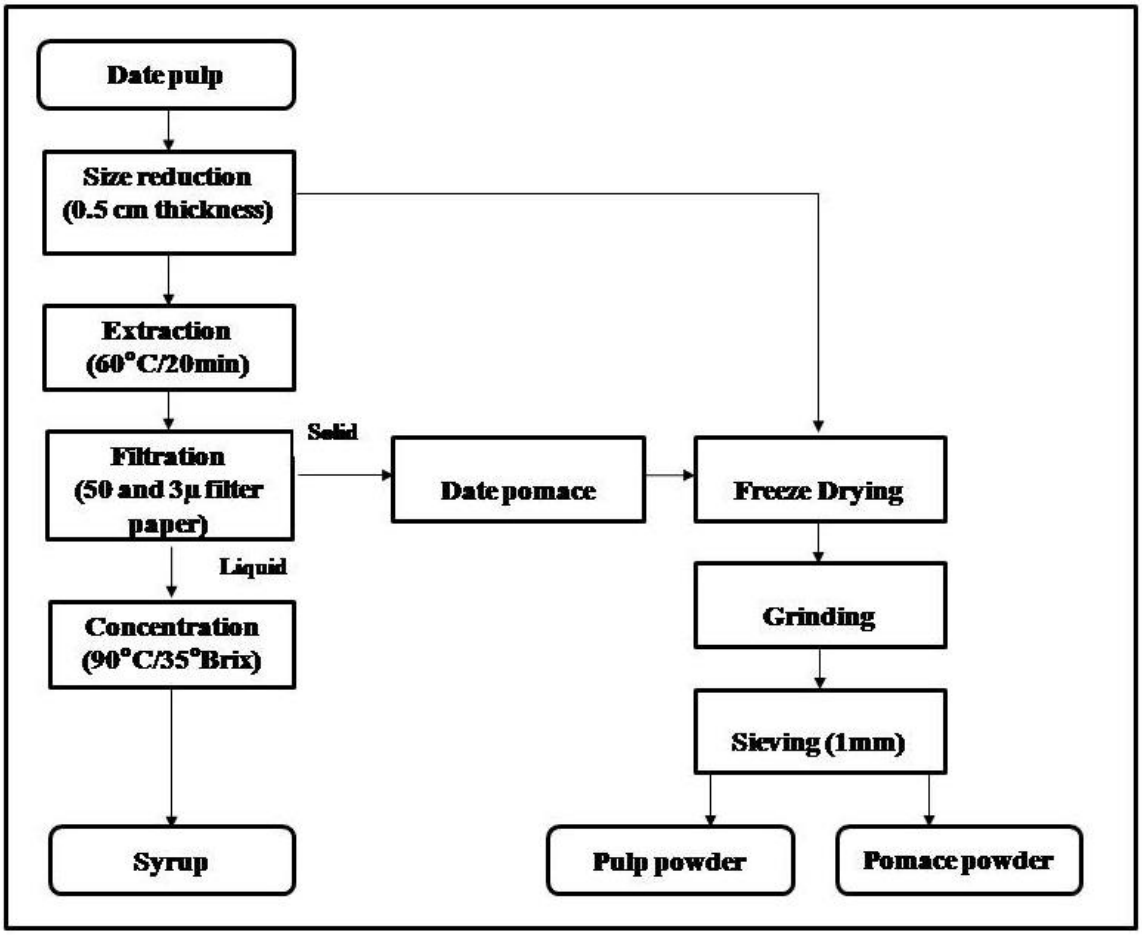

Figure 1. Simplified flowchart of date powders and syrup processing

2.2. Physicochemical and functional analysis of powders and syrup

2.2.1. Physicochemical analysis

For powders, moisture content was determined by drying at 70°C to obtain constant weight in a moisture analyzer, (RADWAG MA 110.R.NS, Poland) and moisture loss expressed as a percentage of (100 kg water / kg wet material) (Goula Athanasia and Adamopoulos Konstantinos, 2004). For syrup, moisture content was obtained by drying at 105°C in a forced convection laboratory oven, (ESCO isotherm, USA) until constant weight (Gurak *et al.*, 2014).

Water activity of date pulp and pomace powders was measured using water activity meter model (rotronic HYGROSCOP BT-

RS1, Gemini BV), set at 24.70 ± 1°C (Caparino *et al.*, 2012). For date syrup, water activity was measured according to Fennir *et al.* (2003) with a slight modification, where samples have the same concentration and prepared without dilution.

For date powders and syrup, ash was determined by combustion of the sample in a muffle furnace, (Nabertherm 30-3000°C, Germany) at 550°C for 8 h (Abbès *et al.*, 2011).

Soluble solids content of date powders and syrup was determined using a digital refractometer, (ATAGO, HSR500, Japan) (Kulkarni *et al.*, 2008).

Titrable acidity was determined by titrating a known quantity of sample with 0.1 N NaOH and expressed as citric acid (Kulkarni *et al.*, 2008).

pH was determined according to standard ISO 10390 (1994); the method consists of preparing a suspension component in five times its volume of distilled water, leave for 5 minutes then rest for at least two hours but no more than 24 hours. pH is measured using a pH meter, (HACH, France) (Kulkarni *et al.*, 2008).

Color: Powders and date syrup were poured into Petri dishes, formed a layer of 10 mm thick and covered with a transparent film. The colorimeter, (KONICA MINOLTA SENSING, INC CR-10, Japan) was calibrated with a standard white ceramic plate before playback (L = 95.97, a= − 0.13, b = − 0.30). L, a, b parameters were measured for all samples. The average values L, a and b were obtained from six readings for each sample (Abonyi *et al.*, 2002).

2.2.2. Functional analysis

Density of syrup was calculated using the following equation:

$$\text{Density} = \frac{\text{weight of syrup volume}}{\text{weight of the same volume of distilled water at 4°C}} \quad (1)$$

Weighing is conducted using a precision balance model (RADWAG, AS 220.R2, Poland) with reading accuracy of 0.0001g and maximum capacity of 220g (Mimouni, 2015).

Water holding capacity (WHC) is determined by the method described by AACC (1995); 2 g of powders put in 200 ml of distilled water, were stirred for 30 seconds with a glass rod. The mixture rested 10 minutes, the stirring operation was repeated five times. Tubes were centrifuged for 20 min at 4000 rev/min in a centrifuge, (SIGMA 6-16 KS, Germany). The supernatant was carefully decanted, then the content of the tube was dried at 45°C for 10 minutes in an oven, (ESCO isotherm, USA) before being weighed, using a standard scale, (RADWAG, AS 220.R2, Poland). The water holding capacity was expressed as percentage increase in the sample's weight (Adepeju *et al.*, 2014).

The index of wettability is measured from the results obtained during the rehydration of powder without stirring. It is expressed as the time in seconds required for a given amount of powder to enter the water through its free surface at rest (Schuck *et al.*, 2012).

Dispersibility: 10 ml of distilled water at 25°C were poured into a beaker of 50 ml. 1 g of powder was added in the beaker. The stopwatch started and the sample stirred vigorously with a spoon for 15 seconds by 25 full movements back and forth across the diameter of the container. Reconstituted honey was poured through a sieve (112 microns). 1 ml of sieved honey was transferred into an aluminum pan weighed and dried for 2 hours in

a vacuum oven 70 ± 1°C model (BINDER, Germany).

Dispersibility of the powder was calculated as follows:

$$D\ (\%) = \frac{(10+m)\ x\ TS}{m\ x\ ((100-MC)/100))} \quad (2)$$

m: amount of powder (g) being used, MC: Moisture content in the powder (db) and TS%: dry matter in of reconstituted honey after it has been passed through the sieve (Koç and Kaymak-Ertekin, 2014).

True density: determined as the ratio of the mass of dry solids (m) to total volume (Vs) of the sample excluding the air ports (Calín-Sánchez *et al.*, 2014):

$$\rho_t = \frac{m}{Vs} \quad (3)$$

Samples were weighted with an analytical balance, (RADWAG, AS 220.R2, Poland), while Vs was measured with a pycnometer model (ISO LAB In 20°C, 10ml BORO 3.3 A, Germany).

Bulk density: Determined as the ratio of solid mass to the bulk volume (Vb). Samples were weighted with the analytical balance, (RADWAG, AS 220.R2, Poland) with reading accuracy of 0.0001g and the bulk volume was measured with an 80 mL graduated container (Calín-Sánchez *et al.*, 2014):

$$\rho b = \frac{m}{Vb} \quad (4)$$

2.2.3. Mathematical modeling of freeze drying kinetics

Moisture ratio (MR) of date (pulp and pomace) during freeze drying was calculated using the following equation:

$$MR = \frac{Mt-Me}{M0-Me} \quad (5)$$

Where: Mt, M0 and Me are moisture content at any time, the initial moisture and the equilibrium moisture (g water / g dry matter), respectively (Erbay and Icier, 2009).

The effective moisture diffusivity can be defined from Fick's second law of diffusion, which describes the movement of moisture within the solid.

$$\frac{\delta M}{\delta t} = D\frac{\delta^2 M}{\delta Z^2} \quad (6)$$

Analytical solution of this law in the case of drying an infinite slab of thin layer, assuming moisture migration being by diffusion, one-dimensional moisture movement, uniform initial moisture distribution, negligible shrinkage, constant moisture diffusivity, and negligible external resistance to heat and mass transfer can be developed in the form of the following equation (Crank, 1975).

$$\frac{M-M_e}{M0-M_e} = \frac{8}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{(2n-1)}\exp\left[-(2n-1)^2\frac{\pi^2}{4}\frac{Dt}{L^2}\right] \quad (7)$$

Where L is the thickness (m); t is the time (min); and D is the effective diffusivity (m^2/s). Simplifying the previous equation by taking the first term of the series solution and assuming M_e equal to 0:

$$MR = \frac{M}{M0} = \frac{8}{\pi^2}\ .\exp\left[-\frac{\pi^2}{4}\frac{Dt}{L^2}\right] \quad (8)$$

Where L the thickness of the sample and D the effective diffusion coefficient.

Taking logarithm on both sides of previous equation gives the following equation (Nag and Dash, 2016):

$$Ln\ MR = \left(Ln\frac{8}{\pi^2}\right) - \frac{\pi^2 Dt}{4L^2} \quad (9)$$

The diffusion coefficient (D) is calculated using the method of slopes.

$$Slope = -\left(\frac{\pi^2 D}{4L^2}\right) \quad (10)$$

Drying kinetics of date pulp and pomace were fitted to five models (Newton, Page, Henderson and Papis, Logarithmic, Wang and Singh). The quality of the adjustment was determined using the coefficient of

determination (R^2), the reduced χ -square (χ^2) (Ergun *et al.,* 2014) and root mean square error (RMSE) as follows:

$$\chi^2 = \frac{\sum_{i=1}^{N} (MRexp(i) - MRpre(i))^2}{N-n} \quad (11)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} (MRpre(i) - MRexp(i))^2}{N}} \quad (12)$$

Where MR_{expi} is the *i*th experimental moisture ratio, MR_{prei} is the *i*th predicted moisture ratio, N is the number of observations, n is the number of constants in drying model (Pardeshi *et al.*, 2009).

2.2.4. Statistical analysis

Analyses were performed with three replications, while color with six and results expressed as the mean ± standard deviation (SD). Results were submitted to the analysis of variance (ANOVA) and means were compared by Tukey's test using Origin Pro 2016 32 Bit software. Differences were considered to be significant at $P < 0.05$.

3.Results and discussions

3.1. Production of powders

100 g of pulp and pomace were freeze dried at a temperature of −40°C and a pressure of 0.12 mbar (Fig 1). After 24h, samples weight became 96.70 g and 63.50 g respectively.

Pulp and pomace samples have an initial water content of 0.11 and 0.45 (kg H_2O/ kg product) respectively. The relative water content of pomace decreases and reaches a level of 0.55 after 12 h. However, pulp reaches a value of 0.94 after 12 h, these results agree with Sahari *et al.* (2008) who studied the effect of initial moisture and date thickness on the drying process.

3.2. Physicochemical properties of powders

Physicochemical properties of date powders are shown in Table 1.

Table 1. Physicochemical properties of pulp and pomace powders

Parameters	Pulp powder	Pomace powder
Moisture (%)	2.50±0.05	7.64±0.22
Water activity	0.17±0.22	0.32±0.02
Ash (g/100g)	0.84±0.04	1.00±0.01
Soluble solids content (°Brix)	60±0.00	59.90±0.17
Titrable acidity (% citric acid)	3.03±0.20	2.57±0.20
pH	6.28±0.05	6.36±0.02
Color (L*)	78.57±0.23	68.9±0.36
Color (a*)	4.41±0.12	7.10±0.09
Color (b*)	22.43±0.29	25.00±0.17

Results are expressed as mean values of three determinations ± SD; analysis of variance (ANOVA) $p < 0.05$ with Tukey's tests.
Color results are expressed as mean values of six determinations ± SD

Moisture content of pulp and pomace powders were 2.50±0.05 and 7.64±0.22% respectively. These results are similar to moisture content values of freeze dried papaya and pineapple, 2 and 7% (wb) respectively (Marques *et al.*, 2011).

However, they were higher than freeze dried marionberries and strawberries cultivars (0.10-0.20%) and corn cultivars (0.70-1.10%) (Asami *et al.*, 2003).

Titratable acidity of pulp and pomace powders were 3.03±0.20, 2.57±0.20 (% citric acid) respectively. They were higher than

freeze-dried papaya pulp powders with a value of 1.38±0.07% citric acid (Canuto *et al.*, 2014).

pH of date powders was almost similar with values of 6.28±0.05 and 6.36±0.02 of pulp and pomace powders respectively. These results were closer to those reported by Mahendran (2010) for freeze dried guava.

Microbiological, chemical and enzymatic stability of food products is related to water activity content (a_w), values of water activity ranging between 0.20 and 0.40 ensure stability of the product (Nur Dirim and Çalişkan, 2012), the a_w of pulp and pomace powders were 0.17±0.22 and 0.32±0.02 respectively. These values were similar to water activity value of freeze dried pumpking puree powder found as 0.20 (Nur Dirim and Çalişkan, 2012). Ashes and total soluble solids have values of 0.84±0.04, 1.00±0.01 (g/100g) and 60±0.00, 59.90±0.17°Brix for pulp and pomace powders respectively. These values were similar to those reported in literature for samples of Mech Degla date powder 1.44±0.20 (g/100g) and 60°Brix.

Food color is a major determinant of product quality and affects consumer preferences. Color may be used as an indicator to predict chemical and quality change due to thermal processing (Valdenegro *et al.*, 2013). Color parameters (L*, a*, b) of pulp and pomace powders were shown in Table 1. Pulp and pomace powders were slightly orange (a*4.41±0.12) and (a*7.10±0.09), slightly yellow (b*22.43±0.29) and (b*25.00±0.17) respectively, the difference in color parameters is due to product composition. Similar L*, a*, and b* values were reported by Sablani *et al.* (2008) for date powder (Mech degla variety).

3.3. Functional properties of powders

Table 2 shows functional properties of pulp and pomace powders. Water holding capacity (WHC), wettability index and dispersibility show the ability of powders to rehydrate.

Table 2. Functional properties of pulp and pomace powders

Parameters	Pulp powder	Pomace powder
Water holding capacity (g water/g dry matter)	0.63±0.23	0.70±0.02
Wettability index (s)	930±0.50	1425±0.33
Dispersibility (%)	91.76±0.21	68.51±0.40
True density (Kg/m^3)	884.96±0.00	454.55±0.00
Bulk density (Kg/m^3)	909.00±0.00	505.05±0.00

Results are expressed as mean values of three determinations ± SD; analysis of variance (ANOVA) $p < 0.05$ with Tukey's tests.

According to Gurak *et al.* (2014), WHC is defined as the amount of water retained by the sample without being subjected to any stress. Pulp powder has a WHC value less than pomace with values of 0.63±0.23 and 0.70±0.02 (g of water / g dry matter) respectively. These results were lower than those obtained by Nguyen (2014) with values of 0.90 to 1.00 (g eau/ g powder) for soy powders produced by atomization and between 3.97 and 6.20 g/g dry fiber for date fiber concentrates varieties (Borchani *et al.*, 2010). The difference is due to composition and varieties. The wettability or the ability of powder to absorb water is one of the most important physical properties related to reconstituting powders (Sarabandi *et al.*, 2014). The obtained results were 930±0.50 (s) for pulp which is lower than 1425±0.33 (s) for pomace. According to Manickavasagan *et al.* (2015), date powder obtained by spray drying has a wettability time ranged between 145.70 and 162.70 (s), dispersibility values of pulp and pomace powders were 91.76±0.21 and 68.51±0.40% respectively. These values are higher than results obtained by Koç and Kaymak-Ertekin (2014), ranging between 51.30 and 100% for spray dried honey powder.

Bulk density of powders is determined by particle density, which is determined by solid density and particle internal porosity, and also by spatial arrangement of particles in the container (Micha, 2005). True and bulk density (Table 2) of powder samples were 884.96±0.00-909.00±0.00 Kg/m^3 and 454.55±0.00 - 505.05±0.00 Kg/m^3 respectively. Pulp powder density was higher than pomace powder and bulk density was also higher than the true density. The difference is justified since powder heap reduces the space between particles, so volume will decrease. The obtained results were similar to Calín-Sánchez *et al.* (2014) results regarding freeze dried chokeberry fruits.

3.4. Physicochemical properties of syrup

Date syrup is an important date by-product and a natural sweetener considered as a suitable replacement of sugar in food products formulation, in order to reduce the harmful effect of sugar, and improving the nutrient properties (Raiesi Ardali *et al.*, 2014). Physicochemical properties of syrup were shown in Table 3.

Table 3. Physicochemical properties of date syrup

Parameters	Date syrup
Moisture (%)	61.81±0.08
Water activity	0.92±0.01
Ash (g/100g)	1.02±0.99
Soluble solids content (°Brix)	35±0.00
Titrable acidity (% citric acid)	0.58±0.20
pH	6.22±0.06
Color (L*)	33.06±0.23
Color (a*)	0.60±0.00
Color (b*)	6.93±0.14
Density (g/ml)	1.20±0.02

Results are expressed as mean values of three determinations ± SD; analysis of variance (ANOVA) $p < 0.05$ with Tukey's tests. Color results are expressed as mean values of six determinations ± SD. Moisture content and water activity of date syrup are 61.81±0.08% and 0.92±0.01 respectively, these results are similar to Ganbi (2012) with values of 74.06±6.10% for water bath date fruits juice (Dibs) and Fennir *et al.* (2003) for Saidi date syrups with values of 0.881. The difference could be due to date varieties. Syrup ash content is 1.02±0.99 (g/100g), this result is lower than Raiesi Ardali and Akbarian (2014) who found a value of 1.69 (g/100g). Total soluble solids, titrable acidity and pH have values of 35±0.00°Brix, 0.58±0.20 (% citric acid) and 6.22±0.06 respectively, which are closer to values reported by Farahnaky *et al.* (2016); El-Sharnouby *et al.* (2014) and Ganbi (2012) respectively. Density has a value of 1.20±0.02 (g/ml) which is similar to Jamshidi Mokhber *et al.* (2008) with a value of 1.35 (g/ml). Color parameters (L*, a*, b) of syrup were shown in Table 3. Syrup was slightly orange (a*0.60±0.00) and slightly yellow (b*6.93 ±0.14). Similar L*, a*, and b* values were reported by Raiesi Ardali and Akbarian (2014); Fennir *et al.* (2003) for date syrup.

Table 4. Model constants and statistical parameters for date pulp and pomace

Date pulp					
Models	**Equations**	**Constants and coefficients**	**R^2**	**χ^2**	**RMSE**
Newton	MR=exp(-kt)	k= 0.00450	0.9403	0.000030	0.00152
Page	MR=exp(-kt^n)	k= 0.00231 n= 1.29878	0.9635	0.000021	0.00390
Henderson and Papis	MR=aexp(-kt)	a=1.00538 k= 0.00512	0.9613	0.000022	0.00401
Logarithmic	MR= aexp(-kt)+c	a=24.13641 k=0.00021 c=-23.13126	0.9619	0.000028	0.00398
Wang and Singh	MR= 1+at+bt^2	a=-0.00443 b=-0.00005	0.9630	0.000027	0.00392
Date pomace					
Models	**Equations**	**Constants and coefficients**	**R^2**	**χ^2**	**RMSE**
Newton	MR=exp(-kt)	k= 0.07042	0.6691	0.009080	0.08811
Page	MR=exp(-kt^n)	k= 0.25645 n= 0.37086	0.9774	0.007420	0.02307
Henderson and Papis	MR=aexp(-kt)	a=0.89177 k=0.05420	0.7824	0.007160	0.07152
Logarithmic	MR= aexp(-kt)+c	a=0.45683 k=0.41041 c= 0.54464	0.9987	0.000052	0.00546
Wang and Singh	MR= 1+at+bt^2	a=-0.10019 b= 0.00566	0.9688	0.001290	0.02710

3.5. Mathematical modeling of freeze drying kinetics

The freeze drying behavior was determined from the loss of samples weight. Total drying time was determined as 12 hours for the pulp and pomace samples. Similar results were obtained by Marques *et al.* (2011) for freeze dried pineapple, guava and mango pulp.

The experimental data were fitted using Origin Pro 2016 32 Bit software to five models (Newton, Page, Henderson and Papis, Logarithmic and Wang and Singh) (Table 4). The correlation coefficient (R^2) was used to define the best model of the drying process for the pulp and pomace date, with χ^2 having the lowest value (Togrul and Pehivan, 2002).

Figure 2 represents the experimental data of water content with the best mathematical model for pulp and pomace date.

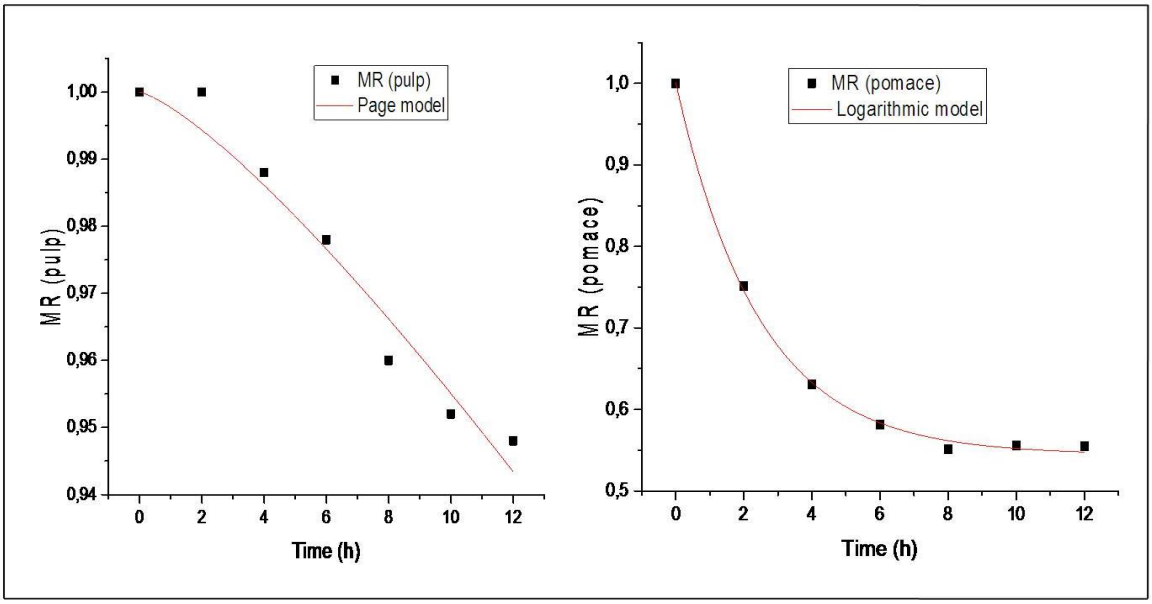

Figure 2. Plot of moisture ratio versus drying time for freeze dried pulp and pomace date (Page and Logarithmic models)

Diffusivity values were ranged between 9.74 × 10^{-11} and 5.15 × 10^{-11} m^2/s for pulp and pomace respectively. These values are within the range reported by Ergun *et al.* (2014) for the foods ranging from 10^{-12} to 10^{-6} (m^2/s).

4. Conclusions

This study investigated the production and characterization of pulp and pomace date powders as well as date syrup from Garn Ghzel variety. Powders were obtained using freeze drying process. The Garn Ghzel variety date shows interesting physicochemical and functional characteristics. The freeze drying process of pulp and pomace date was modeled and best fitted to Page and Logarithmic models respectively.

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