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CURD CHEESE STORAGED IN MODIFIED PACKAGING WITH EXTRACT OF GARLIC AND THYME: VARIATION OF SOME PHYSICO-CHEMICAL PARAMETERS

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ABSTRACT

The chemical changes of homemade curd cheese were evaluated during storage in unmodified and modified packages with extract of garlic (*Allium sativum*) and thyme (*Satureja hortensis*), during 3 and 6 day, at room temperature. White paper and low density polyethylene have been used as packaging materials. The influence of type packaging during storage on titrated acidity, chloride, protein and dry weight content was investigated. Also, a set of measurements was performed on cheese stored at room temperature without being packed, after being treated with thyme or garlic extract by immersion for 10 minutes in the extract. The basic compounds from thyme extract and the antioxidant compounds from garlic extract influence the physico-chemical parameters of curd cheese.

Keywords: *packaging materials, home made curd cheese, garlic extract, thyme extract.*

1. Introduction

Packaging is a factor of great importance effectively protecting the food inside it from light, oxygen and microbial recontamination, thus directly preventing the alteration of food (Moyssiadi et al., 2004; Hoskin, 1988, Bosset et al., 1993; Ravanis & Lewis, 1995; Erickson, 1997; Skibsted, 2000; Borle et al., 2001; Vassila et al., 2002). The light transmittance properties and the permeability to oxygen of plastic containers are disadvantages that influence in general the quality of food (Moyssiadi et al., 2004).

Allium species, mainly onion (*Allium cepa L.*) and garlic (*Allium sativum L.*) are among the oldest cultivated plants, and are used for multiple purposes. They are a rich source of several phytonutrients, and recognized to have significant biological activities. These biological activities are related to the thiosulfinates, volatile sulfur compounds which are responsible for the pungency of these vegetables. The thiosulfinates are formed by the action of the enzyme alliinase from their respective S-alk(en)yl cysteine sulfoxides. However, depending on the *Allium* species, and under differing conditions, thiosulfinates can decompose to form additional sulfur constituents. Due to increased interest into the utilization of natural biologically active compounds and the development of specific packaging, mainly active packaging, the thiosulfinates arouses much interest for the improvement of shelf-life and safety of perishable foods, and for their use as food preservatives and substitutes for chemicals (Benkeblia & Lanzotti, 2007).

Antimicrobials from plants fulfills, the needs of today's consumer looking for wholesome food without chemical preservatives. These can be classified as novel compounds obtained from plants that delay microbial growth of pathogens and spoilage organisms in food. There is growing scientific evidence of the potential use of antimicrobials from plant for the extension of shelf life in food (Zaborowska et. al., 2012, Juneja et al., 2012, Hammer et al., 1999, Ashour & El Astal, 2005). Use of Plant antimicrobials is an

emerging technology that could be used by the industry to extend the storage life of food and overcome these food safety issues.

The aim of the present study is to analyze the changes induced by modified packaging with extract of garlic (*Allium sativum*), and thyme (*Satureja hortensis*) respectively, on following properties of curd cheese: titrated acidity, chloride, protein and dry weight content. Also, curd cheese immersed in the same extracts and stored unwrapped, was subjected to the same analysis above properties.

2. Materials and methods

2.1. Preparation of the homemade curd cheese

Curd cheese preparation was done using 5 liters of freshly milked milk from cow. The milk was heated to 35°C and mixed with 2 ml liquid calf clot from Astro brand of type 1/10.000 SH with a ratio chymosin/pepsin to 80/20. The mixture was left at room temperature for 40-60 minutes for clotting. The clotted milk was cut using a knife and filtered through cheesecloth. Cheese was left to drain in cheesecloth for 6 hours.

2.2. Preparation of the aqueous garlic and thyme extracts

The garlic and dry thyme were buy from supermarket. The Velp solvent extractor was used to obtain the extract of garlic, and thyme respectively. Thus, 5 g of dried thyme or garlic and 60 ml ultrapure water were introduced into the cylinders of extractor. The extractor has been programmed for 3 hours at a extraction temperature of 200 C.

2.3. Preparation of modified packaging with extract of garlic, and thyme respectively

Two types of materials were used for packaging the homemade curd cheese, namely: food film of low density polyethylene from Fino brand and white paper of type 80 mg/sqm. The packaging sheet size was about 150 x 200 mm. A number of 2 sheets for both paper as well as film of low density polyethylene were modified using the extract of garlic and, thyme respectively.

Each sheet was immersed, for one minute, in the fresh garlic extract and, thyme respectively, and then was left at room temperature, for 2 hours. The curd cheese was also immersed in extract of garlic and, thyme respectively, for one minute and was stored unpackaged, at room temperature.

2.4. Experimental conditions

The experiments were performed on 8 series, as follows:

- curd cheese packaged in paper - P
- curd cheese packaged in low density polyethylene film - LDPE
- curd cheese immersed in extract of garlic, stored unpackaged - IFg
- curd cheese packaged in paper modified with extract of garlic - IPg
- curd cheese packaged in low density polyethylene film modified with extract of garlic - ILDPEg
- curd cheese immersed in extract of thyme, stored unpackaged - IFt
- curd cheese packaged in paper modified with extract of thyme - IPt
- curd cheese packaged in low density polyethylene film modified with extract of thyme - ILDPEt

The amount of curd cheese in each experimental series was 50 grams. In the third and sixth day after the start of the experiment, for each experimental series were performed the followings analyses for curd cheese: titrated acidity, chloride, protein and dry weight content. The same analyses were also performed on curd cheese before the start of experiments.

2.5. Assessment of chloride content

The principle of the method (STAS 6354-61) consists in the reaction of silver nitrate with chloride ion followed by titration of the silver nitrate excess with potassium chromate to orange color of silver chromate. In a porcelain capsule weigh out 2 g cheese

and finely grind with 30 ml of hot distilled water. After 15 minutes, the mixture is filtered. In the filtrate is add 0.5 ml of 10% potassium chromate and is titrate with 2.906% silver nitrate until the orange coloration is obtained.

Chloride % = V/m (1)

where,

V_1 - volume of 0.1 N silver nitrate used to titration of sample, mL

m - mass of the sample taken in the analysis, g.

2.6. Assessment of protein content

The steps to assess the protein content using the Kjeldahl were those described by the Kjeldahl's method for determination of protein from milk and milk products.

I - Digestion: 2 g cheese, 7 g anhydrous potassium sulfate (K_2SO_4), 5 g selenium (Se) powder, 10 ml sulphuric acid (H_2SO_4) concentrated 98% and 10 ml hydrogen peroxide (H_2O_2) 35% were added in the digestion tube to be heated for 30 minutes at 420°C. The digestion tubes were left to cool to 50 - 60°C. In each digestion tube was added 50 ml of ammonia free distilated water.

II - Distillation: A digestion tube with a digested sample was placed in the steam distilling unit. 50 ml of 35% NaOH was added by the automatic dispensing device. A collecting Erlenmayer flask containing 25 ml of 4% boric acid (H_3BO_3), was placed in the steam distilling unit. The distillation was carried till a volume of 100 ml of distillate was obtained (about 5 minutes).

III - Titration: In the distillate collected in Erlenmeyer flask was added 10 drops of indicator Toshiro solution and was titrated with 0.2N HCl until the color was change from pale green to pink (1 ml 0.2N HCl = 2.803 mg N-NH_4 because 25 mg N-NH_4 require 8.92 ml of 0.2N HCl).

2.7. Assessment of dry weight content

The analysis was done using standard method (STAS 6344-61) by evaporating water in thermoregulation oven, followed by cooling in desiccator and weighing until constant weight. In a vial is inserted weighing 5 g of sand and glass rod and dried at 105-108 C to constant mass. After cooling, weigh in the vial 2-3 g cheese and mix well with sand using the baguette. Vial is dry in the oven for 2-3 hours at 50-60 C and then at 105-108 C for 4-5 hours. Remove the vial from the oven, cool in the desiccator and weigh. Repeat drying oven until constant weight.

$$W(\%) = [(m-m_1)/m] \times 100 \quad (2)$$

where,

m - product weight taken for analysis, g

m_1 - weight of the product after drying, g

DW (%) = 100 - W

2.8. Titratable acidity assessment

Principle of a method (STAS 6353-61) is to determine acidity by titration, after pre-treatment with the solvent mixture. 10 g homogenized cheese are placed in a porcelain capsule and finely grind with 25 ml distilled water and 1 ml of 2% alcoholic phenolphthalein solution. Titrate with sodium hydroxide solution stirring continuously until the appearance of pink color that persists for 1 minute.

$$\text{Acidity } (°T) = (V/m) \times 100 \quad (3)$$

where,

V - volume of 0.1 N sodium hydroxide solution used for titration, ml

m - weight of the product, g

1°T corresponds to the acidity given of 0.09 g lactic acid in the sample (10 g cheese).

3. Results and disscussion

3.1. DW content modification in time, depending on storage conditions

The DW content of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions are show in Figure 1. From the graph we can see an

increase in the DW content after 6 days of storage in all conditions. This corresponds on the one hand to the drying cheese (as would be expected if keep it unpackaged or packaged in paper) and on the other hand to the decrease of moisture content due to hydrolytic processes occurring during maturation (~14 days or even more, depending on the type of cheese). The hydrolytic processes during maturation refer to the hydrolysis of the proteins to peptides and amino acids and of the glycerides to glycerol and fatty acids.

At 3 days of storage, the evolution of DW content is different for curd cheese packed in LDPE compared to the other storage conditions. Although the drying of curd cheese storaged under these conditions is diminished or even unexpected for only 3 days of storage, the slight decrease of water content equivalent to the slight increase of DW content is due to hydrolytic processes mentioned above. The modification of polythene film with extract of garlic intensify the water elimination due to the rich content of antioxidants, garlic being known as a food rich in phytonutrients (manganese, zinc, selenium, germanium, vitamins A and C and others). These antioxidants easily captures free radicals HO• coming from the water splitting.

In cases of curd cheese packaged in paper and unpackaged, the decrease of DW content after 3 days of storage, although it would be unexpected, is explained by the fact that both paper and curd cheese by immersion in aqueous extract increases their humidity (drying of paper in 12 hours being incomplete).

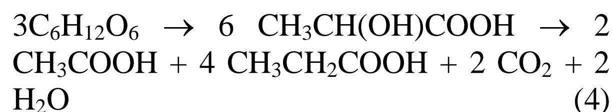
The highest value of DW content has been obtained for storage during 6 days of curd cheese immersed in garlic extract (IFg) when, the water removal is intensified due to due to the antioxidant and volatile properties of garlic extract.

3.2. Titratable acidity modification in time, depending on storage conditions

The titratable acidity of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions are show in Figure 2.

Regarding the titratable acidity, is observed that for all storage conditions both at 3 and 6 days, its value grows relative to baseline due to accumulation of acids during curd cheese maturing.

The acidification of curd cheese is a consequence of lactic fermentation when lactose is converted glucose and galactose, then to lactic acid by lactic bacteria (*Lactococus lactis ssp. lactis* and *Lactobacillus casei*) and subsequently, under the action of microorganisms, in propionic acid, acetic acid and CO_2 (cf. reaction 1).



$$3C_6H_{12}O_6 \rightarrow 6\ CH_3CH(OH)COOH \rightarrow 2\ CH_3COOH + 4\ CH_3CH_2COOH + 2\ CO_2 + 2\ H_2O \qquad (4)$$

Also, the acidity grows due to fats hydrolysis with formation of fatty acids and glycerin, and also due to enzymatic hydrolysis of proteins. Thus, the chymosin present in clot generate α-casein$_{f(1\text{-}23)}$ and the proteinase associated with cell walls of bacteria *Lactococcus* hydrolyses α-casein $_{f(1\text{-}23)}$ forming peptides and finally amino acids.

The slight decrease of the titratable acidity after 3 days storage of the curd cheese immersed in thyme extract (IFt) and, packaged in paper impregnated in thyme extract (IPt) respectively, can be explained by the fact that the thyme extract has high content of thymol, compound with basic character belonging to the class of phenols. In these two cases the extract is retained in the texture of the foodstuff and of the paper leading to the slight decrease of titratable acidity in first three days of storage.

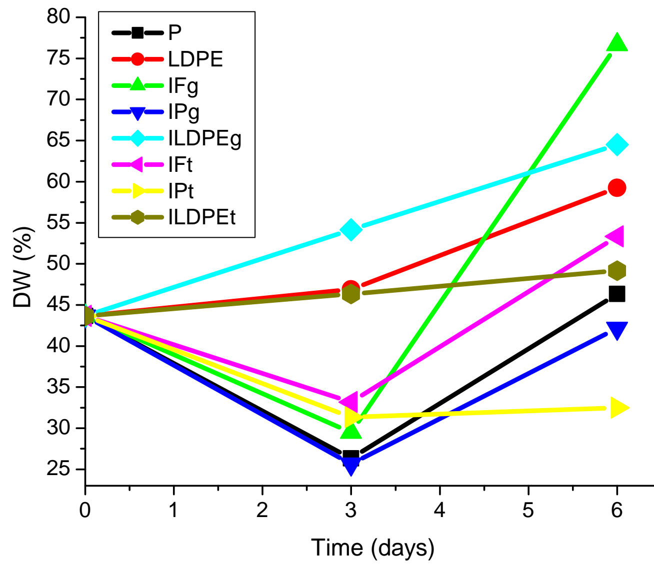


Figure 1. The DW content of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions

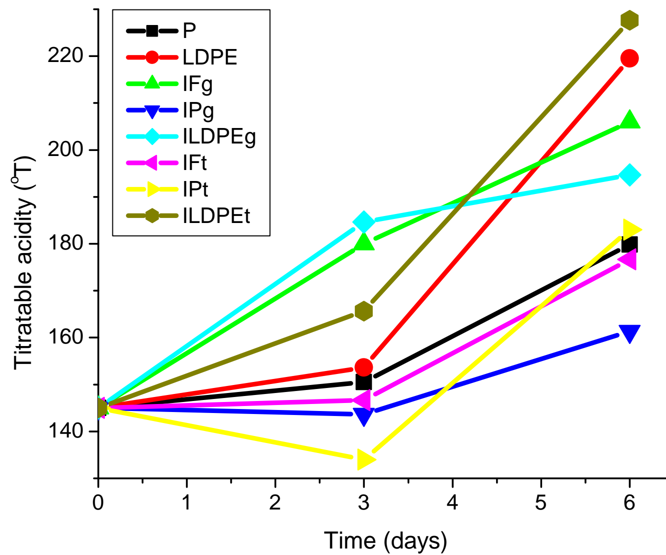


Figure 2. The titratable acidity of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions

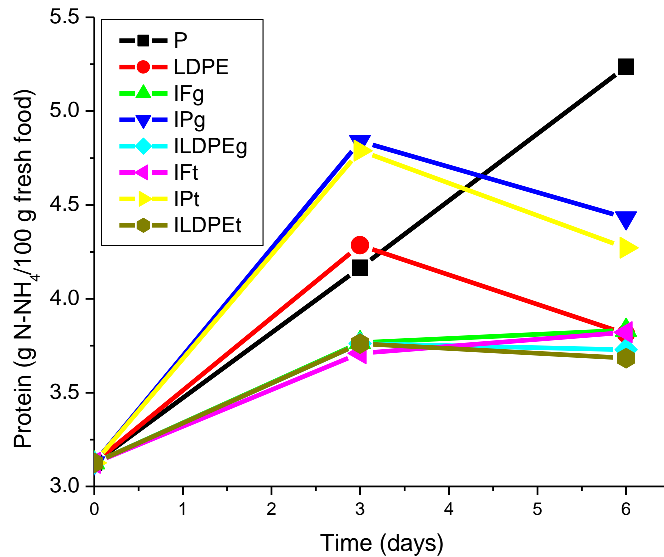


Figure 3. The protein contents expressed as ammoniacal nitrogen of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions

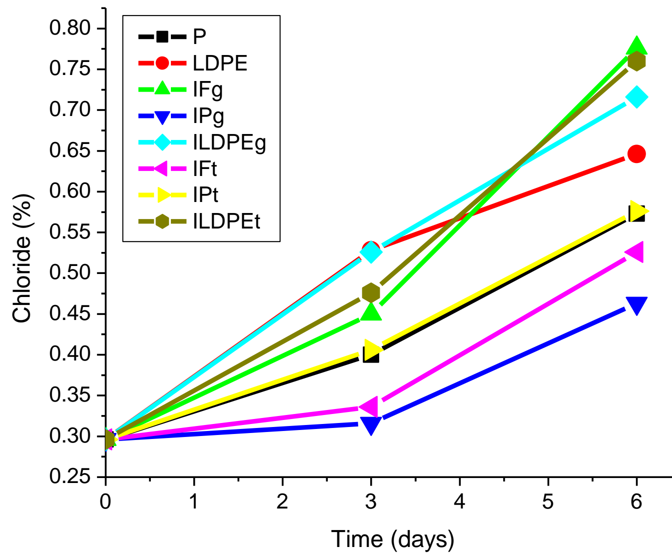


Figure 4. The chloride contents of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions

3.3. Protein content modification in time, depending on storage conditions

The protein contents of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions are show in Figure 3.

In the hydrolysis of casein are obtained intermediate molecular weight peptides which are then degraded by bacterial micro-flora with formation of low molecular weight peptides and amino-acids. In catabolism stage amino-acids undergo

decarboxylation reactions, deamination, transamination, desulphurisation and others. Compounds resulting from these reactions are ammonia, aldehydes, phenols, indole, alcohols and others, all contributing to the cheese flavor. Aammonia formation in amino acid catabolism explain slight decrease in protein content of cheese after 6 days of storage in all conditions (except storage in unmodified paper where hydrolysis processes are slowed down due to low humidity).

3.4. Chloride content modification in time, depending on storage conditions

The chloride contents of curd cheese at the start of experiment and after 3 and 6 days of storage under different conditions are show in Figure 4.

The variation of chloride content in curd cheese is closely related to the change in moisture content of cheese and therefore the content of DW. Thus, chloride content increases during storage.

4. Conclusions

Conclusions on the changes that occur on some physico-chemical parameters of cheese stored in different packaging conditions are:

- the storage in humid conditions lead to increased titratable acidity and slight decrease in DW and chloride content
- the basic compounds present in the extract of thyme lead to slight decrease in titratable acidity
- the presence of garlic extract leads to increased acidity due to hydrolytic process intensification

The study requires further research to be strengthened the results. They refer to optimal concentrations of extracts, finding improved techniques for applying the extracts on package (multilayer) and others research.

5. Acknowledgement

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BIOSENSORS - AN EMERGING TOOL IN FOOD PROCESSING

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ABSTRACT

Recent advances in electronic vision and computer technology have opened the research horizons for greater accuracy in process control, product sorting, and machine operation in food industry. Biosensors are an important alternative in the food industry to ensure the quality and safety of products and process controls with effective, fast and economical methods. Their technology is based on a specific biological recognition element in combination with a transducer for signal processing. Detection of contaminants, verification of product contents, product freshness and monitoring of raw materials conversion are the areas of potential biosensor applications. This article presents an overview on the components of biosensors and describes the most important application areas in food processing industry.

Keywords: *Electronic vision, Biosensors, food,applications, contaminants*,

1.Introduction

A biosensor is defined as a compact analytical device incorporating a biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer. Biosensors have been adapted to detect or measure analytes in on-line systems. In food and agricultural industries, the quality of a product is evaluated through periodic chemical and microbiological analyses which are expensive, slow, need well trained operators and in some cases, require steps of extraction or sample pretreatment, increasing the time of analysis [1].

Biosensors can provide rapid, non-destructive and affordable methods for the quality monitoring of a product. Biosensors have the potential to create an analytical revolution to resolve the problems in the agricultural and the food industries. For quality assessment, grading, and sorting of biological products, several types of electronic sensors are being developed for providing rapid and non-destructive determination of internal qualities. Biosensors offer enormous potential to detect a wide range of analytes in the food industry and environmental monitoring [1].

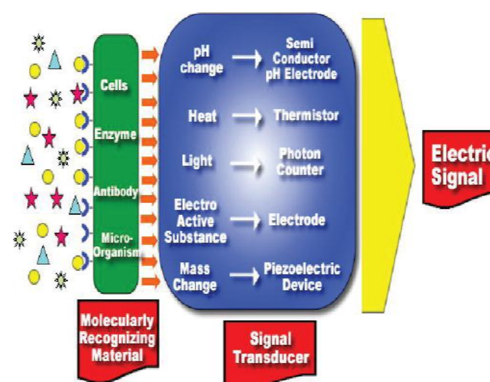


Figure 1: Biosensor [2]

2. Biosensors and its components

Sensor systems are analytical tools combining a chemical, biochemical or biological recognition component with a transducer. The recognition component is capable of selectively interacting with an

analyte directly or indirectly, emitting a signal through the transducer [3].

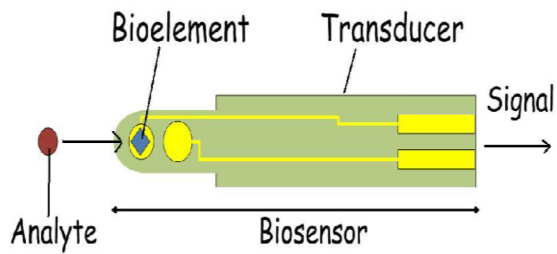


Figure 2: Basic scheme of a biosensor

Biosensor is a compact analytical tool incorporating a biological or biologically derived sensing element either integrated or associated in a physicochemical transducer (Fig. 3)

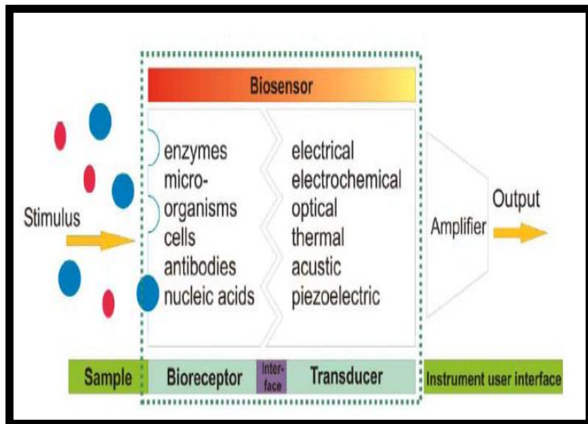


Figure 3: Components of a biosensor[4]

2.1 Biocomponents

As bio-components, an enzyme, antibody, nucleic acid, hormone, cell structure or tissue can be used.

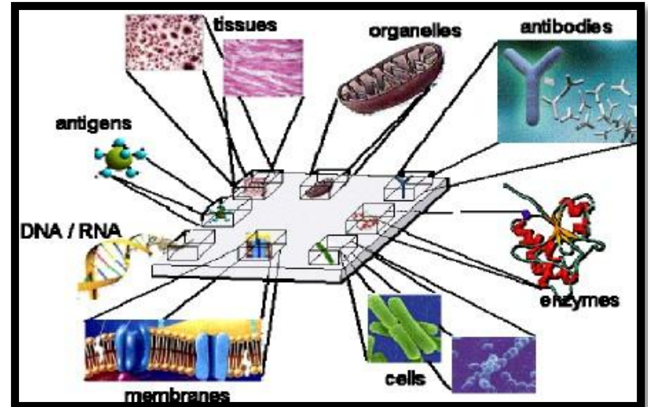


Figure 4: Biocomponents in a biosensor

Its role is to interact specifically with the target analyte and the result of biochemical reaction is consequently transformed through transducer to measurable signal [5].

2.1.1 Enzymes

These biosensors utilize enzymes which are specific for the desired molecules and catalyze generation of the product, which is then directly determined using a transducer. The enzymes are extremely specific in their action (Fig 5). This extremely specific action of the enzymes is the basis of biosensors [5].

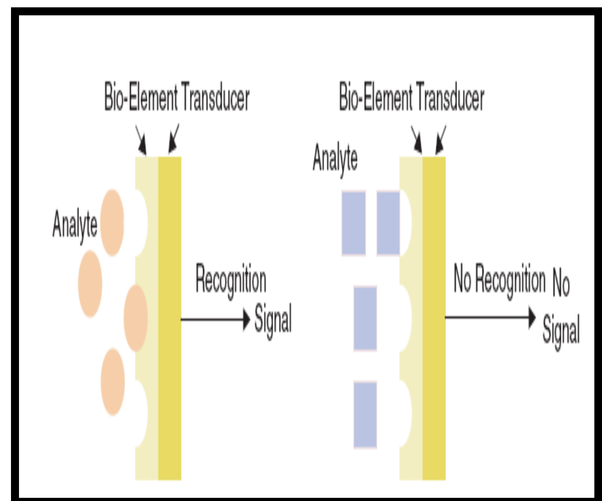


Figure 5: Specificity of enzymes is the basis of biosensors

2.1.2 Nucleic acids

Nucleic acid (NA) based biosensors integrate an NA (natural and biomimetic forms of oligo- and polynucleotides) as biological recognition element. Nowadays, mainly synthetic oligodeoxyribonucleotides (ODNs) are used as probes in the DNA hybridization sensors [5].

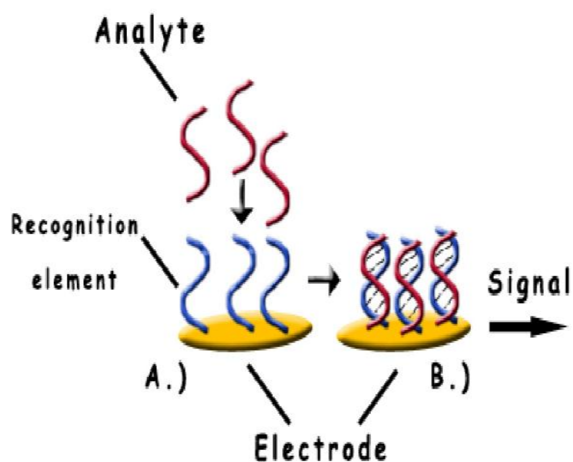


Figure 6: General DNA biosensor scheme

2.1.3 Cells

These bioreceptors are either based on biorecognition by an entire cell/microorganism or a specific cellular component that is capable of specific binding to certain species [5].

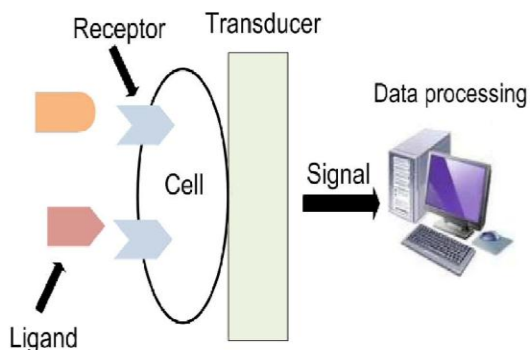


Figure 7: Scheme of cell-based biosensor.

2.1.4 Antibodies

An antibody is a complex biomolecule, made up of hundreds of individual amino acids arranged in a highly ordered sequence. An antigen-specific antibody fits its unique antigen in a highly specific way [5].

2.2 Transducers

Transducer is an analytical tool which provides an output quantity having a given relationship to the input quantity. Depending on the transducing mechanism used, the biosensors can be of many types such as: electrochemical, optical, piezoelectric, thermometric, ion-sensitive, magnetic or acoustic one [5].

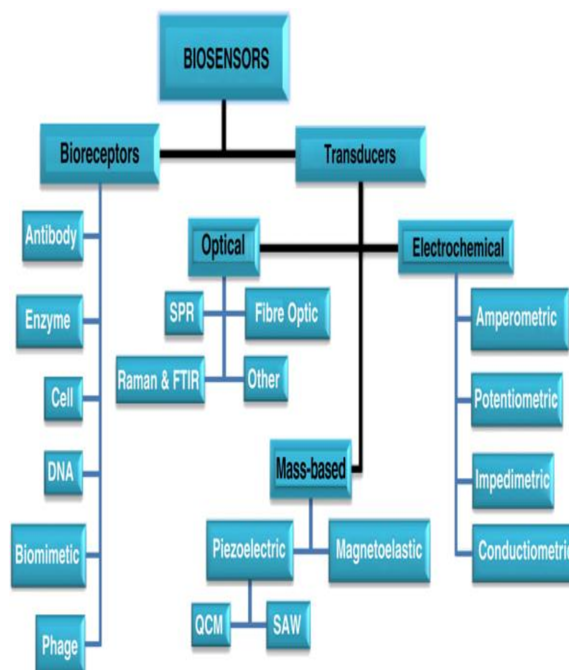


Figure 8: Classification of Biosensors

Very important part of a biosensor fabrication is the immobilization of bio-component.Performance of biosensors with immobilized molecules depends also on factors such as the chemical and physical conditions (pH, temperature and

contaminants), thickness and stability of the materials [5].

3.Biosensors and food industry

Consumers are placing significant importance on the quality of food products. This has forced the agricultural and the food industries to place an increased emphasis on quality monitoring of products. The food industry is benefitting from major advances in the development of enzymatic biosensors with different transduction systems that can be applied in the areas of food safety, quality and process control [5].

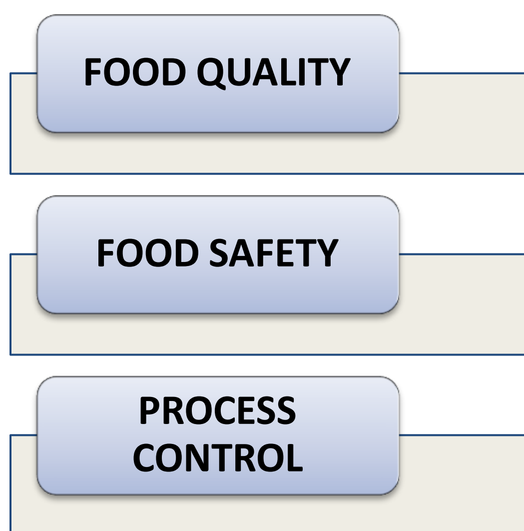


Figure 9: Biosensors and food industry

Novel sensing technologies using biomaterials or nanomaterials can be used to detect quality and safety attributes in packaged foods. These sensing technologies range from rapid non destructive and noncontact to highly specialized microsensing and nanobiosensing structures. Micro- and nano-based sensors that use a variety of transduction mechanisms to sense microbial and biochemical changes in food products are being explored [6].

4. Potential application of biosensors in food processing

- Detection of pathogens
- Sensors to monitor food packaging and shelflife
- Biosensors for food quality/additives control
- Sensory evaluation of food products
- Biosensors and HACCP

4.1 Detection of pathogens

Food borne pathogens pose a risk to food safety and are a threat to the global food supply chain. The detection and identification of pathogens in raw food materials, food products, processing and assembly lines, continue to rely on time consuming conventional culturing techniques. Biosensors have the potential to revolutionize food monitoring by detecting the presence of residues, traces, chemicals, pathogens and toxins quickly [1].

Over the past decade, many improvements have been observed in both conventional and modern methods of pathogenic bacteria detection in foods. Bokken et al. [7] developed a surface plasmon resonance biosensor to detect Salmonella pathogen through antibodies reacting with Salmonella group A, B, D, and E. Another sensor technology was developed using micro-electrophoretic

system (mFFE) that separates and concentrates the analyte in question by several electrophoretic methods [7].

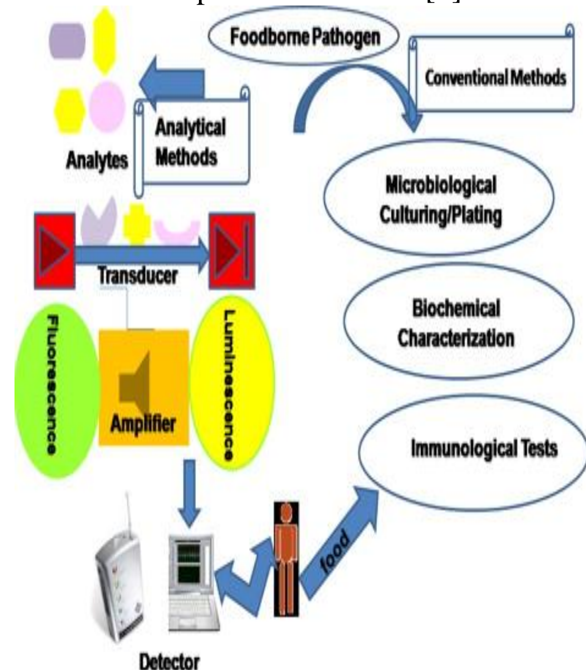


Figure 10: Detection of pathogens

4.2 Sensors to monitor food packaging and shelflife

A variety of innovative platforms are being developed for the detection of biomolecules and microbes that are based on nanotechnology, however most of these are incorporated within devices, and require the extraction of a sample to determine the presence of the target molecule. When considering such systems for food packaging, these are focused on detecting microbial growth. The challenge for such systems is that they must be capable of being integrated within the packaging, provide an easily distinguished response (most likely a colour change), and be cheap to manufacture. It is most likely that the presence of microbial contamination will be detected indirectly by measuring changes in gas composition within the package as a result of microbial growth, using gas sensor technologies. [8].

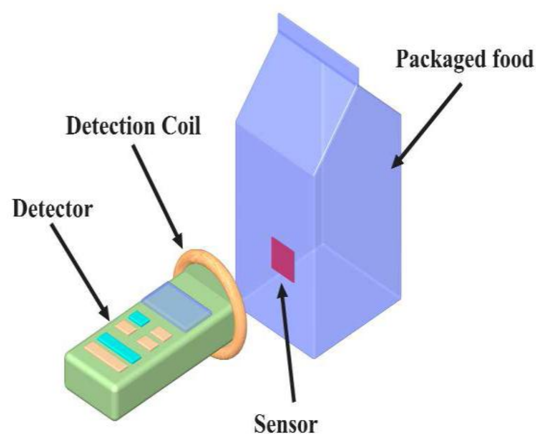


Figure 11 : Sensor in a food package

4.3 Biosensors for food quality/additives control

The concept of food safety involves ensuring the production and marketing of harmless food, with this, ensure the health of the consumer. Based on this need biosensors are used to detect xenobiotic substances, substances external to the food product such as additives and pesticides and components of the food itself like toxins of diverse origins. The development of catalytic biosensors in food additive analysis generally employs enzymes as recognition systems.

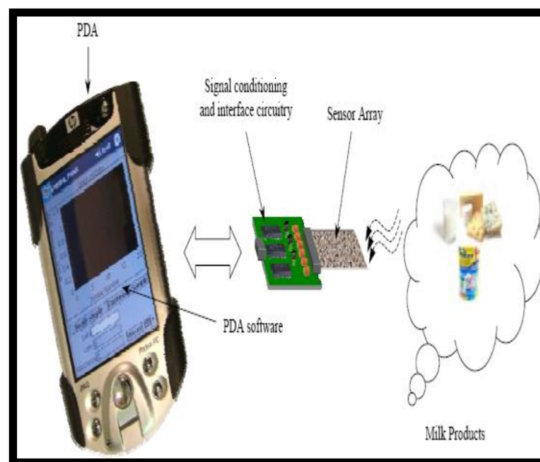


Figure 12 : Nanotechnology-based sensors system

This development is described in several investigations, among these notably are analysis of aspartame with carboxyl esterase, alcohol oxidase[9];analysis of sorbitol with sorbitol dehydrogenase and nicotinamide adenine dinucleotide (NAD+) [10] ; analysis of benzoic acid with tyrosine and analysis of sulphites with sulphite oxidase; all developed with a system of amperometric transduction [6].

4.4 Sensory evaluation of food products

''Electronic noses'' and ''electronic tongues'' are the common names of devices responding to the flavor/odor (volatiles) or taste (solubles) of a product using an array of simple and nonspecific sensors and the pattern-recognition software system [7]. Arrays of gas sensors are termed 'electronic noses' while arrays of liquid sensors are referred to as 'electronic tongues'.

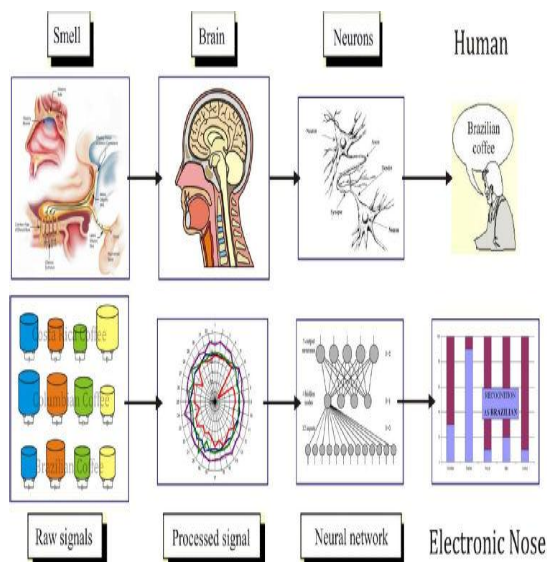


Figure 13: Electronic nose technology

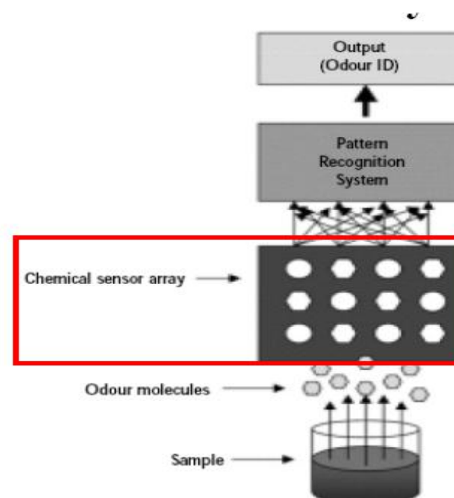


Figure 14: Scheme of Enose

Examples of claimed successful applications include the following:

- Discrimination between single volatile compounds
- Tracking of aroma evolution of ice-stored fish or meat
- Classification of wines
- General raw materials control
- Testing of coffee, soft drinks, and whiskey
- Control of beer quality and faults

Recently, there have been attempts to integrate electronic noses and tongues to obtain improved classifications and/or detection of foods. The nose was comprised of an array of gas sensors with different selectivity patterns while the tongue was based on pulse voltametry.

4.5 Biosensors and HACCP

Timely detection of unsafe foods is the main issue that the food-safety system should address, providing guidance for the design and integration of such system into the existing food safety management structures, i.e., HACCP.

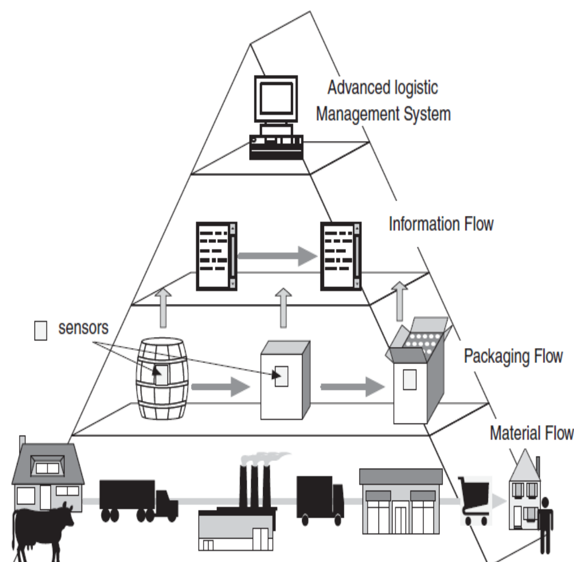


Figure 15: An integration of sensors into information/material flow in distribution network.

The preventive detection of the biohazard can be accomplished by direct measurements with the biosensors, or indirect detection by the process/environment monitoring and control. Such detection is based on the data from physical and chemical sensors, which are reliable and allow scaledown (meaning the possibility of easy integration into the existing information carriers). The HACCP system for food-safety management is designed to identify health hazards and to establish strategies to prevent, eliminate, or reduce their occurrence.

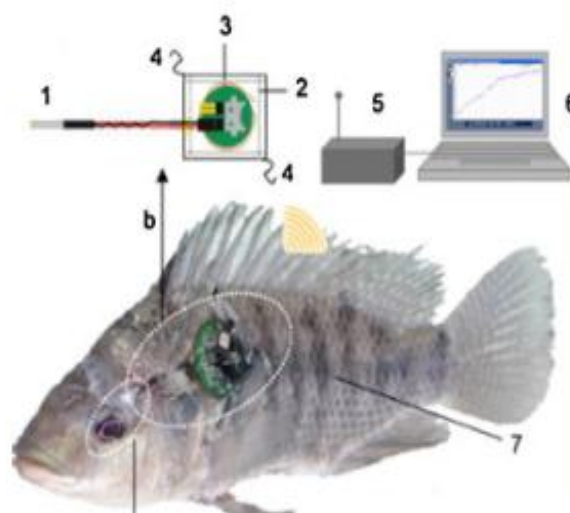


Figure 16: The tethered biotransducer with externally located power, electronics and communications components [11]

5. Nanobiosensors in food analysis

On the development of biosensor, nanotechnology play an important role. Kinds of approaches are done about nanoscience and nanotechnology advance for bioanalyses.

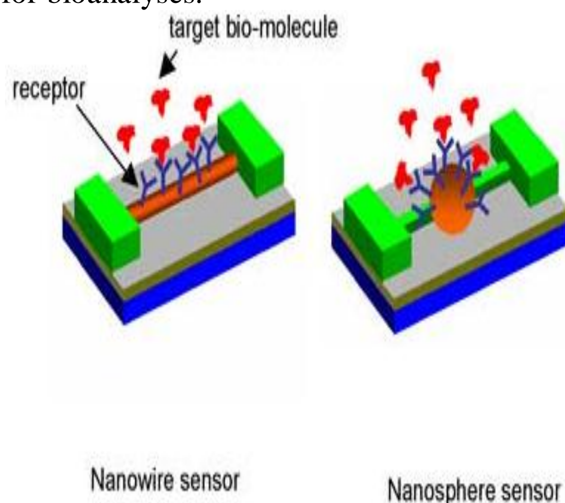


Figure 17 Nanobiosensors

This kind of nanobiosensors could be used to detect toxins, nutrients, pathogens, allergens, heavy metals etc [12]. Nanomaterials, such as carbon nanotubes, metal nanoparticles, nanowires, nanocomposite and nanostructurated materials are playing an increasing role in the design of sensing and biosensing systems with interest for applications in food analysis. Furthermore, these nanobiosystems are also bringing advantages in terms of the design of novel food detection strategies [13].

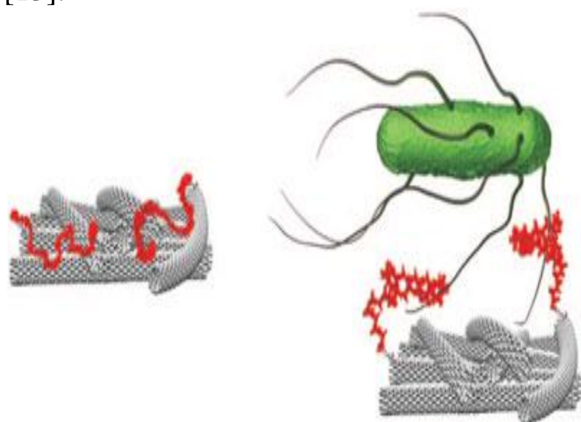
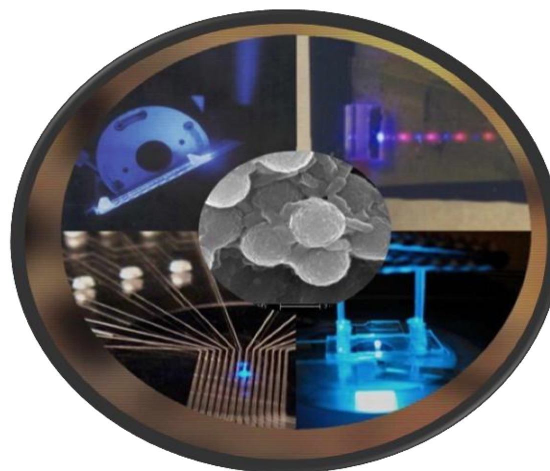


Figure 18 :Biosensor with Nanocarbontube [14]

An aptamer attached to an electrode coated with single-walled carbon nanotubes interacts selectively with bacteria (Figure 14) . The resulting electrochemical response is highly accurate and reproducible and starts at ultralow bacteria concentrations, providing a simple, selective method for pathogen detection [14].

6. Outlook

Food chain can be contaminated by a number of ways: chemicals present in the environment; the improper use of agrichemicals such as antibiotics and pesticides; the illegal use of growth promoting compounds in animal production; by products of food processing techniques; naturally occurring shellfish toxins and naturally occurring toxic fungal metabolites (mycotoxins).



The food industry needs suitable analytical methods for process and quality control; that is, methods that are rapid, reliable, specific and cost-effective in their provision of information about physical and chemical characteristics of food. The biosensor offers a technology that can be used in the laboratory to analyse large numbers of samples for the presence of chemical contaminants. However, very few biosensors play a prominent role in food processing or quality control. Considerable effort must be made to develop biosensors that are inexpensive, reliable, and robust enough to operate under realistic conditions. Such a technique would offer a great commercial advantage to food processing and food manufacturing sector.

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COMBINED USE OF PAPER OR LDPE AND NATURAL EXTRACTS FROM *SATUREJA HORTENSIS* AND *ALLIUM SATIVUM* FOR THE PRESERVATION OF THE SUMMER SALAME

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ABSTRACT

The study aims to establish if the packages based on paper or LDPE modified with extract of thyme and garlic are efficient in preserving the summer salame during storage, as compared with the unmodified packages. Eight different types of packages based on paper or LDPE impregnated in the aqueous extracts of thyme and garlic were prepared and used to test their preservation activity during the storage of the summer salame, a Romanian meat product, at room temperature and light. The eight types of packages are as follows: product impregnated in extract of thyme (IP thyme) and garlic (IP garlic), summer salame packaged in unmodified paper (H) and LDPE (LDPE), summer salame packaged in paper impregnated in extract of thyme (IH thyme) and in extract of garlic (IH garlic), summer salame packaged in LDPE impregnated in extract of thyme (ILDPE thyme) and in extract of garlic (ILDPE garlic). At 0, 3 and 6 days, the following physico-chemical parameters were monitored during storage: acidity, pH, water, salt and protein content. Additionally, the changes of the organoleptic characteristics were established. It was observed that the most reduced decrease of the acidity and protein content during storage occured in the summer salame impregnated in the thyme extract and in the salame packaged in the paper impregnated in the garlic extract. Moreover, the organoleptic characteristics of the samples impregnated in the thyme and garlic extract, excepting those in packages based on LDPE were not significantly changed during storage, at room temperature and under light.

Keywords: *summer salame, paper, LDPE, thyme, garlic*

1. Introduction

Food packaging has been traditionally defined as a passive barrier that delays environment effects on food products [1]. However, trends in current research involve the development of packaging materials that can positively interact with the environment and food, playing an active role in preservation. To date, active packaging is a novel food biopreservation technique for extending the shelf life of food products [2].

Garlic (*Allium sativum L.*) is one of the world's oldest medicines and has been employed for flavouring and as a medical herb due to its diverse biological activities, including antimicrobian and antioxidant effects [3]. Garlic is composed mainly of fructose-containing carbohydrates and sulfur compounds.

Additionally, thyme (*Satureja hortensis*) is well known for its antimicrobial activity and antioxidant effects.

The reason for using the low-density polyethylene film (LDPE) as basing package is the fact that the polymers are effective vehicles for the active substance s [4], giving the possibility to incorporate different antimicrobial additives.

Paper was used as based packaging due to the fact that it is the most common type of package. Due to the porosity, it allows the transport of the active substances to the investigated food samples.

The study aims to establish if the packages based on paper or LDPE modified with extract of thyme and garlic are efficient in preserving the summer salame during storage, as compared with the unmodified packages.

2. Materials and methods

2.1. Packages preparation

The extracts of garlic and thyme were obtained by extraction in ultrapure water, as follows: 5 grams of dry vegetal mater were introduced in the cotton flask inside the extractor. In the solvent vessel were introduced 60 ml of ultrapure water (obtained from the Thermoscientific device). The working temperature is 200^0C. The extraction procces occured in the Velp extractor and consists in three stages. The first is the immersion of the vegetal materials in the solvent and it takes 3 hours, the second stage is washing and its takes 20 minutes and the third is recovery of the unreacted solvent and it takes 5 minutes. The reason for choosing the ultrapure water as solvent is the non-toxicity, even if the extraction efficiency is lower than that occuring when an organic solvent would be used (clorophorm, diethyleter).

The as obtained extracts were used to packages preparation. The paper (approximativelly 400 cm^2) was introduced in 50 ml extract of garlic or thyme. After 10 minutes of immersion, the modified paper is dried in air, at room temperature. The same procedure was applied also for preparation of the packages based on LDPE. The LDPE film was bought in Kaufland's supermarket in Baia Mare. Kaufland is a chain of supermarkets owned by Lidl & Schwartz, a prominent German company with wide and diversified presence in many Central and Eastern European countries, including Romania.

2.2. Experimental tests

In the each prepared package, an amount of 60 grams of summer salame was deposited, at rooms temperature and in the presence of the light. At 3 and 6 days, sensorial and physic-chemical analyses were realized.

The summer salame was purchased from the Ferma Zootehnica market, a chain of markets specialized in commercialization of meat and meat products from Maramures County, Romania.

2.3. Physico-chemical analyses

2.3.1. Acidity measurements

A quantity of 10 g sample was mixed with ultra pure water (10 ml) and 40 ml water at 35-40^0C and two drops of phenolphthalein solution 1% (S.C. Chemical Company S.A. Iasi, Romania) were then added. The mixture was titrated with solution 0.1N NaOH (S.C. Chemical Company S.A. Iasi, Romania), until the pink colour of the solution persisted for at least one minute. The acidity was calculated using the formula:

$$Acidity\ (acidity\ degrees) = V \times 10 \quad (1)$$

where: *V* – volume of NaOH 0.1 N solution used to titration (ml), *10* – sample mass (g)

2.3.2. pH

A sample of 5 g was mixed togheter with 30 mL ultrapure water and then filtered. In the obtained filtrate the pH was measured using the Inolab pH 730 pH-meter.

2.3.3. Chlorine content

A sample of 5 g was mixed with 15 ml acetone and then 50 ml of ultrapure and a small quantity of sodium carbonate were added. The mixture was filtered and the entirely quantity of extract was added to 0.5 ml of potassium chromate 10% (S.C. Chemical Company S.A. Iaşi, România) were added. The mixture was titrated with 2.906% solution of silver nitrate (S.C. Chemical Company S.A. Iaşi, România) until the color changed in red-brown **[5]**(STAS 961-56) .

The chlorine content was calculated using the formula:

$$NaCl\ (\%) = \frac{V}{m} \quad (2)$$

where: V – volume of $AgNO_3$ solution used to titration (ml).
m – sample mass (g)

2.3.4. Water content

A sample of 5 g (G) was mixed with calcined cuartz sand and the mixture is weight (G_1). The mixture was then treated with 5 ml of ethanol (S.C. Chemical Company S.A. Iaşi, România) and introduced in the Binder oven at 40...60°C, for 2 h and then at 105...110°C, until the weigh is constant **[5]** (STAS 961-56). After cooling the mixture is weigh again (G_2). The percentage of dry mass is calculted with the formula

$$\textit{water content}\ (\%) = \frac{G_1 - G_2}{G} \times 100 \quad (3)$$

when : G_1 - mass of food sample and sand before drying (g),

G_2 - mass of food sample and sand after drying (g)

G - mass of food sample (g)

2.3.5. Protein content

The content of protein was determinated using the Kheldahl system and consists in three steps.The first step is mineralization (digestion) when the proteic nitrogen is transformed in inorganic nitrogen, occuring in the digestion system VELP scientifica. An amount of 1 g of salame was mixed with 7 g K_2SO_4, 5 mg Se powder, 10 ml concentrated H_2SO_4 and 10 ml H_2O_2 35%. The digestion took place at 420^{0}C for 20 minutes. The second step is distilation, using the distilator VELP scientifica. The distillation was peformed in NaOH 35%, for 5 minutes. Finally, the titration step consisted in titration of the inorganic nitrogen resulted in the ditillation step in the presence of Toshiro reagent, with HCl 0.2N, until the green color changes in pink or violet.

The amount of the proteic nitrogen was calculated taking in consideration that 60 mg of nitrogen require 21.4 ml of 0.2N HCl.

2.3.6. Organoleptic characteristics

Consisted in establishing the variations of color, flavour, aspect and consistence of the food samples deposited in studied packages, at different time ranges.

2.3.7. Statistical analyses

Each experiment was repeated three times and the higher value of the standard deviation (SD), calculated using Microsoft Excel, did not exceed 0.5%.

The aim of the statistical analyses was to establish a possible correlation between the physico-chemical parameters and time, for each type of package taken into consideration.

3. Results and disscussion

3.1.Acidity

The acidity decreased during storage for the samples packaged in all the investigated packages (Figures 1 and 2). This behaviour is explained by two processes: first, reducing of the population of acidic microorganisms bacteria due to the antimicrobial activity of the extracts of thyme and garlic and second, the UV-blocking property of the packages reduces or cuts off the permeability of oxygen, responsible for the generation of organic acids [6, 7].

The lower decrease of acidity occured in the sample impregnated with extract of thyme and garlic, suggesting that this type of preservation of the most efficient for the minimum variation of the acidity.

3.2. pH

The pH values of the samples stored in the investigated packages slightly increase, suggesting the reduction of the processes generating the acidic subtances (Figures 3 and 4). Moreover, the pH indicates the strength of the acidic groups, as compared with the acidity parameter that measures the number of the acidic groups.

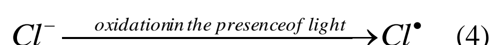
The results of pH are corelated with those obtained for the acidity. The increase of pH means the decrease of the acidity.

The most reduced increasing of the pH occured in the sample impregnated in the

extract of thyme and garlic, results similar with those obtained for acidity. The very close values of pH for the samples deposited in the packages with extract og garlic demonstrates that this type of extract did not influence in a significant manner the physico-chemical characteristics of the summer salame duting storage.

3.3. Chlorine content

After 6 days of storage, the chlorine content decrease in the same manner for all the samples (Figures 5 and 6). This reducement of the chlorine content can be explained by the photolysis process that occurs during the storage, meaning the oxidation of the chlorine ions to chlorine radicals, and subsequently generation of the molecular chlorine (reactions 1 and 2) [6, 7].



$$Cl^- \xrightarrow{oxidation\,in\,the\,presence\,of\,light} Cl^\bullet \quad (4)$$

$$2\,Cl^\bullet \rightarrow Cl_2 \uparrow \quad (5)$$

Again, one observes that the most reduced decrease occured in the sample impregnated with extract og thyme and garlic.

3.4. Protein content

The protein content decreases in all cases, probably due to the degradation of the proteic components by photolysis, in the presence of light [6, 7] (Figures 7 and 8). The most reduced decreases one observes for the sample impregnated in the extract of thyme and for the those packaged in the paper modified with both types of extract. This suggests that the direct contact between the salame and extract is more efficient in preserving the physisco-chemical characteristics of the sample as compared with the using of the impregnated packages.

The most accentuated decrease of the protein content occured in the food deposited in the packages based on LDPE, unmodified and impregnated with thyme extract. Figure 8 shows that the most accentuated decrease occured in the sample impregnated with garlic extract.

3.5. Water content

The water content decreases for the samples package din extract of thyme, excepting the sample packaged in LDPE (Figure 9). The increase of the water content for the other samples can be explained by low permeability of the packages and moreover, by the fact that the extract of thyme enhance this property.

In contrast, Figure 10 shows that the water content of the samples packaged in all the packages excepting impregnated LDPE decreases. This suggests the reducing of the barrier of permeability of the packages in the presence of the extract of garlic.

3.6. Correlation between the physico-chemical parameters

In table 1 are presented the correlation coefficients between acidity and chlorine, pH, protein and water contents, for each type of the extract used. The correlation coefficient is a measure of the degree of influence between the parameters in a pair. A value closely to 1 suggest a significant positive dependence between the parameters.

Table 1. *Correlation coefficients between the physico-chemical parameters*

THYME	acidity-chlorine	acidity-pH	acidity-protein	acidity-water
IP	0,62	-0,72	0,62	0,66
IH	0,68	-0,96	0,61	0,98
H	0,94	-0,92	0,59	0,84
ILDPE	1,00	-0,84	0,95	0,90
LDPE	0,94	-0,87	0,66	0,41

GARLIC	acidity-chlorine	acidity-pH	acidity-protein	acidity-water
IP	0,67	-0,98	0,65	0,98
IH	0,77	-1,00	0,64	0,84
H	0,80	-0,88	0,60	0,75
ILDPE	0,98	-1,00	0,94	0,49
LDPE	0,99	-0,98	0,98	0,99

It can be observed that, when the extract of thyme is used, the higher correlation coefficients are obtaining between acidity and chlorine, in the case of

IDPE. For all types of packages, the highest correlation coefficients are obtained for the following pairs: acidity-chlorine, acidity-pH and acidity-water, meaning that the values of acidity are significant influenced by these parameters. As we expect, the dependence between acidity and pH is inverse proportional.

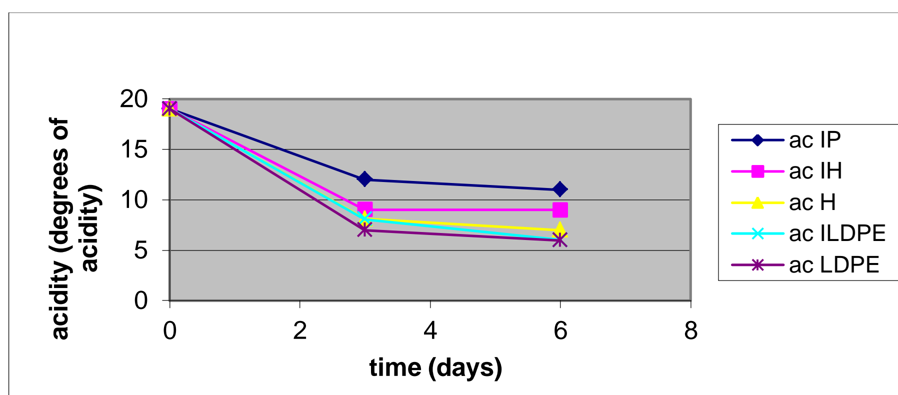


Figure 1. Variation of the acidity of summer salame stored in packages impregnated with extract from thyme (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

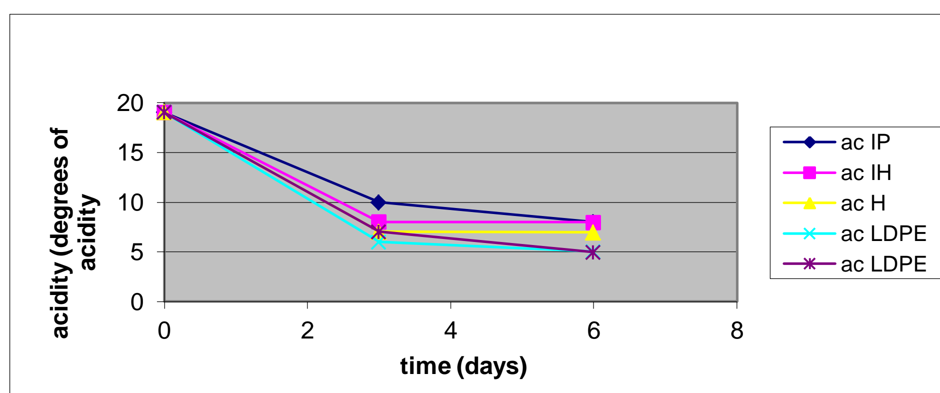


Figure 2. Variation of the acidity of summer salame stored in packages impregnated with extract from garlic (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

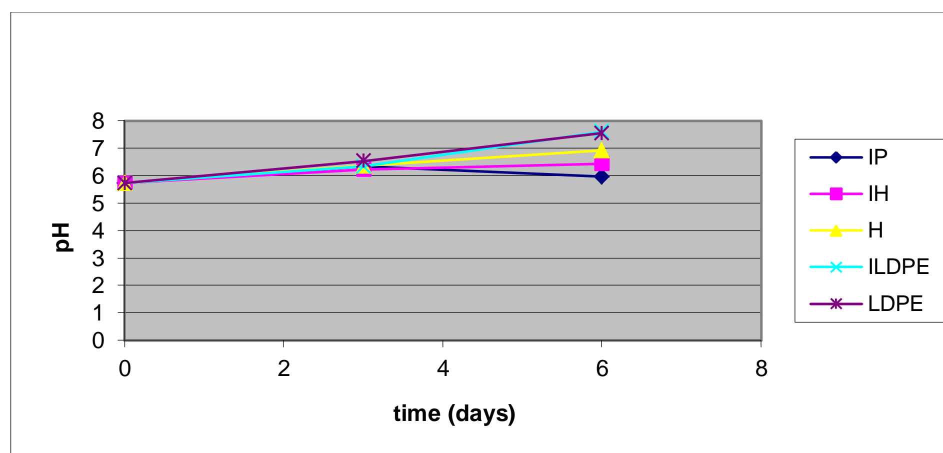


Figure 3. Variation of the pH of summer salame stored in packages impregnated with extract from thyme (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

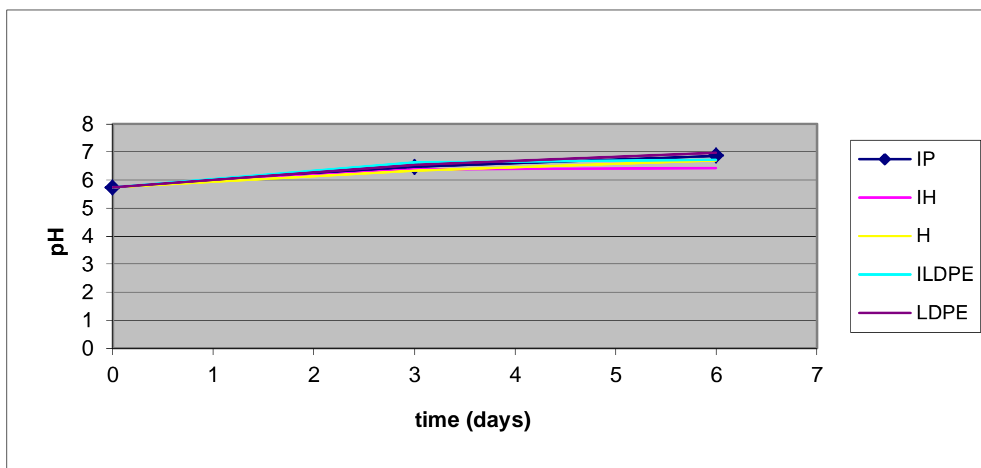


Figure 4. Variation of the pH of summer salame stored in packages impregnated with extract from garlic (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

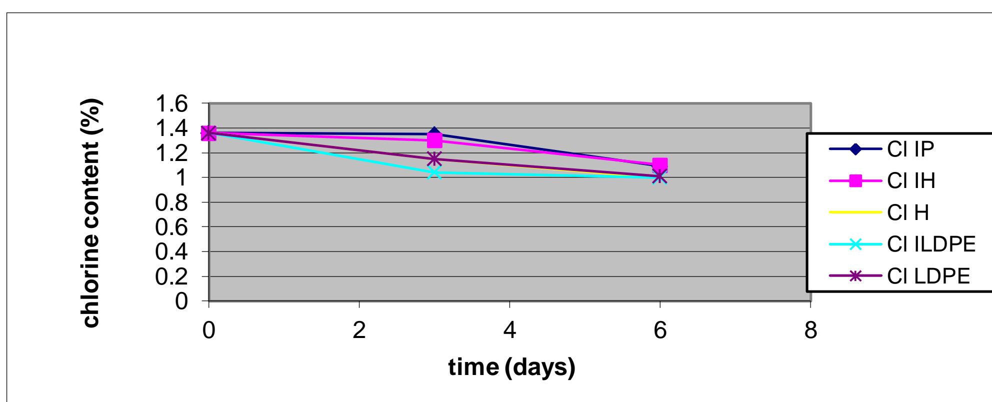


Figure 5. Variation of the chlorine content of summer salame stored in packages impregnated with extract from thyme (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

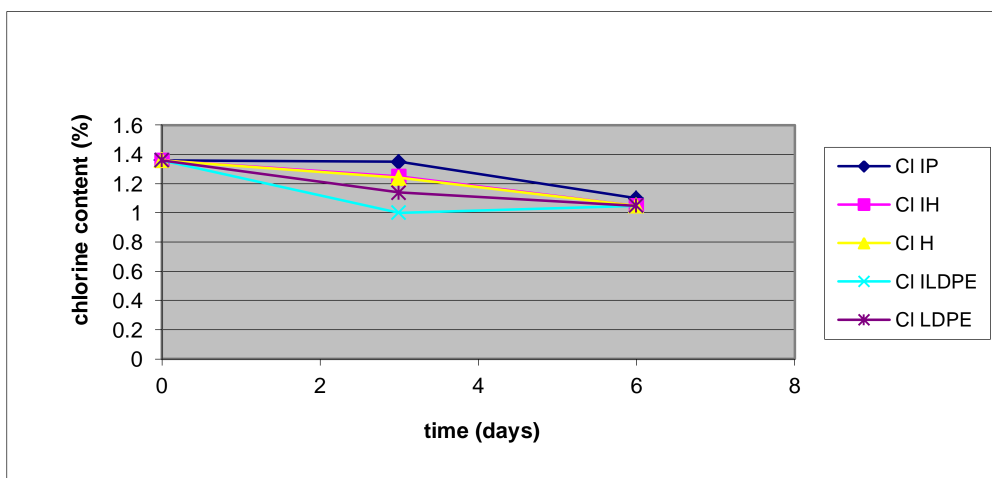


Figure 6. Variation of the chlorine content of summer salame stored in packages impregnated with extract from garlic (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

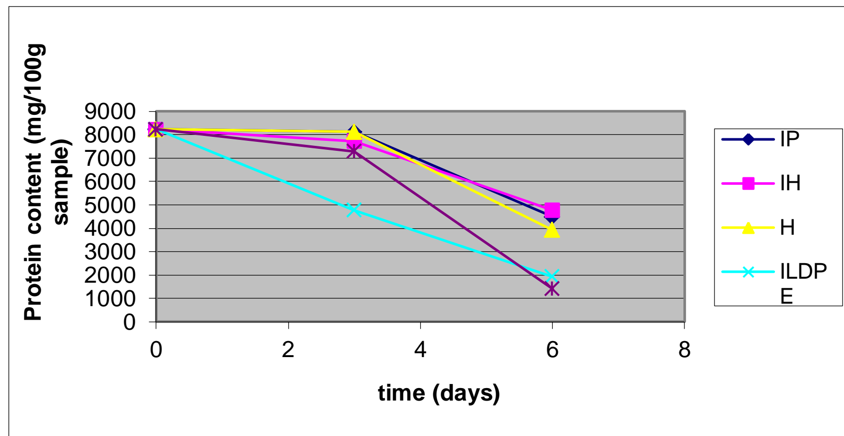


Figure 7. Variation of the protein content of summer salame stored in packages impregnated with extract from thyme (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

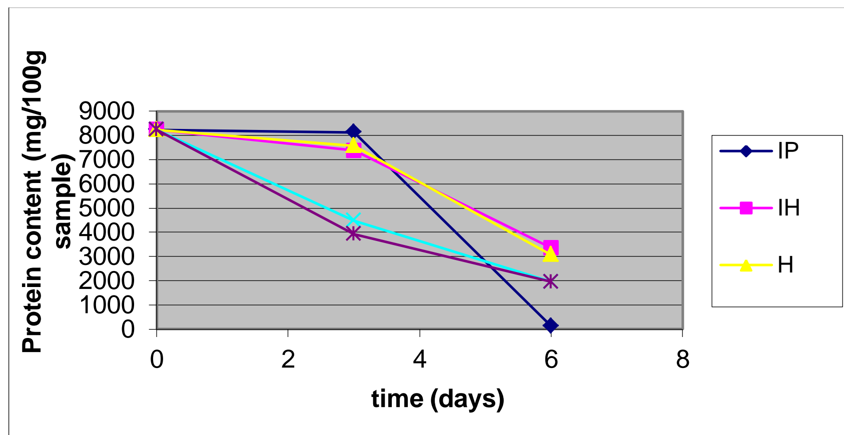


Figure 8. Variation of the protein content of summer salame stored in packages impregnated with extract from garlic (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

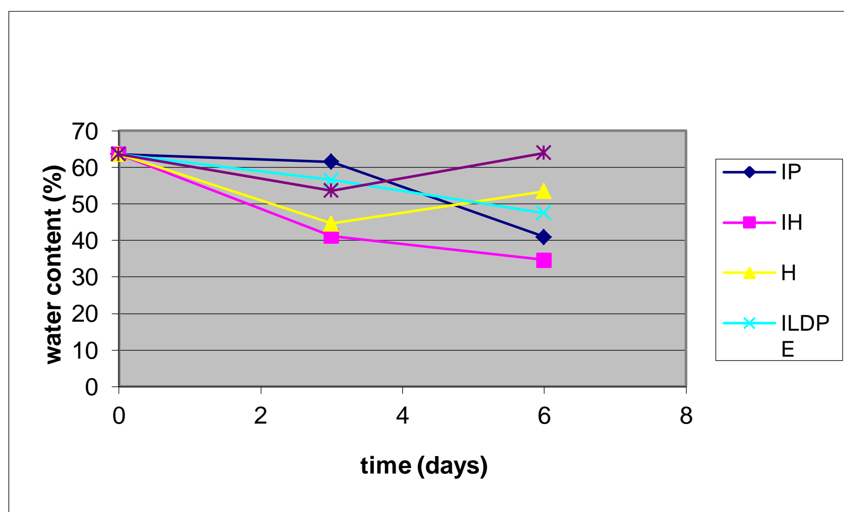


Figure 9. Variation of the water content of summer salame stored in packages impregnated with extract from thyme (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

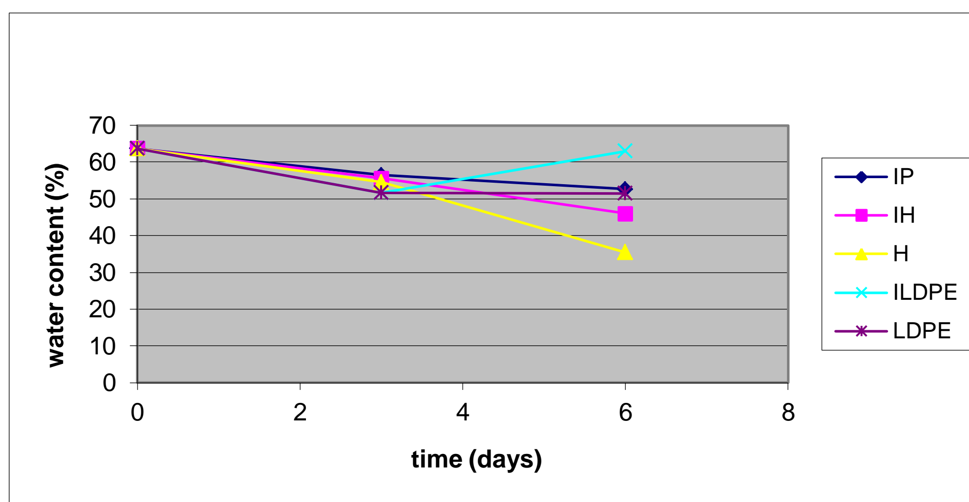


Figure 10. Variation of the water content of summer salame stored in packages impregnated with extract from garlic (IP – impregnated salame, IH – impregnated paper, H – paper, ILDPE – impregnated LDPE)

In the case of garlic extract, the highest correlation coeficients were obtained for the pair acidity-pH. For the pair acidity-chlorine, the highest values were obtained in the case of paper, impregnated and non-impregnated LDPE packages.

For the pair acidity-protei, the highest values were obtained in the case of impregnated and non-impregnated LDPE packages and for the pair acidity-water, the highest values were obtained in the case of impregnated product, impregnated paper, paper and impregnated LDPE packages.

3.7. Organoleptic characterisation

Extract of garlic

IP – the color has become darker, the flavour and aspect are similar with the reference sample, but is feels the garlic flavour, solid consistence

IH and H– the color of the core is similar with that of the reference, but the shell is becoming darker, the flavour and aspect are normal, similar with that of the reference

ILDPE – the color is green-yellow, the flavour and the aspect are altered

LDPE – the color is pink-green, the flavour is normal without significant changes, the aspect present a little trend of alteration

Extract of thyme

IP – the color has become darker, the flavour is similar with the reference sample, solid consistence

IH and H– the color of the core is similar with that of the reference, but the shell is becoming darker, the flavour and aspect are normal, similar with that of the reference

ILDPE – LDPE – the color is pink-green, the flavour is deep altered, pungent

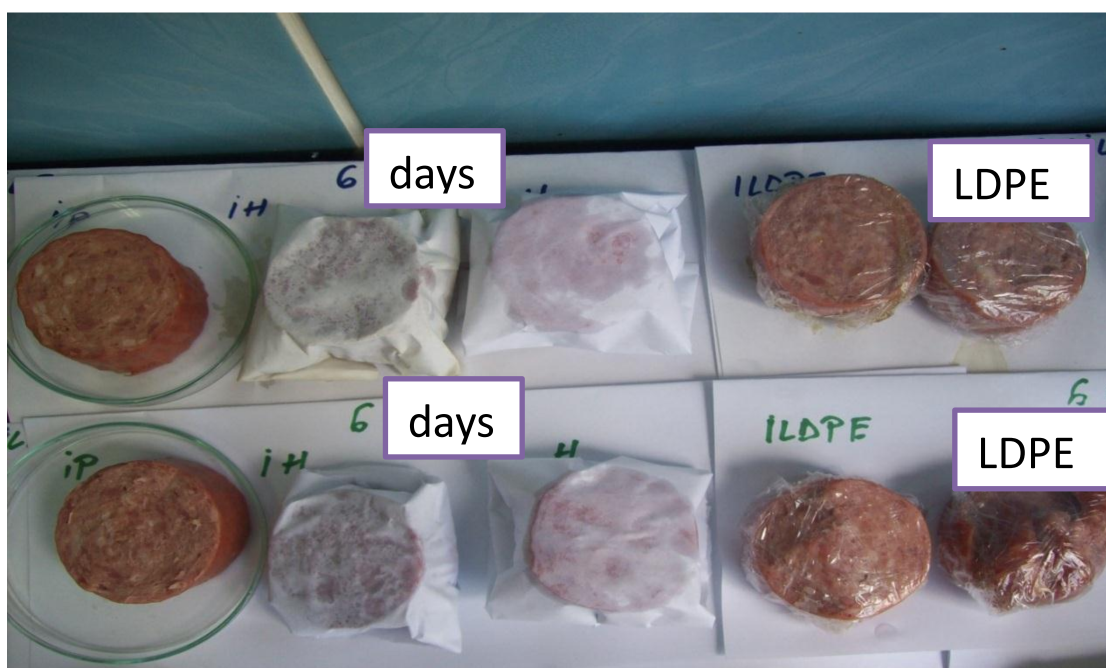


Figure 11. Macroscopic view of the summer salame deposited in the packages for 6 days

4. Conclussions

The study aims to establish if the packages based on paper or LDPE modified with extract of thyme and garlic are efficient in preserving the summer salame during storage, as compared with the unmodified packages.

It was observed that the most reduced decrease of the acidity and protein content during storage occured in the summer salame impregnated in the thyme extract and in the salame packaged in the paper impregnated in the garlic extract. Moreover, the organoleptic characteristics of the samples impregnated in thyme and garlic extract and deposited in paper impregnated with those extracts were not significantly changed during storage for 6 days, at room temperature and in the presence of light, as compared with those deposited in packages based on LDPE.

In conclusion, the modifyng of the paper with extracts of garlic and thyme is an alternative to preservate the quality of the summer salame in terms of acidity, chlorine and protein contents as compared with the conventional packages.

5. Acknowledgements

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BUILDING THE METHOD TO DETERMINE THE RATE OF FREEZING WATER IN PENAEUS MONODON OF THE FREEZING PROCESS

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ABSTRACT

The method of determination the rate of freezing water in Penaeus Monodon of freezing process was established on base the equation of energy balance in warming up process Penaeus Monodon after freezing to determine specific heat of Penaeus Monodon. The result obtained was built the mathematical model (19) to determine the rate of freezing water according to the freezing temperature of Penaeus Monodon. The results indicated that when water was completely frozen (ω = 1 or 100%), the optimal freezing temperature of Penaeus Monodon was -22.00^0C.

Keywords: *Food freezing, The freezing process of Penaeus Monodon, The method determining rate of freezing water.*

1.Introduction

The freezing technology for using in processing and preservation of foods were very important problems that has ever attracted considerable attention, it ensured food security for the world, [1], [2]. The problem posed here is how to determine the optimal freezing temperature and the optimal freezing time of food to save energy for the freezing process. Currently, there are two ways to determine the optimum freezing temperature and freezing time of a product, [3], [4]:

- Determining the time of the freezing process in order that the center temperature of foods reach freezing point. It means that water inside the product completely crystallized, [5], [6].
- Determining the time of the freezing process in order that the product temperature reachs the optimal freezing temperature. When water is completely frozen (ω = 1 or 100%), [6], [7], [8].

There were many research on mathematical modeling about the rate of freezing water of flat – shaped cattle meat (Plank R et al, 1913), frozen velocity of water inside flat – shaped fish fillet (Lame, Clapeiron, Shijov G.B, 1931), rate of freezing water in wet materials (Plank, Veinik, 1937; Raoult, 1958; Sbijov G.B,1967; Golovkin N.A, 1972; Luikov, A.V,1974; Dennis R. Hledman,1999). However, mathematical modeling of these authors was not suitable for determining rate of freezing water in *Penaeus Monodon* in ĐBSCL of Vietnam because experimental results showed that error between the mathematical model and experimental data was higher than 38.45% [8], [9], [10], [11].

Because of water in food always contents dissolving compounds. Therefore, crystallization temperature, latent heat of freezing of water and other thermophysical parameters constantly change during the freezing process [10], [11]. These are the main causes of error between the mathematical model with experimental data. In case of large error, it will not allow the use of mathematical modeling to determine the technological mode, [12], [13].

For this reason, the problem posed here was finding a new method to determine the rate of freezing water according to the freezing temperature of *Penaeus Monodon* and to determine the optimal freezing temperature of *Penaeus Monodon* in freezing process.

Nomenclature

- $\omega \in [0,1]$: rate of freezing water
- ω_E: rate of freezing water determined by experimental method
- $W_a = 0.7467 = 74.67\%$: initial moisture of *Penaeus Monodon*.
- $c_n = 4184.7 + 1.74T$ (J/(kg.K): Specific heat of water
- $c_{nd} = 2090 + 7.79T$ (J/(kg.K)): Specific heat of ice
- $c_{ck} = 1805.36 + 1.91T$ (J/(kg.K)): Specific heat of the dry matter inside penaeus monodon.
- c ($J.kg^{-1}.K^{-1}$) Specific heat of *Penaeus Monodon* when water is crystallized
- $c_1 = 380$ ($J.kg^{-1}.K^{-1}$): Specific heat of copper.
- G (kg): weight of *Penaeus Monodon* sample
- $G_1 = 0.125$ (kg): weight of copper box in equipment determine specific heat moist material.
- $T_{kt} = -1.21^0C$: freezing temperature of water inside *Penaeus Monodon*.
- $T_p = 25^0C$: room temperature.
- T_F (^0C): temperature of *Penaeus Monodon* when water completely crystillized.
- $T_d = T_1 = T_2 = T_3$ (^0C): initial temperature of *Penaeus Monodon* sample.
- $T_c = T_1' = T_2' = T_3'$ (^0C): temperature of *Penaeus Monodon* sample after supplying energy
- $T = (T_d + T_c)/2$ (^0C): average temperature of *Penaeus Monodon*.
- $r_{nc} = L = -0.000021T^2 + 1.054T + 333601.5$ (J/kg): Latent heat of freezing of water
- U (V): number of voltmeter.
- I (A): number of amperemeter.
- τ (s): heat supply time.
- $\varphi = 0.1101$: the loss of heat coefficient

1. BUILDING THE METHOD TO DETERMINE THE RATE OF FREEZING WATER

1.1. The basic concepts

- The freezing process of *Penaeus Monodon* has three stages (Fig.1), [1-2], [19]:

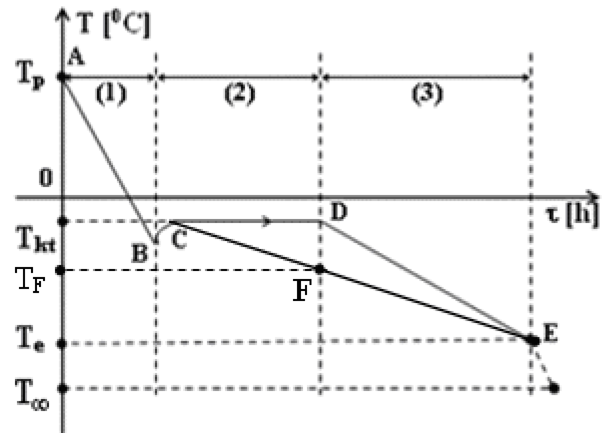

Fig 1. Penaeus monodon frozen process

AB: cooling stage; BC: extreme cold stage; CD: crystalline water in humidity materials stage of theory; CF: crystalline water in humidity materials stage of actuality; DE, FE: thermal equilibrium stage.

In figure 1, if the process carried out from A to E (A → B → C → F → E), it would be called the cooling and the freezing process. Whereas, if the carried out from E to A (E → F → C → B → A), it would be called the melting and the warm up process [11], [15].

a) Cooling stage: reduce *Penaeus Monodon* temperature from the initial temperature $T_P = 25^0C$ (room temperature) to the freezing temperature of water inside the *Penaeus Monodon* $T_{kt} = -1.21^0C$ before freezing the *Penaeus Monodon,* [11], [16], [17].

b) Freezing stage: crystallize water inside the *Penaeus Monodon* in environment with temperatures of $T_e = -45^oC$. This stage finished when the water inside the *Penaeus Monodon* completely crystallized ($\omega = 1$ or 100%). At this point, the optimal freezing temperature of *Penaeus Monodon* is T_F (^oC), [17], [18].

c) Energy balance stage: reducing the temperature of *Penaeus Monodon* from T_F (^oC) to the final temperature T_e (^oC) with $T_e \leq T_F$, [17], [18].

It is obvious that *Penaeus Monodon* is frozen to reach the freezing temperature of T_E (^0C), in figure 1, after heat supply to carry out the melting and the warm up process to determine specific heat of *Penaeus Monodon*. And via the determination of specific heat of the *Penaeus Monodon* will build the new method to detremine rate of water freezing inside *Penaeus Monodon*.

1.2. Building the method to determine rate of freezing water

This method was built on base the energy balance equation in warming up process *Penaeus Monodon* after the freezing process to determine specific heat of *Penaeus Monodon* by the experiment. The results obtained could be applied to determine the optimal freezing temperature of *Penaeus Monodon* of the freezing process ($T = T_F$). When temperature of *Penaeus Monodon* reached the optimal freezing temperature, the rate of freezing water in *Penaeus Monodon* was 100% ($\omega = 1$), [11], [18].

- The rate of freezing water (ω) inside *Penaeus Monodon* was defined as follow, [11]:

$$\omega = \frac{G_{db}}{G_n} \qquad (1)$$

Where: G_{db}, G_n – amount of crystallized water and total water inside *Penaeus Monodon*, kg.

-The rate of freezing water ω ($0 \le \omega \le 1$) was determined via the determination of specific heat of the *Penaeus Monodon* with equipment in Fig 3.

The *Penaeus Monodon* was put into the copper box of equipment in Fig 3. The freezing process was carried out until the temperature of *Penaeus Monodon* in the copper box reached -40.5^{0}C after putting this box in the system of freeze DL-3 (Fig 2) and before determining the specific heat of the *Penaeus Monodon*.

The heat was supplied by the electric resistance ($Q = UI\tau$, J) to determine specific heat of the *Penaeus Monodon*. It was divided into three parts as follows:

$$Q = Q_s + Q_{cu} + Q_{dn} \qquad (2)$$

Where:

Q_s (J): the loss of heat pass the heat-insulated surrounding area of equipment in Fig 3.

Q_{cu} (J): the heat warm up the copper box in equipment in Fig 3.

$$Q_{cu} = G_1 c_1 (T_c - T_d), \text{ (J)} \qquad (3)$$

Q_{dn} (J): the heat warm up *Penaeus Monodon* to put into the copper box of equipment in Fig 3.

$$Q_{dn} = Gc(T_c - T_d), \text{ (J)} \qquad (4)$$

From (2) can write as follows:

$$Q_s = Q - (Q_{cu} + Q_{dn})$$

$$\varphi = \frac{Q_s}{Q} = 1 - \frac{(G_1 c_1 + Gc)}{UI\tau}(T_c - T_d) \qquad (5)$$

Where: φ is the loss of heat coefficient, it was determined by the denatured protein, the specific heat of the denatured protein was $c_0 = c = 2045.1$ J/(kg.K).

The heat was supplied by electric resistance after putting $G_0 = G = 0.112$ kg denatured protein into the copper box of equipment in Fig 3 with $T_d = 28.85^0C$ until the time reached $\tau = 80s$ to determine $U = 60V$; $I = 0.25A$; $T_c = 32.51^0C$. From (5), the loss of heat coefficient determined $\varphi = 0.1101$.

From (4) can write as follow:

$Q_{dn} = (1 - \varphi)Q - Q_{cu} = (1 - \varphi)UI\tau - G_1 c_1 (T_c - T_d)$, (J) (6)

The heat warm up *Penaeus Monodon* to determine specific heat. It was divided into four parts as follows:

$$Q_{dn} = Gc(T_c - T_d) = Q_1 + Q_2 + Q_3 + Q_4, \text{ (J)} \qquad (7)$$

Where:

Q_1 (J): the heat warm up to thaw a part of the crystallize water inside the *Penaeus Monodon*.

$$Q_1 = LW_a(1 - \omega)G, \text{ (J)} \qquad (8)$$

Q_2 (J): the heat warm up to vary temperature of the crystallize water inside the *Penaeus Monodon* form T_d to T_c.

$$Q_2 = c_{nd}GW_a\omega(T_c - T_d), (J) \quad (9)$$

Q_3 (J): the heat warm up to vary temperature of water after thawing inside the *Penaeus Monodon* form T_d to T_c.

$$Q_3 = c_nG\, W_a(1 - \omega)(T_c - T_d), (J) \quad (10)$$

Q_4 (J): the heat warm up to temperature of the matter inside the *Penaeus Monodon* form T_d to T_c.

$$Q_4 = c_{ck}G(1 - W_a)(T_c - T_d), (J) \quad (11)$$

Substituting (8), (9), (10) and (11) into (7) found:

$$c = c_nW_a(1-\omega) + c_{nd}W_a\omega + c_{ck}(1-W_a) + \frac{LW_a(1-\omega)}{(T_c - T_d)} \quad (12)$$

- When $T > T_{kt}$, $\omega = 0$, $Q_1 = 0$, $Q_2 = 0$ and

$$c = c_nW_a + c_{ck}(1 - W_a) \quad (13)$$

- When $T_F < T \le T_{kt}$, $0 \le \omega \le 1$ and

$$c = c_nW_a(1-\omega) + c_{nd}W_a\omega + c_{ck}(1-W_a) + \frac{LW_a(1-\omega)}{(T_c - T_d)} \quad (14)$$

- When $T \le T_F$, $\omega = 1$, $Q_1 = 0$, $Q_3 = 0$ and

$$c = c_{nd}W_a + c_{ck}(1 - W_a) \quad (15)$$

From equation (14), the rate of freezing water can be written as follow:

$$\omega = \frac{c - \left(\left(c_nW_a + c_{ck}(1-W_a)\right)(T_c - T_d) + LW_a\right)}{\left((c_{nd} - c_n)(T_c - T_d) + L\right)W_a} \quad (16)$$

Where:

$$c = \frac{(1-\varphi)UI\tau - c_1G_1(T_c - T_d)}{G(T_c - T_d)} = \frac{(1-\varphi)UI\tau}{G(T_c - T_d)} - \frac{c_1G_1}{G}, (J/(Kg.K)) \quad (17)$$

From equation (16) and (17), the rate of freezing water was written as follow [11, 18]:

$$\omega = \frac{(1-\varphi)UI\tau - LW_aG}{\left((c_{nd} - c_n)(T_c - T_d) - L\right)W_aG} - \frac{\left[G\left(c_nW_a + c_{ck}(1-W_a)\right) + c_1G_1\right](T_c - T_d)}{\left((c_{nd} - c_n)(T_c - T_d) - L\right)W_aG} \quad (18)$$

Where: T (^{0}C) – average temperature of *Penaeus Monodon*, $T = (T_d + T_c)/2$.

With: $\phi_1 = LW_aG$;

$\phi_2 = (c_nW_a + c_{ck}(1-W_a))G + c_1G_1$;

$\phi_3 = GW_a(c_{nd} - c_n)$

Therefore :

$$\omega = \frac{(1-\varphi)UI\tau - \phi_1 - \phi_2(T_c - T_d)}{\phi_3(T_c - T_d) - \phi_1} \quad (19)$$

•Where: c_1, G_1: specific heat and weight of flat-shaped copper in equipment (Fig. 3); G: weight of *Penaeus Monodon* sample; $T_d = T_1 = T_2 = T_3$: initial temperature of *Penaeus Monodon* sample. $T_c = T_1' = T_2' = T_3'$: temperature of *Penaeus Monodon* sample after supplying energy. U: number of voltmeter, I: number of amperermeter, τ: energy supply time.

•The equipment (Fig.3) was surrounded by heat-insulated material to ensure that almost energy from electric resistance transmit to *Penaeus Monodon* sample and a little the loss of heat to surrounding ($\varphi = 0.1101 = 11.01\%$).

2. Materials and method

2.1. Materials

Penaeus Monodon is grown in the Flat country of Mekong river (ĐBSCL) of Vietnam [17].

Penaeus Monodon of approximately (40 ÷ 50) body/kg is cut off head, and removed cover [17].

2.2. Apparatus

Equipments used to determine specific heat of *Penaeus Monodon* are listed [18]:

- Determining weigh of *Penaeus Monodon* by Satoriusbasic Type BA310S: range

scale (0 ÷ 350)g, error: ± 0.1g = ± 0.0001 kg, [18].

- Determining temperature of *Penaeus Monodon* by Dual Digital Thermometer: range scale (-50 ÷ 70)^{0}C, error ± 0.05^{0}C, [18].

- DL-3 Freezing System (Fig. 2) could reduce the temperature of environment to (-50 ÷ -45)^{o}C . The temperature profile is measured by the automatic control system PLC, [18].

Fig 2. The Systems of freeze DL-3, the temperature of freezing enviroment (-50 ÷-45)

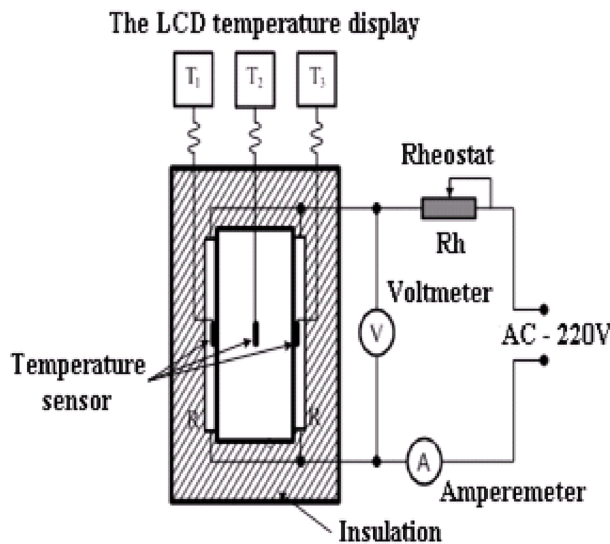

Fig 3. Principle diagram of the equipment determine the specific heat of moist material

- Equipment used to identify specific heat was shown in Fig.3. The equipment includes a Voltmeter (range scale: (0 ÷ 110)V, error: ± 1V), an amperemeter (range scale: (0 ÷ 2)A, error: ± 10mA) and an automatic timer (error: ± 0.001s). The Voltmeter is used to measure the potential difference of resistance (R). The Amperemeter is used to determine the current intensity which pass through 2 resistances (R) (Fig.3).

2.3. Methods

- To determine the rate of freezing water, the experiment was carried out through 5 steps as follow, [18]:

  - **Step 1**: *Penaeus Monodon* was placed in the copper box of equipment in Fig.3, weighed the *Penaeus Monodon* samples were G (kg), the samples were freezed the by the system of freeze DL-3 (Fig 2) until the average temperature of the samples reached – 40°C, [18].

  - **Step 2**: Place the copper box content *Penaeus Monodon* samples into the equipment in Fig.3. The initial temperature of *Penaeus Monodon* (T_d = T_1 = T_2 = T_3, ^{0}C) was determined. The samples were then supplied with energy from the resistance. Parameters such as U(V), I(A) and energy supply time τ (s) were determined. Subsequently, the system stopped supplying energy. Temperature of fillet samples increased from (T_1, T_2, T_3) to (T_1', T_2', T_3'). When energy balance occurred, T_c = T_1' = T_2' = T_3' (^{0}C), [18].

  - **Step 3:** calculate the average temperature of the samples $T = (T_d + T_c)/2$, determine c_n, c_{nd}, c_{ck} with T, [18].

  - **Step 4**: calculate ϕ_1, ϕ_2, ϕ_3, [18].

  - **Step 5**: substituting ϕ_1, ϕ_2, ϕ_3 into the equation (19) to determine relationship between rate of freezing water inside *Penaeus Monodon* sample and average temperature T, [18].

- The results obtained were represented in Table 1 and Table 2.

- From the experimental datas in Table 1 and Table 2 were determined the rate of freezing

water according to the freezing temperature of *Penaeus Monodon* and optimal freezing temperature of *Penaeus Monodon*.

4. Results and discussion

4.1. Determining the rate of freezing water according to the freezing temperature of Penaeus Monodon by the mathematical model (19)

Experiments were carried out according to five steps in section 3.3. With:

$G_1 = 0.125$ kg; $c_1 = 380$J/(kg.K); $G = 0.112$ kg.

$c_n = 4184.7 + 1.74T$ (J/(kg.K)

$c_{nd} = 2090 + 7.79T$ (J/(kg.K))

$c_{ck} = 1805.36 + 1.91T$ (J/(kg.K))

$L = -0.000021T^2 + 1.054T + 333601.5$ (J/kg)

$T = (T_d + T_c)/2$; $\varphi = 0.1101$.

The results have determined T_d, T_c, ϕ_1, ϕ_2, ϕ_3, U, I and τ and were presented in Table 1 and Table 2.

Table 1. The experimental value of T_d, T_c, T, ϕ_1, ϕ_2, ϕ_3 of of penaeus monodon

T_d (^{0}C)	T_c (^{0}C)	T (^{0}C)	ϕ_1	ϕ_2	ϕ_3
-2	2	0	27899.10	448.69	-175.18
-2.5	-1.5	-2	27898.92	448.29	-176.19
-4.5	-3.5	-4	27898.75	447.89	-177.20
-6.5	-5.5	-6	27898.57	447.49	-178.22
-8.5	-7.5	-8	27898.39	447.09	-179.23
-10.5	-9.5	-10	27898.22	446.69	-180.24
-12.5	-11.5	-12	27898.04	446.29	-181.25
-14.5	-13.5	-14	27897.87	445.89	-182.26
-16.5	-15.5	-16	27897.69	445.49	-183.28
-18.5	-17.5	-18	27897.51	445.09	-184.29
-20.5	-19.5	-20	27897.34	444.69	-185.30
-22.5	-21.5	-22	27897.16	444.29	-186.31
-24.5	-23.5	-24	27896.98	443.89	-187.32
-26.5	-25.5	-26	27896.81	443.49	-188.34
-28.5	-27.5	-28	27896.63	443.09	-189.35
-30.5	-29.5	-30	27896.45	442.69	-190.36
-32.5	-31.5	-32	27896.28	442.29	-191.37
-34.5	-33.5	-34	27896.10	441.90	-192.38
-36.5	-35.5	-36	27895.93	441.50	-193.40
-38.5	-37.5	-38	27895.75	441.10	-194.41
-40.5	-39.5	-40	27895.57	440.70	-195.42

Table 2. The experimental value of T, U, I and τ of of penaeus monodon and the rate of freezing water with freezing temperature of penaeus monodon

T (^{0}C)	U (V)	I (A)	τ (s)	ω_E
0	120	2.50	111.24	0.0000
-2	120	2.50	90.01	0.1539
-4	120	2.50	75.51	0.2917
-6	100	2.50	68.39	0.4677
-8	100	2.50	48.94	0.6218
-10	90	2.50	36.74	0.7475
-12	90	2.50	28.59	0.8056
-14	80	2.50	19.80	0.8839
-16	80	0.25	94.26	0.9496
-18	60	0.25	42.89	0.9889
-20	60	0.25	22.38	0.9986
-22	60	0.25	19.33	1.0000
-24	60	0.25	19.22	1.0000
-26	60	0.25	19.12	1.0000
-28	60	0.25	19.01	1.0000
-30	60	0.25	18.91	1.0000
-32	60	0.25	18.80	1.0000
-34	60	0.25	18.69	1.0000
-36	60	0.25	18.59	1.0000
-38	60	0.25	18.48	1.0000
-40	60	0.25	18.38	1.0000

From Table 1 and Table 2, substituting value of T_d, T_c, ϕ_1, ϕ_2, ϕ_3, U, I and τ into the equation (19) to deternine the rate of freezing water with the temperature of *Penaeus Monodon* and were presented in Table 2.

4.2. Determining the optimal freezing temperature of Penaeus Monodon

It was obvious that mathematical model (19) was built by the energy balance equation. In there, parameters of mathematical model (19) were determined by the experiment. From results in Table 1 and Table 2 was determined the optimal freezing temperature of *Penaeus Monodon* in order that water inside *Penaeus Monodon* was completely crystallized.

When temperature of *Penaeus Monodon* was -22.00^{o}C ($T = T_F = -22.00^0$C). By the mathematical model (19), the rate of freezing water inside *Penaeus Monodon* was determined 100% (ω_E = 100% or 1). Therefore, mathematical model (19) can be not only used to set up parameters for the operation of the freezing system but also to determine technological mode in freezing process of

Penaeus Monodon which grown in the Flat country of Mekong river, Vietnam.

Currently, factories often reduce the freezing temperature of *Penaeus Monodon* to (-18 ÷ -16)^{0}C for using in preservation. In such low temperature, microorganisms test showed that microorganisms could not grow, reproduce and most of the microorganisms are inactivated. However, the optimal freezing temperature of *Penaeus Monodon* has not exactly determined yet.

Different with the technological freezing for using preservation only need the rate of freezing water inside *Penaeus Monodon* to reach over 86%. Whereas, the technological freezing of *Penaeus Monodon* for using in the freeze drying need to have to reach 100%. Therefore, the optimal freezing temperature of *Penaeus Monodon* must be determined in order that the water in *Penaeus Monodon* was completely crystallized. The results showed that the optimal freezing temperature of *Penaeus Monodon* was – 22.00^{o}C and corresponding to ω_E = 100% or 1 (see in Table 2).

These results were not only suitable to large-scale process but also a technological solution for factories to improve the freezing process, saving energy costs when the freezing system is operated, [6], [16].

In addition, results obtained were able to apply for building the mathematical model to describe about relationship the rate of freezing water inside *Penaeus Monodon* and the freezing temperature of *Penaeus Monodon*, it can see this result in [18].

5.Conclusions

- The new method was built to determine the rate of freezing water according to the freezing temperature of *Penaeus Monodon* by the energy balance equation of determining specific heat of *Penaeus Monodon*.

- From the energy balance equation has established the mathematical model (19) which was completely compatible with experiment. The results obtained also determined the optimal freezing temperature of *Penaeus Monodon* which grow in ĐBSCL, Vietnam. It was completely compatible with large-scale process.

- Calculating the mathematical (19) determined technological mode for the freezing process of *Penaeus Monodon* in ĐBSCL, Vietnam.

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EFFECT OF VARRYING PULP CONCENTRATION ON SENSORY QUALITY OF NONI (*MORINDA CITRIFOLIA L.*) - TOFFEE BLENDED WITH PAPAYA AND GUAVA PULP

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ABSTRACT

Noni fruit has globally accepted as a source of more than 150 nutracueticals. Amongst various noni based products. Noni juice is having significant commercial values. However, the pulp after extraction of Noni juice is also rich in various nutraceuticals but yet underutilized. In present investigation, the efforts were made to judge the suitability of noni pulp in preparation of toffees. Toffee was prepared by blending noni pulp with papaya and guava pulp. Different levels of incorporation of noni pulp were made in order to assess the suitability of noni pulp and its effects on the organoleptic quality characteristics of toffee. The results revealed that Noni toffees could be successfully prepared by utilizing noni pulp. The toffee containing 90 per cent of noni pulp with 10 per cent of papaya pulp and 93 per cent noni pulp and 7 per cent guava pulp was found to show significantly higher overall organoleptic acceptability. On the basis of present investigation, it seems that noni pulp could be successfully utilized in preparation of toffees while blends of different fruit pulps could be used in enhancing the consumer acceptability of Noni pulp based toffees.

Keywords: *Morinda citrifolia, Noni pulp, blend, toffee, sensorial quality. Neutraceuticle*

1.Introduction

Practical Applications:

Noni juice can be more popularized in the form of RTS, beverages, squash, concentrated liquid, flavoured beverages, fortified beverages, mixed or blended juice, wine etc. Pulp can be successfully exploited in preparation of fruit leather bar, pulp concentrate, herbal prash, puree, jam, spread, mixed fruit pulp, mix-fruit prash, powder, sauce, chutney, toffee etc. The fruit powder can be reconstituted with water and later concentrated or used as base for RTS, squash, flavoured beverages, fruit drink etc. It may contain added flavouring substances, honey or sugar for taste modification. The powder can be encapsulated or used for tablet making. It have a broad range of therapeutic effects, it's juice is equally effective for diabetics and hypertension (Yanine *et al.*, 2006). Recently, the fruit juice is in high demand as food supplement or alternative herbal medicine for different kind of illnesses.

Morinda citrifolia Linn. – an Indian mulberry or "Noni" in English, and "Yor" in Thai, is the plant that can be used as a raw material for nutraceutical and functional food products (Pranee et al. 2005). Noni made a remarkable transition from traditional herbal medicine to modern natural remedy over the last few decades. Noni grows extensively throughout the Pacific and is widely used as herbal medicine in almost all Pacific island communities. Noni juice has become increasingly popular in recent years as a health food drink in western and Asian countries where there appears to be substantial market (Murukesen, 2007). Noni has been used in folk medicine for over 2000 years, it's every part i.e. roots, stem, bark, leaves, flowers and fruit is utilized in various combinations for herbal remedies (Tabrah and Eveleth, 1966). It have a broad range of therapeutic effects, it's juice is equally effective for diabetics and hypertension (Yanine *et al.*, 2006). Recently, the fruit juice is in high demand as food supplement or alternative herbal

medicine for different kind of illnesses. With the increasing demand, both the harvesting and processing of Noni fruits got more importance for the product preparation.

Noni juice can be more popularized in the form of RTS, beverages, squash, concentrated liquid, flavoured beverages, fortified beverages, mixed or blended juice, wine etc while the pulp is yet to find its suitability in value added products. Hence, the present investigation was carried out to utilize noni fruit pulp in preparation of preparation of toffees. Different levels of papaya and guava pulp were added separately with the objective of enhancing the organoleptic properties of noni pulp based toffee.

2. Materials and methods

Raw Materials

Noni fruits were collected from college orchard. The fruits were washed, cleaned and used for the experiment. All the other ingredients were obtained from the local market of Parbhani (MS) India.

Standardization for preparation of Noni Toffee by blending with Papaya Pulp:

The Noni pulp extracted from well matured Noni fruits having TSS 8°bx with acidity 1.28 %, was standardized by blending with Papaya pulp having TSS 11^{0} .Toffees were prepared using 95%,93% and 90% Noni pulp and 5%, 7% and 10% papaya pulp respectively.

Table.1 Preparation of Noni Toffee by blending with Papaya Pulp

Sr. No.	Treatments	Noni plup (%)	Papaya pulp (%)
1	(Control)T_1	100	--
2	T_2	95	5
3	T_3	93	7
4	T_4	90	10

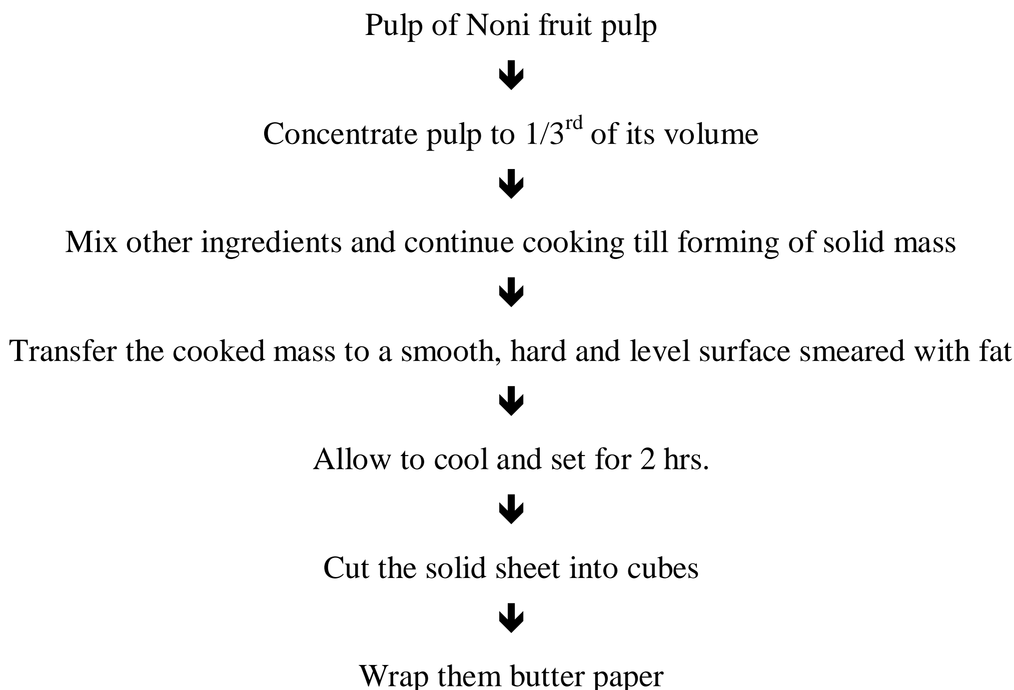

Figure1.Flow sheet for Preparation of Noni fruit toffee

Standardization for preparation of Noni -toffee by blending with Guava Pulp:

Noni –guava toffee is prepared using Noni pulp having TSS 8^0 brix with acidity 1.28 % and Guava pulp having TSS 12^0 brix with acidity 0.48 %. The different combinations of Noni-Guava pulp were used(Table-1) for standardization i.e. Guava pulp is 5%, 7% and 10% and Noni pulp is 95%, 93% and 90% respectively.

Table 2. Preparation of Noni Toffee by blending with Guava Pulp

Sr. No.	Treatments	Noni pulp (%)	Guava pulp (%)
1	T_1	100	--
2	T_2	95	5
3	T_3	93	7
4	T_4	90	10

Sensorial Analysis: Sensory analysis of prepared product was performed by using standard method (Amerine *et al.*, 1987).

Statistical analysis: The analysis of variance of the data obtained was done by using Completely Randomized Design (CRD) for different treatments as per the methods given by Panse and Sukhatme (1967). The analysis of variance revealed at significance of $P < 0.05$ level, S.E. and C.D. at 5 % level is mentioned wherever required.

3. Results and discussion

Organoleptic evaluation of Noni-Papaya Toffee:

The average score values of organoleptic evaluation of Noni-Papaya Toffee are presented in (Table-3). It is evident from the values (Table-2) that sample T_3 was found organolepically superior for attributes like colour, flavor, taste and consistency as compared to other sample T_1, T_2 and T_4. Moreover, sample T3 has got highest score for overall acceptability indicating very much liked by the panel members.

Table 3. Organoleptic evaluation of Noni-Papaya Toffee.

Sr. No	Sample code	Appearance	Color	Flavor	Taste	Consistency	Overall acceptability
1	T_1	4.2	4.3	3.9	4.0	4.6	4.5
2	T_2	6.0	6.3	5.9	6.4	6.1	6.0
3	T_3	7.0	7.4	7.2	7.8	7.7	7.8
4	T_4	7.1	7.0	7.2	7.5	7.3	7.5
SE $\pm$		0.07	0.14	0.13	0.15	0.14	0.13
CD at 5%		0.25	0.41	0.38	0.43	0.37	0.37

Organoleptic evaluation of Noni – Guava Toffee

Organoleptic evaluation of Noni – Guava Toffee summarized in table-4. Table 4 revealed the sensory evaluation of Noni-Guava Toffee, and Noni Toffee prepared was used as control. As the Noni plup was having unpleasant aroma and taste. It was blended with Guava pulp at 5%, 7%, 10% concentration. Sample T_3 found organoleptically better as compared to samples T_1, T_2, and T_4 and scored high for overall acceptability and was very much liked by all the panel members.

Table 4. Organoleptic evaluation of Noni – Guava Toffee:

Sr. No	Sample code	Appearance	Color	Flavor	Taste	Consistency	Overall acceptability
1	T_1	4.2	4.3	3.9	4.0	4.6	4.5
2	T_2	6.2	6.8	6.6	6.7	6.7	6.7
3	T_3	7.3	7.7	7.2	7.1	7.4	7.9
4	T_4	7.1	7.2	7.0	7.1	7.2	7.4
SE ±		0.12	0.14	0.12	0.19	0.16	0.14
CD at 5%		0.34	0.38	0.32	0.54	0.45	0.41

5.Conclusion

The Noni fruits are edible but don't have palatable taste and flavour to make them organoleptically accepted and hence in the present investigation attempt have been made to prepare Toffee using Noni fruits blending with Papaya and guava pulp separately. The toffee containing 90 per cent of noni pulp with 10 per cent of papaya pulp and 93 per cent of noni pulp with 7 per cent of Guava pulp respectively found to show significantly higher overall organoleptic acceptability.

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DETERMINATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OIL ISOLATED FROM *ROSMARINUS OFFICINALIS L.*

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ABSTRACT

The present work reports on the evaluation of chemical composition and antioxidant and antimicrobial activities of rosemary essential oil, used in traditional medicine: *Rosmarinus officinalis* L. The principal components of essential oil was: 1.8-cineole (24.5%), camphene (13.38%), α-pinene (12.9%), β-pinene (6.95%). Essential oil showed antioxidant activity either by DPPH radical scavenging method. Antimicrobial activity of essential oil was examined against *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* by agar diffusion method. The essential oil showed antibacterial activity against all of the tested Gram (-) bacteria and had no effect on the entire tested Gram (+) bacteria. Having in account the important antioxidant and antimicrobial properties observed in present work, we consider that rosemary essential oil might be useful on pharmaceutical and food industry as natural antibiotic and food preservative.

Keywords : *Rosmarinus officinali, rosemary essential oil, antimicrobial activity*

1.Introduction

Rosemary (*Rosmarinus officinalis* L.) is an aromatic, medicinal and condiment plant that belongs to the Family Labiatae. It is widely spread in Algeria and broadly used in traditional medicine. Rosemary is selected because it is of interest as a preservative due to its antioxidative characteristics and it is used in the pharmaceutical, food and cosmetic industries.[1]

2. Materials and Methods

Chemicals, reagents and plant materials. Chemicals and reagents were supported by Sigma-Aldrich (Steinheim, Germany). Leaves dried of *Rosmarinus officinalis* L., Labiatae, were bought from a store natural plants (AdNatura Cluj-Napoca).

Distillation method. The herb material (100 g) was submitted to water distillation for 4 h using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers, and stored at +4°C until analysed by GC-MS.

Gas chromatography-mass spectrometry (GC-MS). The essential oil was analyzed using a gas chromatography (GC) system[2] equipped with a flame ionization detector and Rtx-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The column temperature was programmed from 50°C (2 min) to 150°C at 4°C/min and until to 250°C at 10°C/min. The interface temperature was 250°C. The flow rate of carrier gas (helium) was 2.8 ml/min. The MS conditions were: the ionization voltage 70 eV and the electron emission current 100 µA. The components were identified by comparing their mass spectral data with those in the WILEY and the NIST mass spectra libraries.

Microbial strains. In order to evaluate the activity of the essential oil of

Rosmarinus officinalis, the following microorganisms were used: *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*.

Antimicrobial screening. The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oil.[3] The above-mentioned bacteria were cultured on the Nutrient broth at 37 ± 0.1°C. Each suspension of the tested microorganism (1×10^6 CFU/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with undiluted rosemary essential oil (10 µl, 20 µl) and placed on the incubated plates. The plates were kept at room temperature for 30 min and than incubated at 37°C for 20 h. The results reading was carried out by measuring the diameters of the inhibition zones, clear growth in mm. In addition, reference antibiotic discs such as penicillin G and chloramphenicol were used for comparison under the same conditions as in the essential oil experiment. All tests of the inhibitory activity were carried out in duplicates and the developing inhibition zones were compared with those of reference discs. The essential oil was also subjected to the sterility test and was found to be free of microorganisms.

DPPH radical scavenging activity. The antiradical activity of the extract was assessed on the basis of the radical-scavenging effect of the stable 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical. A series of essential oil concentration in the same extraction solvent was prepared (20, 30, 50, 70, 100 mg/ml). Then, 30 µl of essential oil at different concentrations was mixed with 950 µl ethanol and 20 µl of 3.8 mM DPPH in ethanol. The disappearance of DPPH was read spectrophotometrically at 517 nm after 600 s of incubation at room temperature in the dark. The measurements were performed in triplicate and the results were averaged. Free radical scavenging capacity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

$$I(\%) = (1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}) * 100 \quad (1)$$

From the obtained values, the IC_{50} (defined as the concentration of extract at which 50% of maximum scavenging activity was recorded) was calculated for each extract.[4]

3. Results and Discussion

Hydrodistillation of the *Rosmarinus officinalis* leaves yielded 1.34% of essential oil (w/w, based on the dried weight of the leaves). The results obtained are similar to those reported in the literature where the yield was 0.5–2% (w/w, based on the dried weight of the leaves).[5]

The chemical composition of the hydrodistilled rosemary essential oil is shown in Table 1. GC-MS analyses revealed that the major component was 1.8-cineole (24.5%), while α-thujene (0.22%) and linalool (0.25%) were minor main components. Other compounds identified in the oil obtained were camphene, α-pinene, β-pinene, verberone, borneol, camphor, limonene, α-phellandrene, γ-terpinene, α-terpinene. The rosemary oil consisted 1.8-cineole determines the commercial value of the oil and its importance as a raw material for different industries.

Table 1. Chemical composition of the leaf essential oil of *Rosmarinus officinalis*

	Compound	**MW**	**RT (min)**	**A (%)**
1	α-thujene	136	5.47	0.22
2	α-pinene	136	6.03	12.9
3	camphene	136	6.58	13.38
4	β-pinene	136	7.85	6.95
5	α -phelandrene	136	8.76	0.98
6	α -terpinene	136	9.85	0.86
7	para-cymene	132	10.63	1.27
8	limonene	136	12.06	1.32
9	1,8-cineole	154	13.41	24.5
10	γ -terpinene	136	14.45	0.86
11	linalool	154	14.87	0.25
12	borneol	154	17.02	2.86
13	camphor	152	18.63	1.48
14	verberone	150	20.43	3.6
15	bornyl acetate	196	21.75	0.90
16	geranyl acetate	196	24.74	0.25

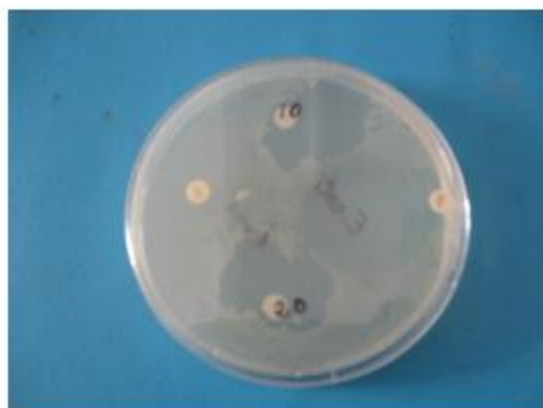
MW- molecular weight; RT- Retention time (min); A- Area GC-MS (%).

Table 2. Antimicrobial activity of the *Rosmarinus officinalis* essential oil and some standard antibiotics against Gram-positive and Gram-negative bacteria

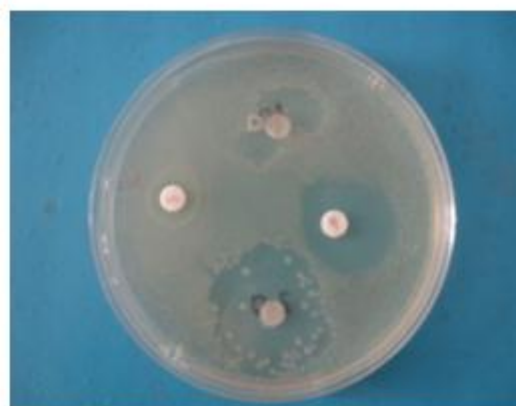
Microorganism	**Standard antibiotics (µg)**		**Rosmarinus officinalis oil (µl)**	
	Penicillin G	Chloramphenicol	10	20
	Inhibition zone (mm)			
Klebsiella pneumoniae	15	20	25	30
Escherichia coli	24	30	20	30
Staphylococcus aureus	25	30	20	20
Enterococcus faecalis	40	15	10	10

Antimicrobial activity. The antimicrobial plate diffusion assay for *Rosmarinus officinalis* essential oil, as summarised in the Table 2 showed that different microorganisms tested had different susceptibility to the same essential oil. Rosemary oil was very potent against all the selected microorganisms except *Stapylococcus aureus* and *Enterococcus faecalis* (Figure 1).

Gram - negative

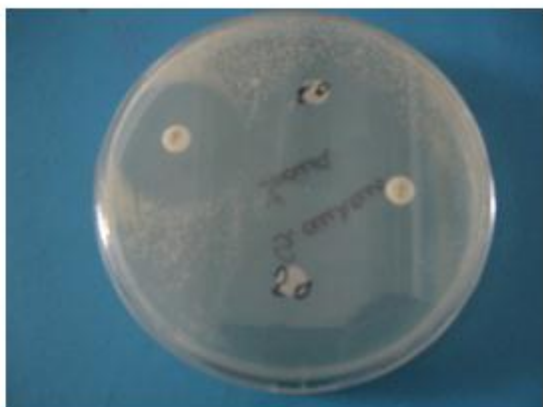


Escherichia coli

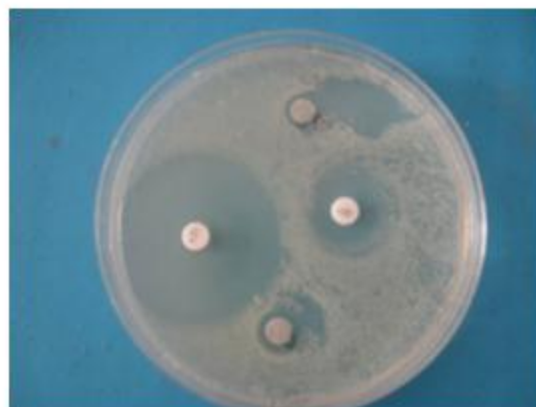


Klebsiella pneumoniae

Gram - pozitive



Staphylococcus aureus



Enterococcus faecalis

Figure 1. Inhibition zones for Gram (-) bacteria and Gram (+) bacteria - agar diffusion method for Rosmarinus officinalis essential oil

Antioxidant activity. The antioxidant activity of the ethanol extract was measured spectrophotometrically through DPPH free radical scavenging. The essential oil of *Rosmarinus officinalis* possesses free radical scavenging and antioxidant properties.

In this study, the DPPH method was selected to evaluate the antioxidant activity of the rosemary essential oil because it is one of the most effective methods for evaluating the concentration of radical scavenging materials that are very active in the chain breaking mechanism. The IC_{50} value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period, is a parameter widely used to measure antioxidant activity; a smaller IC_{50} value corresponds to a higher antioxidant activity of the plant extract.

In the Figure 2. we observed the decrease of DPPH free radical absorbance measured at 517 nm according to the concentration of rosemary essential oil. At increasing the essential oil concentration increases the amount of DPPH* reduced by antioxidant.

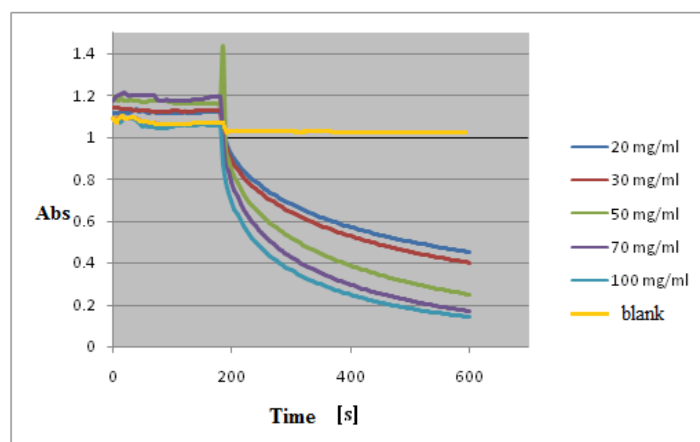


Figure 2. The decrease of DPPH absorbance 3,8 mM according with different concentrations of essential oil*

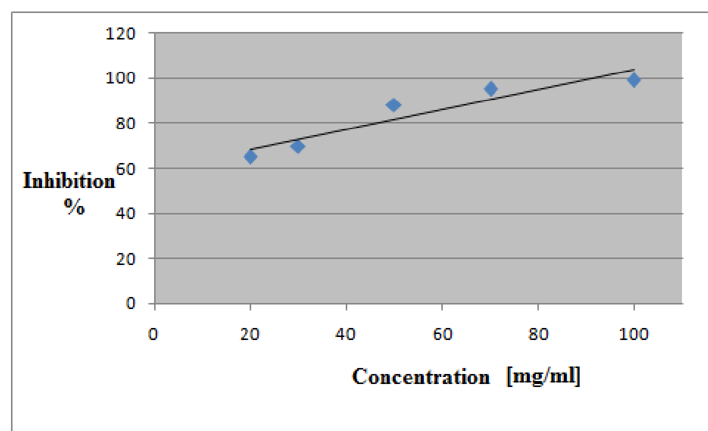


Figure 3. Antiradical activity of the rosemary essential oil

In the Figure 3. is presented the degree of inhibition I(%) depending on the concentration of essential oil. We observed that a dose-response relationship is found in

the DPPH radical scavenging activity; the activity increased as the concentration increased.

4.Conclusions

Our results suggest that the essential oil of *Rosmarinus officinalis* L. may be used as a natural antioxidant and as antimicrobial agents. The antimicrobial activity of rosemary essential oil could be associated with the presence of 1.8-cineole and linalool. 1.8-Cineole as well as linalool are well-known substances with pronounced antimicrobial properties.[6,7,8]

Although the antibacterial activities of the essential oils from many herb species have been extensively surveyed[9], their antimicrobial mechanisms have not been reported in great details. Since the active antimicrobial compounds of essential oils are terpenes and phenolics in nature, it seems reasonable to suppose that their modes of action might be similar to those of other phenolic compounds.[10] Any individual essential oil contains complex mixtures of such compounds, however, little is known about the effect of the interaction between the individual constituents on the antimicrobial activity. Interactions between the constituents may lead to additive, synergistic, or antagonistic effects.[11]

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TOTAL PHENOLICS, LYCOPENE AND ANTIOXIDANT ACTIVITY OF HYDROPONICALLY CULTURED TOMATO SANDIN F1

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ABSTRACT

Total phenols, flavonoids, non-flavonoids and lycopene content have been evaluated in hydroponically cultured tomato fruits cv. Sandin F1 in a five stages ripening experiment. Antiradical activity (DPPH) and the ferric reducing antioxidant power (FRAP) were also assessed. Total phenolics, flavonoids and non-flavonoid phenolics concentration on the initial ripening stage of the fruit (100% green color) was 170 mg (GAE), 138 mg (GAE) and 32 mg (GAE) per kilo fresh matter respectively. The levels of total phenolics, flavonoids and non flavonoid phenolics during fruit ripening were 42%, 48% and 38% of the initial ripening stage of the fruit. Lycopene fruit concentration was 5mg/Kg (GAE) at the initial ripening stage and gradually was raised at 132 mg/Kg (GAE) when fruit reached full maturity stage (100% red color). The values of DPPH assay at the initial ripening stage and those of FRAP assay were 70 μmol DPPH/ kg fresh matter and 210 μmol FRAP/kg fresh matter respectively. During fruit ripening, DPPH and FRAP assay values were found to be 12fold and 20fold times raised respectively. There were correlation between lycopene and DPPH values (r^2= 0.6798) and lycopene and FRAP values (r^2= 0.7048) but not between total phenols and values of DPPH or FRAP.

Keywords: *Tomato, Total phenols, Non-flavonoid phenols, Flavonoid phenols, Lycopene, DPPH activity, FRAP activity*

1. Introduction

Tomato consists a natural reservoir for nutrients such as folate, vitamin C, and potassium, carotenoids and phenolics [1, 2]. The two latter compounds support and protect plant tissues, functioning as defence molecules in immunity, attractants for insect polinators, allelopathic agents and ultra violet (UV) protectants[3]. Varietal, agricultural, technological, and environmental factors influence the content of lycopene and carotene content in different cultivars of tomato [4]. Based on its structure, lycopene is a long-chain unsaturated carotenoid which reflects the basis of the red colour of ripened tomato fruits [5]. It is the most powerful antioxidant member of the carotenoid family and synegistically with vitamins C and E helps to detoxify free radicals. In general, diets rich in carotenoids are associated with low risk probability in some diseases, such as lung, bladder and cervical diseases, skin cancers and disorders of the digestive tract [6]. Undoubtfully, there is a global interest in lycopene since it was discover of it potential against prostate cancer [7]. Furthermore, lycopene also protects from degradation ß-carotene, which is a source of vitamin A [8]. Free radicals are highly reactive compounds that play an essential role in many biological processes, both beneficial and deleterious; however excessive presence of free radicals induce cellular ageing and susceptibility to diseases [9]. Due to its multi- and inter-disciplinary interest, oxidative stress emerges a strong interest in plant cellular and tissue settings. Furthermore, tomato cultural techniques and cropping systems always affect the levels of antioxidant content [10]. Therefore, this work is focused on: (1) total phenols, flavonoids, nonflavonoids and lycopene content determination in tomato fruit at different ripening stages and (2) antiradical activity

(DPPH) and Ferric reducing antioxidant power (FRAP) evaluation of fruit at the same ripening stages.

2. Material and Methods

Tomato plants (var. Sandin F1) was cultivated in hydroponic system during October 2010 to May 2011 at the Technological Education Institute of Larisa, Greece. For experimental purposes, drip irrigation technique was used in order to supply the open hydroponic system. The crop substrate consisted from cocconut (table 1). The crop was fertilized through a stably chemical nutritive solution at the rates of 58.9 ml/min for 3 minutes, repetitively 4 times a day.

Nutrients were kept separately according to their chemical properties in three tanks. The nutritive solution was prepared automatically mixing equal solution quantity from each tank. The final nutritive solution consisted of Ca^{++}= 169 meq/L, K^{+}=253.4 meq/L, Mg^{++}= 64.8 meq/L, NH_4^{+}= 18.3 meq/L, H^{+}=112 meq/L, Fe^{++}=0.6 meq/L, NO_3^{-}=281.3 meq/L, PO_4^{---}= 143.3 meq/L and SO_4^{--}= 193.5 meq/L.

The changes in the content of total phenols (TP), nonflavonoid (NFP), flavonoid phenols (FP), lycopene, the antiradical activity (DPPH) and ferric redusing antioxidant power (FRAP) were assessed during five fruit ripening stages: 1) green, 2) pink (50% pink colour), 3) light red (50% red colour), 4) red (90% red colour) 5) over ripening stage. Three tomato fruit with uniform size, shape and color were harvested for analysis at each maturity sage.

The annual production was 100 tn/ha (3.5 Kg/plant) with mean fruit weight 0.25 Kg.

2.1 Extract preparation: For phenols determination, 80% of ethanol was added in test tubes which contained 10g of fruit sample. Test tubes were kept for 1 h in dark at room temperature. After centrifugation the extracts were brought to 20 ml with aqueous ethanol and used for further chemical analysis [11].

2.2 Determination of total phenols content.

Total phenols (TP) content were determined with the Folin-Ciocalteu reagent according to the method of Singleton and Rossi [12] and were expressed as gallic acid equivalent (GAE).

2.3 Determination of phenol fractions. The non-flavonoid phenols (NFP) were determined with the F.C. reagent after the removal of flavonoid phenols (FP) with formaldehyde according to the method of Kramling and Singleton [13]. FP content was determined as a difference between the content of TP and NFP and was expressed as gallic acid equivalent (GAE).

2.4 Determination of antioxidant activity. The antiradical activity of the ethanol extracts was determined according to the method of Brand-Williams [14] using the stable free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH). The activity was evaluated in μmol DPPH/kg sample. The ferric reducing antioxidant power (FRAP) was evaluated according to the method of Benzie and Strain [15] and was expressed as μmol FRAP reagent/kg sample.

2.5 Determination of Lycopene. The lycopene content was determined in fruit extract taken from 0.5 g homogenized fruit sample using 20 ml of acetone-ethanol-hexane in volume ratio 1:1:2, according to Ravelo-Perrez method [16]. The result was expressed in $mg.kg^{-1}$ of fresh matter.

2.6 Statistical analysis. The experiment was completely randomized with four replications. Data analysis was made using the MINITAB statistical package. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences between treatments were found.

3. Results and discussion

The concentration of the total phenols in tomato fruits increased from 170 mg GAE / kg to 242 mg GAE / kg of fresh matter between green and over ripening stage. Total phenols were marginally increased at the levels of 40% of the initial ripening stage (100% green color). There was an increase in total phenols from the first to third ripening stage (50% red color) while in the next stages phenolic content decreased. Many reports showed that there are small changes in the composition of phenolic during tomato fruit ripening while the content of total phenols reduced after maturity stage [17, 18]. Reduction of chlorogenic acid and increase of naringen in concentration during ripening of tomatoes has been reported in these studies.

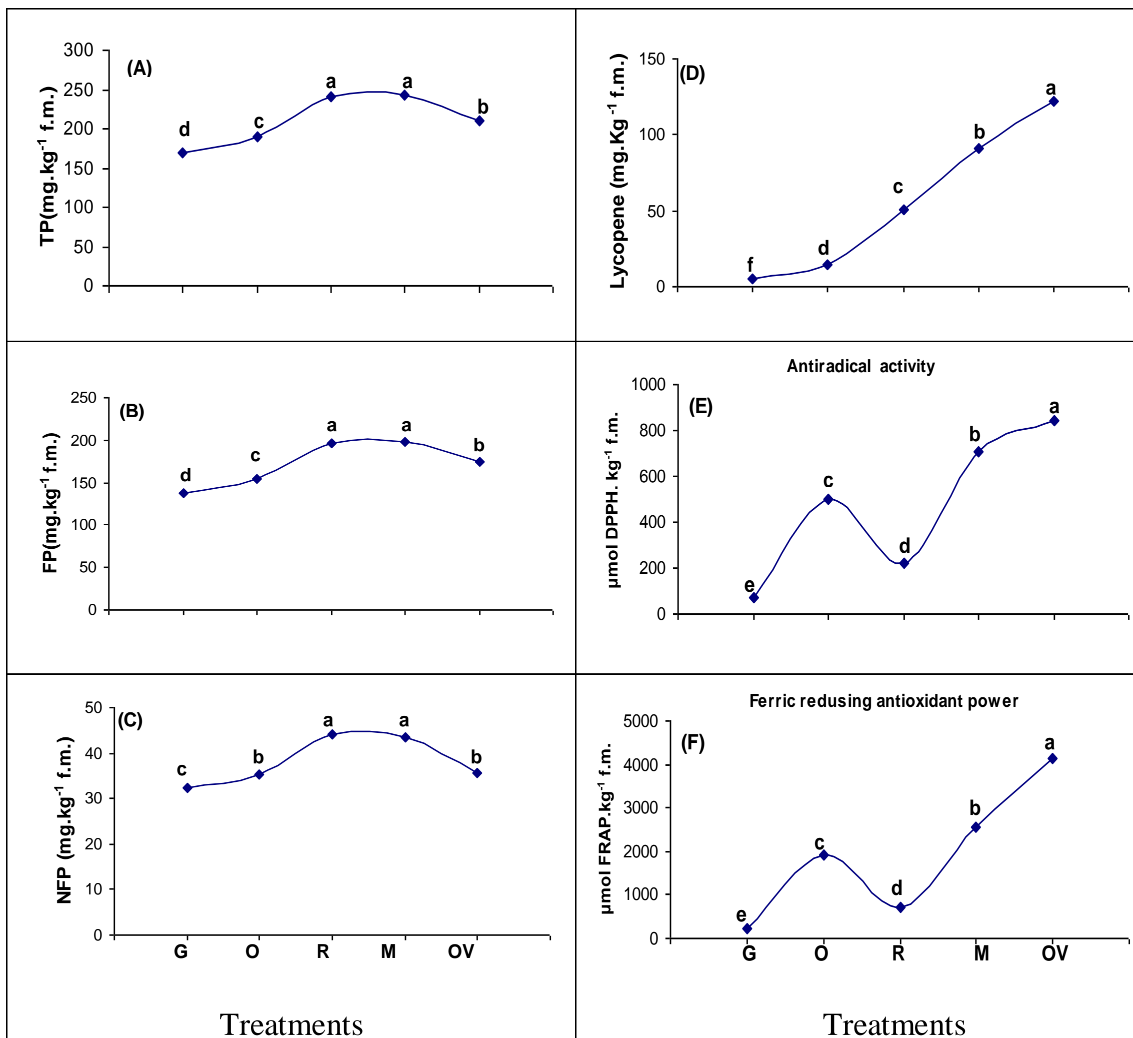

Figure 1. Changes through tomato fruit ripening stages: (A) TP, (B) FP, (C) NFP, (D) lycopene, (E) DPPH activity, (F) FRAP activity. G: green, O: pink, R: light red, M: red and OV: over ripe stage.

The phenolic fraction (NFP and FP) composition had small change during fruit maturation (Fig. 1). The NFP content varied from 32.3 to 44.16 mg GAE / kg of fresh matter, while the FP content varied from 137.7 to 198.44 mg GAE / kg fresh matter.

The non-flavonoid phenols increased during maturation up to the red stage (50% red color). Although, the chlorogenic acid

has been mentioned to reduced, in this work the increase on NFP was due to the synthesis of other NFP substances. The flavonoid phenols content increased which may be due to increased naringenin or other flavonoids. In over-ripening stage there was a decrease in NFP and FP content. Total phenols during ripening constituted by 18-20% of non-flavonoids.

The lycopene increased from 5 to 132 mg kg^{-1} of fresh matter during tomato ripening. In the first two maturity stages tomatoes had very small quantity of lycopene, while the red stage had great quantity. The lycopene synthesis continued during over-ripe stage (132 mg kg-1) which come in argument with other research finding [19].

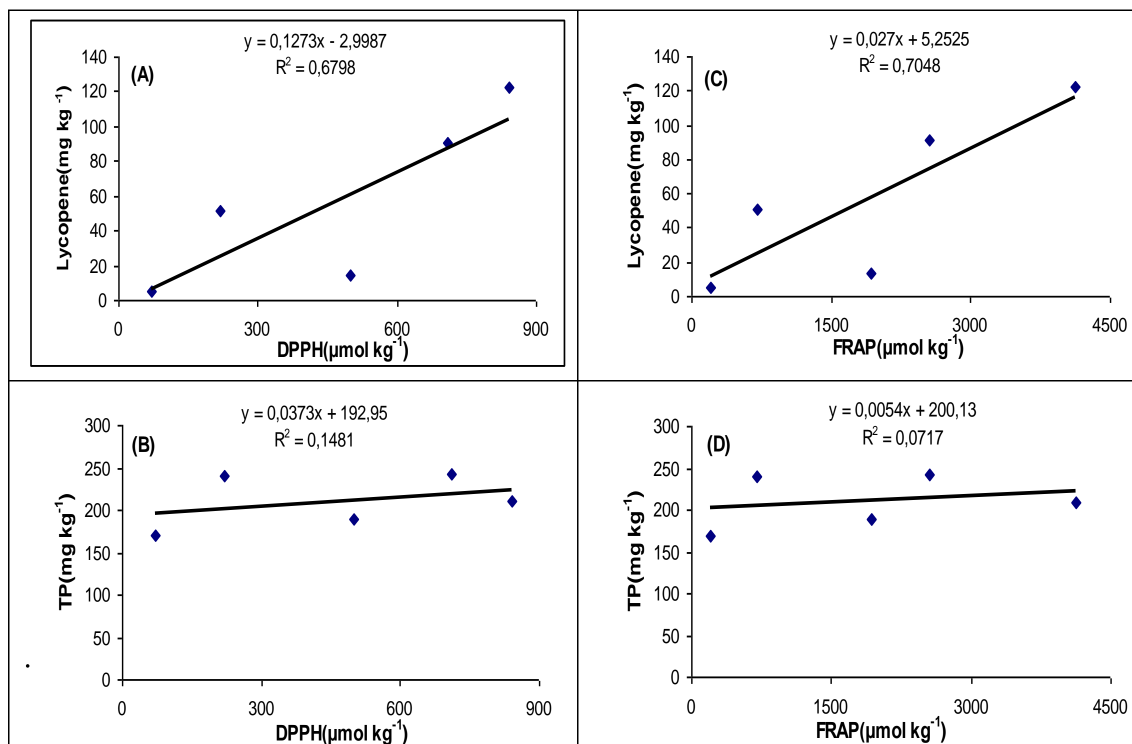

Figure 2. Correlation between antiradical (DPPH) and antioxidant (FRAP) activity with lycopene and TP.

Table 1. Chemical properties of the coco palm substrate and the nutrient solution were used in experiment.

Chemical Property	Coco palm (fresh mass)	Nutrient solution influx	Nutrient solution efflux	Irrigation water
Initial pH	6.7	6.1	6.1	7.6
Mean pH value	6.46±0.20	6,12±0.22	6.33±0.37	
Final pH	6.1	5.9	5.9	
Initial Electrical conductivity, (dS m^{-1})	0,68	2.25	2.35	0.5
Mean values of electrical conductivity (dSm^{-1})	0.63±0.18	2.10±0.16	2.72±0.40	
Final Electrical conductivity (dS m^{-1})	0.65	2.1	2.4	

The determination of the antiradical activity in ethanol tomato extracts was carried out by using the stable free radical DPPH, through its decolourisation in the presence of antioxidant compounds. The antiradical activity is expressed as μmols DPPH/kg of fresh matter. The antiradical activity of tomato fruits during ripening varied from 70 to 840 μmol DPPH/ kg of fresh matter, with the latter value to characterize the over-ripening stage (Fig. 1). The antiradical activity was correlated with lycopene and total phenols content and the following significance levels were obtained (r^2) = 0.6798 and 0.148,1 respectively (Fig. 2)

The results of the FRAP antioxidative assay during fruit maturity showed that Ferric reducing antioxidant power (FRAP) varied from 210 to 4120 μmol FRAP/kg of fresh matter. The antioxidant FRAP activity was correlated with the quantity of lycopene and TP and the following significance levels were obtained (r^2) = 0.7048 and 0.0717, respectively (Fig 2).

High correlation between lycopene and antioxidant FRAP activity during ripening of tomato has reported in other studies [20, 21].

Research studies has shown that lycopene and major phenolic components of tomato such as ferulic acid and caffeic acids are characterized by high antioxidant and antiradical activity, in contrast to chlorogenic acid that characterized by low antioxidant and antiradical activity [22]. The antiradical activity (DPPH) and the antioxidant activity (FRAP) in tomatoes obtained by the combination of all individual antioxidant compounds activity found in tomatoes.

4. Conclusions

During early ripening stages of hydroponically cultivated tomato the total phenols and phenolic fractions are in high quantity in fruits while the lycopene content is negligible. From green to over-mature stage the composition of total phenols, NFT and FP increased by 40%, 35% and 45% respectively due to chlorogenic acid reduction and to lycopene and naringenin increase by 17-fold of initial quantity. In the over-ripening stage TP, NFP, FP decreased while lycopene content increased. The DPPH assay and the Ferric reducing antioxidant power (FRAP) increased up to over-ripening fruit stage while the amount of total phenols decreased. This is due to increased of lycopene and antioxidant compounds that have synergistic activity with lycopene.

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COMPARISON OF ANTIOXIDANT PROPERTIES BETWEEN RED AND YELLOW FLESH WATERMELON RINDS BY DIFFERENT EXTRACTION CONDITIONS

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ABSTRACT

This study aimed to assess the total phenolic content (TPC) and antioxidant activities of watermelon rinds from two cultivars, red-flesh and yellow-flesh extracted based on different extraction media used, water to solvent ratio, and extraction time. Aqueous acetone (90%) and ethanol (30%) had yielded relatively highest TPC and antioxidant activities for red and yellow watermelon rind extracts, respectively. With ideal aqueous solvents application, high TPC and antioxidant activities were found for 5 h extraction of red watermelon rind and 2 h extraction of yellow watermelon rind. The TPC and antioxidant activities were significantly different between red and yellow watermelon rinds that extracted using different percentage of aqueous solvents and extraction times but with some exception. The values of TPC and antioxidant activities were linear against the concentrations of the extracts used. Therefore, this optimized extraction method is useful for future studies of antioxidant extraction of watermelon rinds.

Keywords: *antioxidant activity, total phenolic content, FRAP, DPPH, watermelon*

1. Introduction

Watermelons, *Citrullus lanatus* are found laden with vitamins A and C, beta-carotene, lycopene, iron, zinc, potassium, and others. These antioxidants aid in maintaining the freshness and radiance of our skin. Red flesh watermelon is found to have a high concentration of lycopene than other varieties such as tomatoes [1].

The lycopene found in watermelon may helps in scavenging free radicals apart from containing as high as 90% of water that make a choice for thirst quenching. Watermelon is also easily cultivated and widely consumed, which in turn, it owns potential to serve as alternative dietary antioxidants [2].

Main source of antioxidants come from a wide variety of fruits and vegetables, tea, and wine. Some fruits have high phenolic compounds and other antioxidant vitamins and minerals and received great attention on the last decade. Interestingly, the peels and seed fractions of some fruits contain higher antioxidant activity than the pulp fractions itself [3]. Thus, many researchers have focused on extraction of antioxidants from plants and agro-industrial by-products.

Extraction is a pivotal step in obtaining the bioactive compounds from plants [4], where its procedure depends on the type of antioxidants to be extracted. Differences in the extract yields may be due to a variation in the availability of extractable components associated with the diverse chemical composition in the tested plants.

In order to increase yield of antioxidative compounds, extraction conditions such as polarity of solvents, particle size, extraction time, temperature, pH, and existence of interfering substances are the major concerns.

Therefore, this study aims to evaluate the effects of various extraction media, percentage of organic portion, and extraction time on total phenolic contents and antioxidant activities of the rinds of red- and yellow-flesh watermelon.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade ethanol, hexane, chloroform, L-ascorbic acid and Tween 40 were purchased from Fisher Scientific (Leicestershire, UK). Butylated hydroxyanisole (BHA), (±)-α-tocopherol, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) powder, 2,4,6-tris(2-pyridyl)-s-(triazine) (TPTZ), and ß-carotene were obtained from Sigma Chemicals (MO, USA). Potassium persulfate and 2,2,-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Fluka (Munich, Germany). Trolox, linoleic acid, and gallic acid were obtained from Acros Organics (New Jersey, USA), while other chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Sample preparation

Three fruits of each red-flesh watermelon (RW) and yellow-flesh watermelon (YW) were purchased from a supermarket in Kuala Lumpur, Malaysia. The watermelons were cut with flesh removed. The rinds were further cut into smaller pieces (about 0.5 cm^3) and dispersed on a drying pan which has been covered with aluminum foil to avoid contamination. The purpose of drying is to inactivate the enzymes polyphenol oxidases that lead to significant changes in the composition of phytochemicals. The rinds were measured as wet weight before putting it into the convection oven (UNB400, Memmert, Germany) at 45°C for 24 h. The weight after drying was recorded as dry weight. By applying this drying condition, the samples of RW and YW rinds had 83.8% and 80.87% of moisture.

The dried rinds of watermelon were milled at Forest Research Institute of Malaysia (FRIM) using QUADRO® COMIL miller (Quadro Engineering, Ontario, Canada) at 3873 rpm into powder form of 813 µm in size. Small size particles allow greater yields during extraction. Sample powders (10 g) were then vacuum-packed (DZQ400/500, Zhejian, China) into nylon-linear low-density polyethylene (LDPE) (Flexoprint, Selangor, Malaysia) pouches. The samples were wrapped with aluminum foil to avoid sunlight exposure and stored in dark at –20˚C.

2.3. Sample extraction

Extraction of phenolic compounds in red and yellow watermelon was performed based on two separate steps. In the first step, dried sample powder of 10 g was weighed and added with 200 ml of five different extraction media (100% distilled water, 100% ethanol, 100% methanol, 100% hexane, and 100% acetone). Secondly, five different percentage of aqueous organic solvent (10%, 30%, 50%, 70%, and 90% v/v) were used to extract the phenolic compounds in the dried sample powder based on the chosen extraction media in the first step.

In the second step, the mixtures were then shaken at 150 rpm based on five different extraction time (1, 2, 3, 4, and 5 h) at ambient temperature. The mixtures were then filtered with Whatman No. 1 filter paper. The extracts were collected and concentrated using a rotary evaporator (Rotavapour R-200, BUCHI, Switzerland) at 40˚C with a rotation speed of 40 rpm and further dried using liquid nitrogen.

2.4. Total phenolic content assay

Total phenolic content of watermelon rind was determined using the method as described by Ferreira et al. [5]. Briefly, 1 ml of sample extract was added with 4 ml of Folin-Ciocalteu's solution (diluted previously with 10-fold dilution) and was allowed to stand for 3 min at room temperature. Then, 5 ml of 7.5% sodium carbonate solution was added into the mixture. The mixture was shaken vigorously and incubated for 30 min at room temperature in dark. The absorbance was measured using spectrophotometer (Secomam, Alès Gard, France) at 765 nm against blank (distilled water). The

analysis of each sample was performed in triplicate. Total phenolic content of the sample extract was calculated based on using a calibration curve using gallic acid (0.2–25 μg/ml) and expressed as mg gallic acid equivalents (μg GAE/g extract). The calibration equation of gallic acid was $y = 0.0165x - 0.0003$ ($R^2 = 0.99$).

2.5. 2,2-Diphenyl-1-picrylhydrazyl assay

Scavenging ability of the samples against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was measured based on the method described by Yang et al. [6] and Xu and Chang [7] with slight modifications. Briefly, 2 ml of sample extract was added into a test tube containing 0.5 ml DPPH solution (7.8 mg of DPPH powder in 100 ml of ethanol). The aliquot was shaken vigorously and allowed to stand for 30 min at room temperature in dark. The absorbance of the resulted decolorized mixture was measured at 517 nm against blank that contain ethanol only. The ability of the sample extract to scavenge the DPPH free radicals was calculated based on equation (1).

$$Scavenging\ activity\ (\%) = [1 - \frac{Absorbance_{sample}}{Absorbance_{control}}] \times 100 \quad (1)$$

The percentage of scavenged DPPH was plotted against the natural logarithm (Ln) of sample concentration to calculate the EC_{50} values (mg/ml).

2.6. Ferric reducing antioxidant power assay (FRAP)

Ferric ion reducing power was measured based on a method described by Xu and Chang [7] and Biglari et al. [8] with slight modifications. Briefly, a freshly prepared FRAP reagent [mixture of 2.5 ml of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl, 2.5 ml of 20 mM ferric tricholride hexahydrate ($FeCl_3.6H_2O$), and 25 ml of 0.3 M acetate buffer at pH 3.6] was pre-incubated in water bath at 37^oC. FRAP reagent (3 ml) was added with 100 μL of sample extract and 300 μL of distilled water. The mixture was vortex and incubated for 4 min. The absorbance was measured at 593 nm against blank. The FRAP value was calculated based on the absorbance change and expressed as Trolox equivalent (TE) antioxidant capacity (μg TE/g extract) using a calibration curve of $y = 0.0057x - 0.0214$ ($R^2 = 0.99$).

2.7. Statistical analysis

All data obtained were analyzed using Statistical Packages for Social Sciences (SPSS) version 17.0 and expressed as mean ± standard deviation of triplicate measurements. The results were analyzed using independent sample t-test and one-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons. The significant value was set as $P<0.05$.

3. Results and discussion

3.1. Effect of extraction media

This study was initially performed based on different extraction media in selecting the best solvent to further extract total phenolics using different percentage of aqueous solvent principle. TPC and antioxidant activities of both RW and YW rinds extracted using five different extraction media are presented in Figure 1. Results show that the RW and YW rinds that were extracted using different extraction media had low TPC, except YW rind that were extracted by 100% ethanol. The ethanol extract of YW rind had TPC >10 times higher than the other extracts studied. The RW rinds that were extracted using 100% acetone had the highest TPC and significantly higher ($P<0.05$) than the rest of the extraction media used. For all extraction media studied, TPC was significantly different between the RW and YW rind extracts except RW and YW for methanol extract using DPPH assay.

The antioxidant activities of the RW and YW rind extracts also revealed similar result as of TPC. Extracted using 100% acetone, RW rind extract showed

non-significantly highest ($P \geq 0.05$) antioxidant activities (based on DPPH and FRAP assays) than other extraction media (Figure 1). The YW rind extracted using 100% ethanol had significantly highest ($P < 0.05$) antioxidant activities. Besides, the antioxidant activities of the studied extracts were significantly different between RW and YW rinds except for the scavenging activity (%) of methanol extracts. Based on the results obtained from DPPH and FRAP assays, 100% acetone and 100% ethanol were the best extraction medium used for the RW and YW, respectively. As shown in Table 1, the R^2 values of watermelon rind extracts concentrations for TPC and antioxidant activities that extracted using these extraction media were ranged from 0.956 to 0.999 (high linearity).

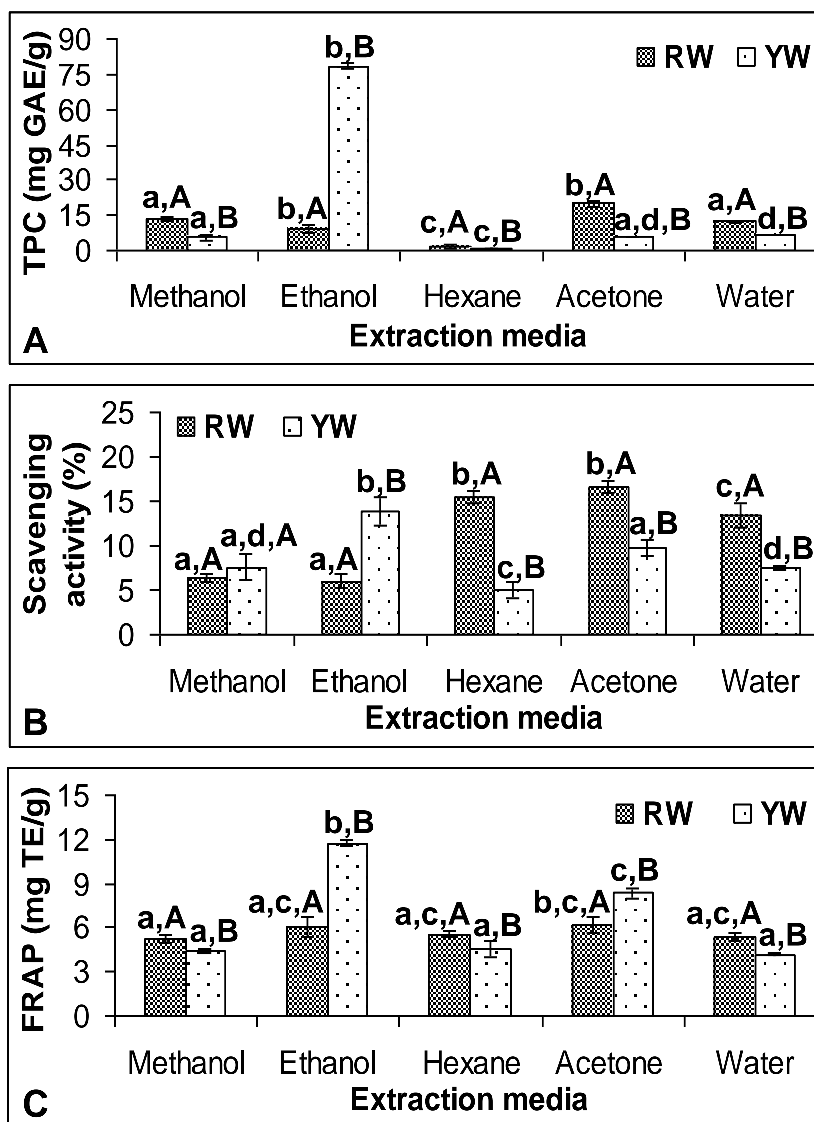

Figure 1. (A) Total phenolic content; (B) DPPH radical scavenging activity; and (C) FRAP (Trolox equivalent, TE) of RW and YW rind extracts extracted using different extraction media. Similar lower case letters show no significant difference in the mean values of each extract between the extraction times ($P \geq 0.05$), while similar upper case letters show no significant difference in the mean values between RW and YW rind extracts ($P \geq 0.05$).

Extraction medium is one of the factors that affect the TPC and antioxidant activity of the extracts studied. Chanda and Nagani [9] reported that the types of extraction solvent can influence the TPC and antioxidant activities of an extract when the temperature and time of extraction are fixed under the same condition. Fernandez et al. [10] claimed that since the antioxidant activity is depending on the type of antioxidant and antioxidant assay, it is required to have comparative studies in selecting the suitable type of extraction medium. Besides, appropriate ratio of solvent and other aspects of extraction medium used need to be considered. These are including practical (boiling point, density, and stability), economic (cost), and toxicology aspects.

Table 1. *Correlation coefficient (R^2) and slope (M) for total phenolics and antioxidant capacity estimation in red watermelon (RW) and yellow watermelon (YW) rind extracts based on different extraction media*

Sample	Extraction media	TPC		DPPH		FRAP	
		R^2	*M*	R^2	*M*	R^2	*M*
RW rind	Methanol	0.999	0.0139	0.967	0.0434	0.998	0.0133
	Ethanol	0.994	0.0073	0.963	0.0642	0.995	0.0133
	Hexane	0.990	0.0061	0.969	0.0762	0.993	0.0087
	Acetone	0.989	0.0073	0.966	0.0716	0.991	0.0278
	Water	0.999	0.0113	0.969	0.0735	0.993	0.0149
YW rind	Methanol	0.994	0.0059	0.967	0.0417	0.986	0.0067
	Ethanol	0.994	0.0083	0.979	0.0786	0.965	0.0395
	Hexane	0.987	0.0005	0.999	0.0410	0.956	0.0065
	Acetone	0.986	0.0122	0.962	0.0692	0.993	0.0145
	Water	0.999	0.0057	0.967	0.0431	0.983	0.0071

M represents the power of antioxidant concentrations in each extracts, which the studied samples with larger M will have higher total phenolics/stronger antioxidant capacity with even slight increase in the extract concentration applied.

Water, alcohols, acetone, and ether are commonly used to extract phenolic compounds in plants owing to the broad range of polarities. Extraction yield and antioxidant activity of the studied extracts are highly dependent on the solvent polarity [11, 12], in which it increases the phenolic solubility [13]. Mixture of solvents with wide range of polarity are required in extraction process as the antioxidant compounds in plants are of diverse structure, composition, and physiochemical properties that lead to the differences in their behavior [14].

In extracting the water-soluble (hydrophilic) antioxidants, water or different buffers are frequently used while acetone or chloroform is used in extracting non-water soluble antioxidants [15]. As plant materials contained phenolic compounds with varying degree of solubility in accordance to their molecular weight, degree of esterification, acylation, and glycosylation [16], polar solvents such as methanol, ethanol, and acetone are typically used for extraction. These solvents are either solely used or mixed with water to extract the phenolic compounds in plant sample [17].

3.2. Effect of solvent percentages

The chosen extraction media such as acetone and ethanol were applied in extraction of phenolic compounds based on five different percentages of solvent (10, 30, 50, 70, and 90%). The best percentage of aqueous solvents chosen was further applied in extraction of phenolic

compounds in the watermelon based on different extraction time. Results show that TPC and antioxidant activities of the RW and YW rinds extracted based on different percentage of solvent were varied (Figure 2). The RW rind extracted using 50% acetone had the lowest TPC and antioxidant activities. Highest TPC was found for the RW rind extracted using 10% acetone. For DPPH and FRAP assays, RW rinds extracted with 90% and 70% acetone had the highest antioxidant activities, respectively. YW rind extracted using 30% ethanol had the significantly highest ($P<0.05$) TPC and antioxidant activities (based on DPPH and FRAP assays).

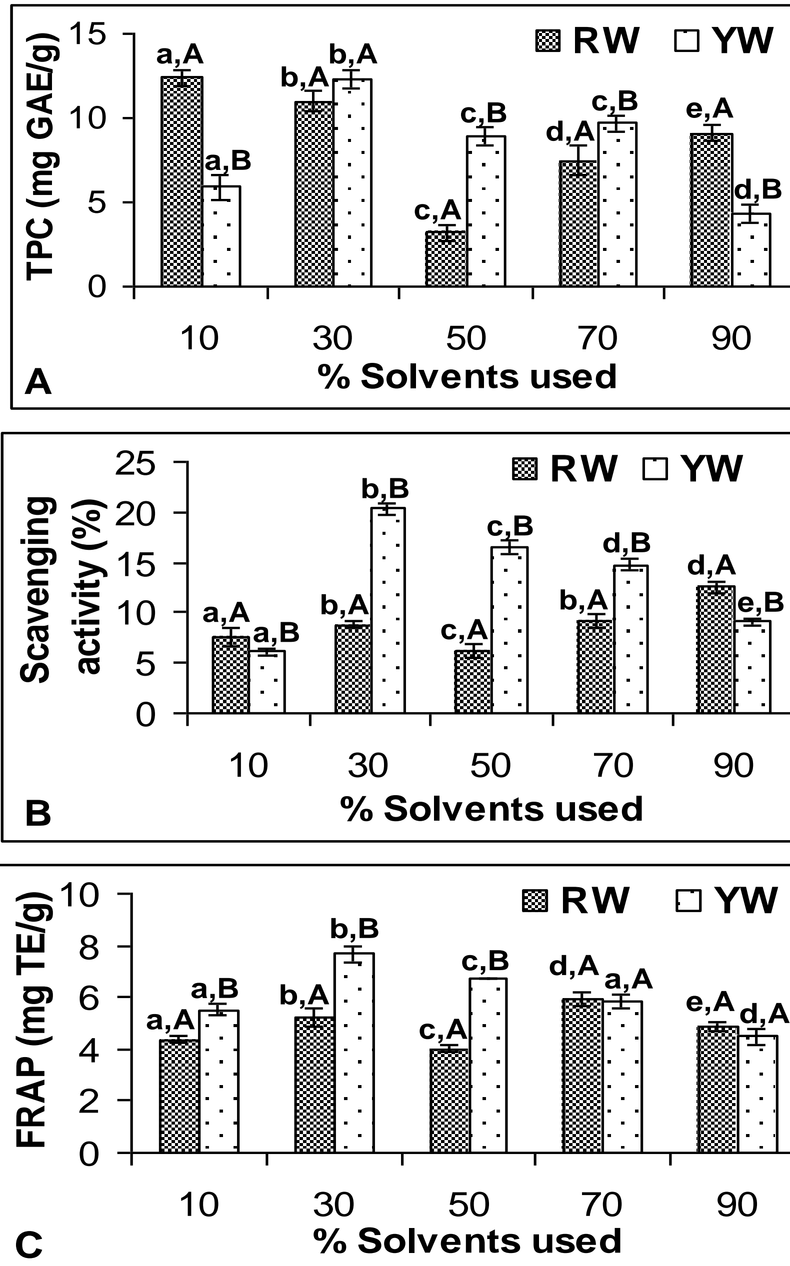

Figure 2. (A) Total phenolic content; (B) DPPH radical scavenging activity; and (C) FRAP (Trolox equivalent, TE) of RW and YW rind extracts based on different percentage of solvents used (10–90%), where RW and YW rinds were extracted using aqueous acetone and aqueous ethanol, respectively. Similar lower case letters show no significant difference in the mean values of each extract between the extraction times ($P\geq0.05$), while similar upper case letters show no significant difference in the mean values between RW and YW rind extracts ($P\geq0.05$).

TPC and antioxidant activity of FRAP assay were found to be the lowest in YW rind extracted using 90% ethanol, while the percentage of scavenging activity (DPPH assay) was the lowest in YW rind extracted using 10% ethanol. All these extracts had significantly lowest TPC and antioxidant activities (*P*<0.05). On the other hand, the TPC of the studied watermelon rinds were significantly different between red and yellow varieties, except for the watermelon rinds (both red and yellow) extracted using 30% of the selected solvents. The antioxidant activities of FRAP assay for both RW and YW rinds extracted using different percentage of solvent were all significantly different, except for extraction using 70% and 90% solvents (acetone and ethanol for RW and YW rinds, respectively). Hence, this study revealed that 90% of acetone and 30% ethanol are the best aqueous solvents for extraction of RW and YW rinds, respectively. Besides, the linearity of watermelon rind extracts concentrations for TPC and antioxidant activities extracted with different percentages of aqueous solvents was high (Table 2).

Table 2. *Correlation coefficient (R^2) for total phenolics and antioxidant capacity estimation in red watermelon (RW) and yellow watermelon (YW) rind extracts based on different percentage of aqueous solvent and different extraction time*

Sample		**TPC**	**DPPH**	**FRAP**		**TPC**	**DPPH**	**FRAP**
	Hour				***% Acetone***			
RW rind	1	0.999	0.956	0.989	10	0.965	0.964	0.973
	2	0.999	0.917	0.988	30	0.994	0.959	0.988
	3	0.999	0.929	0.997	50	0.992	0.965	0.997
	4	0.999	0.919	0.940	70	0.995	0.963	0.961
	5	0.999	0.930	0.997	90	0.998	0.968	0.999
	Hour				***% Ethanol***			
YW rind	1	0.994	0.945	0.911	10	0.997	0.984	0.988
	2	0.996	0.967	0.983	30	0.999	0.944	0.980
	3	0.994	0.987	0.999	50	0.999	0.936	0.994
	4	0.998	0.973	0.918	70	0.999	0.956	0.980
	5	0.996	0.971	0.992	90	0.999	0.973	0.998

Influenced of aqueous organic solvent ratio is another major factor in the extraction of antioxidative compounds. Turkmen et al. [18] and Yilmaz and Toledo [19] ascertained that phenolic compounds are strongly dependent on the type of the solvent as well as the ratio of water to solvent used. Rødtjer et al. [20] revealed that 70% of solvent-water mixture (binary solvent system) is more efficient in yielding phenolics including complex mixtures of phenolic compounds than the pure solvent. Aqueous ethanol and methanol are more efficient extraction media for obtaining higher antioxidant compounds. Spigno et al. [21] also indicated that mixture of alcohol and water yielded higher amount phenolic compounds as compared to mono-component solvent system, where these will facilitate the extraction of polyphenols.

Higher solvent polarity is able to extract phenolic substances from both highest polarity substances to lowest polarity substances [22]. Hemwimon et al. [23] revealed that water plays an important role in assisting and inducing the swelling of plant's cell walls in order to increase the contact of surface area with the solvent

thereby enhancing the phenolics extraction efficiency. Besides, acetone is the least polar solvent and has higher efficiency in degrading the cell wall thus allowing phenolics and other antioxidant compounds to be released [11].

3.3. Effect of extraction time

RW and YW rinds were extracted using 90% acetone and 30% ethanol for respectively in a second step of extraction that based on five different extraction times. Results show that TPC in RW was found to decrease as increased in extraction time. As shown in Figure 3, extraction from 3–5 h using 90% acetone for RW rind extract had a significant decreased in TPC as compared to 1 h and 2 h extractions. For YW rinds, the TPC was increased significantly at 2 h extraction and decreased from 3 h of extraction. Although longer extraction time seems to have degradation trend for TPC, TPC of RW extracted using 90% acetone increased significantly at 5 h extraction compared to 4 h extraction.

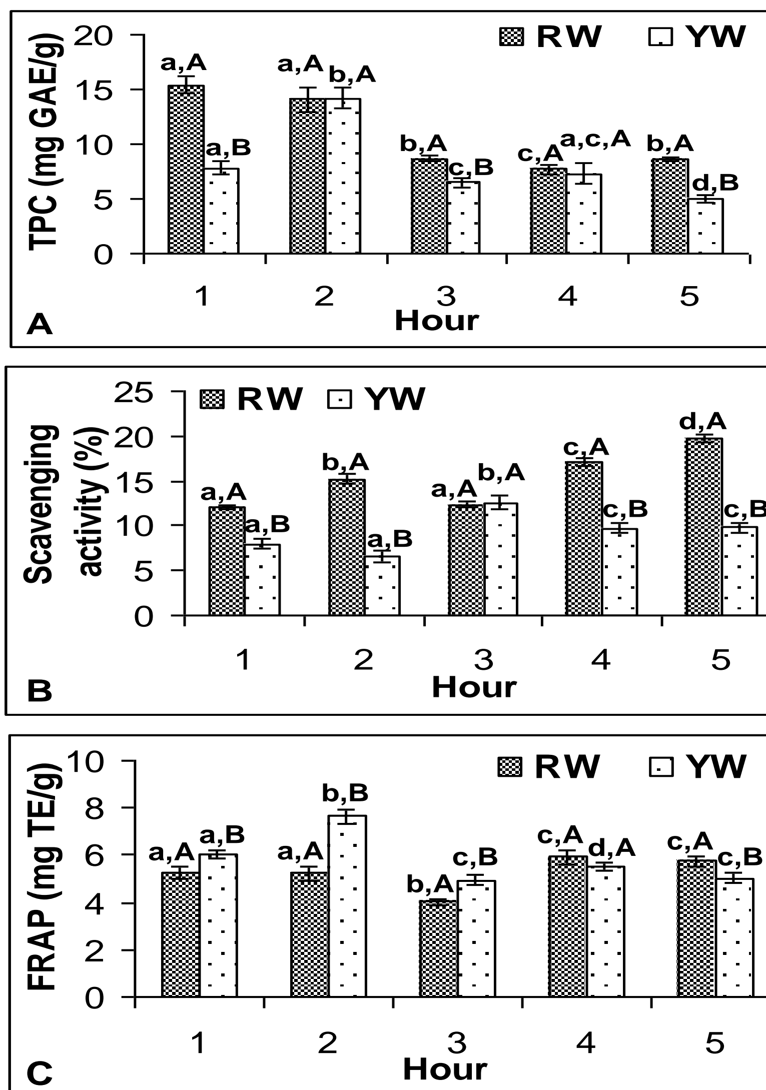

Figure 3. (A) Total phenolic content; (B) DPPH radical scavenging activity; and (C) FRAP (Trolox equivalent, TE) of RW and YW rind extracts based on different extraction time (1–5 h) by using 90% acetone and 30% ethanol, respectively. Results are expressed as per gram extract. Similar lower case letters show no significant difference in the mean values of each extract between the extraction times (P≥0.05), while similar upper case letters show no significant difference in the mean values between RW and YW rind extracts (P≥0.05)

The antioxidant activities of watermelon rind extracts had a fluctuated trend during prolonged extraction duration. RW rinds extracted with 90% acetone showed an increasing trend of scavenging activity (%) analyzed by DPPH assay. However, at 2 h extraction, the scavenging activity (%) increased drastically but reduced at 3 h extraction. The scavenging activity (%) of YW rind extracted using 30% ethanol did not show any drastic change. Similarly, the antioxidant activity for both watermelon rind extracts did not show any significant change for FRAP assay.

Comparing the TPC and antioxidant activities between the two varieties of watermelon rinds, significant differences ($P<0.05$) in the TPC and antioxidant activities were found between RW and YW rind extracts with some exception. During 2 h and 4 h extractions, the results show no significant differences in TPC between RW and YW rind extracts. The antioxidant activities assessed using DPPH and FRAP assays show no significant changes between the RW and YW rind extracts that were extracted using aqueous solvent for 3 h and 4 h extraction, respectively. Generally, the RW rinds extracted using 90% acetone had TPC higher than YW rinds extracted using 30% acetone. Conversely, at a shorter extraction duration (1–3 h), the antioxidant activity (FRAP assay) of RW rind extracts were significantly lower than the YW rind extracts. A high linearity was also found for the extract concentrations with R^2 values ranged between 0.936 and 0.999 (Table 2).

Extraction time is known to affect the efficiency of the aqueous solvent to extract the phenolic compounds. The duration of extraction can be varied from one to another sample with different molecular weights and chemical structures of phenolic compounds. For example, the recovery for molecule with higher molecular weight may require longer duration as compared to those with lower molecular weights [24]. The extraction time may range from few minutes as conducted on roasted wheat germ [25] to few hours or even a few days for some other samples [26]. In economical perspective, a shorter extraction time is preferred as it saves cost and time. Degradation of phenolic compounds by oxidation may occur during longer extraction time, thus attributed to the lower antioxidant activity.

Al-Farsi and Lee [27] reported that one hour treatment is optimal for the phenolic extraction in date seed. Chew et al. [28] affirmed that recovery of phenolics was ideal during the first hour of extraction. Some studies found prolonged extraction may not yield more phenolics [29, 30]. Indeed, lengthen in the extraction time causes degradation, oxidation, and hydrolysis of phenolic compounds [13], which also attributed to the lower total phenolic content.

On the other hand, a study disclosed that longer extraction duration (6 h) yielded a maximum amount of total phenolics in lemon peel [31]. Spigno and Faveri [32] explained that the increased in total phenolic contents was due to the polymers or wall-bound phenolics retained in cells being extracted out during the long maceration time. Despite, longer extraction time imposed not much difference in the amount of phenolic compounds extracted compared to shorter time [17].

4. Conclusions

This study evaluated the effects of extraction parameters on TPC and antioxidant activities in RW and YW rinds. Among the studied extraction media, acetone and ethanol were the best extraction solvents for obtaining highest TPC and antioxidant activities in RW and YW rinds, respectively. Aqueous acetone and ethanol used in extraction of watermelon rinds have increased the scavenging activity. More extraction parameters are needed to be considered in future studies.

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DEVELOPMENT AND STABILITY OF BIO - FOOD COLOUR FROM BEETROOT BY SPRAY DRYING

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ABSTRACT

A study was conducted to develop natural food colour powder from beetroot by using spray dryer. The colour was extracted with water and solvents viz., acetone and ethanol. The experimental results showed that the colour value was better, when extracted with 3 % acetone (B_2), 3 % acetone and 0.1 % citric acid (B_3) and 100 % water (B_1). All the filtrates were spray dried at different drying air temperature (180, 200 and 220ºC) and flow rate (25 and 30 ml / min) using 10, 15 and 20 % maltodextrin (MD). The yield recovery was maximum at 200ºC with a flow rate of 25 ml / min at 20 % maltodextrin. The stability of the colour was analysed by exposing the colour powders in various concentrations of pH, preservative and temperatures followed by packed in aluminium pouch (P_1), brown pet bottle (P_2) and brown glass bottle (P_3). The packed samples were stored in room (R_1) and refrigeration (R_2) temperatures for 3 months and analyzed their colour intensity (L* a* b*). The experimental results revealed that the addition of preservative, increasing pH, temperature and storage periods had affected the stability of colour. The samples packed in P_3 had better colour retention than P_2 and P_1 during storage. Colour intensity of developed lollipop, jujubes and icing with B_3 had best colour strength and sensory attributes next to carmoesin than B_1 and B_2.

Keywords: *Beetroot, Spray Drying, Beetroot Colour Powder, Preservatives, Stability, Confectionery Products*

1.Introduction

Colour is one of the first characteristics perceived by the sense and is indispensable for rapid identification and ultimate acceptance of any food products (Sampathu *et al.,* 1981; Pattnaik *et al.,* 1997; Aparnathi *et al.,* 1999). Commonly in food industries, colours are used to enhance (or) restore the original appearance of food products (Kakali Roy *et al.,* 2004; Nuzhet and Ferruh, 2006). Synthetic food colourants face an uncertain future on the point of safety-in-use. Therefore, attention of food industry has turned to the availability of suitable natural colourant alternatives (Aparnathi *et al.,* 1999). Natural colours are generally extracted from fruits, vegetables, seeds, roots and micro-organisms and they are often called "bio-colours" because of their biological origin (Pattnaik *et al.,* 1997; Nadia and Tariq, 2002; Rekha *et al.,* 2007). Natural food colours not only enhance the appearance and flavour of the foods, but also protect them from oxidation by enzymes. Many natural colourants may be nutritionally important antioxidants and their presence in the diet reduces the risk of cardiovascular disease, cancer and other diseases associated with aging (Reddy *et al.,* 2005; Pszezola, 1998; Rao and Agarwal, 1999; Mazza, 2000). Many natural pigments have less stability during extraction, processing, storage and incorporation into food (Pattnaik *et al.,* 1997). However, the natural food colour show greater resistance and stability when exposed to oxidation, changes in temperature, pH and other factors compared with artificial food colour agents (. Francis, 1989; Hong and Wrolstad, 1990; She *et al.,* 1992; Fabre *et al.,* 1993). Natural food colour from beetroot is an important emerging food colour and has maximum stability at pH range from 4 to 5, but with increased in temperature and exposure time, the colour degradation is more (Pattnaik *et al.,* 1997; Rekha *et al.,* 2007).

Objectives: To standardize appropriate spray drying techniques for development and stabilisation of natural food colour powder from beetroot. To study the possible utilisation of developed bio-food colour powder in confectionary products instead of synthetic food colour powder.
To study the storage stability of developed bio-food colour powder

2. Materials and methods

Standardisation of extraction of beetroot colour:

Beetroot was used for the extraction of colours using different solvents viz., water, ethanol and acetone as given in Table.1. Beetroot (Ooty 1) was cleaned, peeled and cut into small cubes. It was pulped in a pulper and the sample was used for extraction of the colour. One gram of the pulp was mixed with 5 ml of solvent and kept the mixers for 30 minutes (Complete extraction) and filtered through Whatmann No1 filter paper. The colour was read in the Spectronic 20 colorimeter at 476nm. Based on the colour value, acetone at 3 per cent concentration (B_2), 3 per cent acetone and 0.1 per cent citric acid (B_3) and 100 per cent water (B_1) extractions were selected.

Table 1.Colour extraction media

Extractionmedia	Concentrations (%)
Water	100
Acetone	1,2,3,4, and 5
Ethanol	1,2,3,4, and 5

Standardisation of drying parameters for the production of beetroot colour powder using spray dryer:

The edible portion of the beetroot was cut into small pieces and made into pulp by adding 3 per cent acetone in the proportion of 4:1 (beetroot: acetone w/v). The pulp was kept at room temperature for 30 minutes to dissolve the pigments in the solvent. After 30 minutes, the pulp was filtered by using muslin cloth. To this solvent mixture maltodextin at different levels (10, 15 and 20 %) and 0.01 per cent anticaking agent (calcium stearate) were added. The residue was filtered again through muslin cloth and the filtrate was spray dried at an inlet drying air temperature of 180, 200 and 220 ºC at a feed rate of 25 and 30 ml / min. The colour powder recovery from beetroot is presented in Table (2).

Based on the results, the process parameters viz., inlet drying air temperature, feed rate and maltodextrin were optimized as 200ºC, 25 ml / min and 20 per cent maltodextrin, respectively for the production of beetroot colour powders B_1 (100% water extraction), B_2 (3% acetone extraction) and B_3 (3% acetone extraction and 0.1% citric acid) in spray drying method.

Table 2. Effect of drying air temperature, feed rate and maltodextrin (MD) on spray dried beetroot powder recovery

Drying temperature (^{0}C)	Feed rate (ml/min)	Powder recovery (g/100 ml)		
		Maltodextrin (%) (MD)		
		10	15	20
180	25	6.4	8.6	12.5
	30	6.1	7.9	10.3
200	25	7.7	10.1	14.6
	30	6.7	8.2	12.1
220	25	7.5	9.5	13.7
	30	6.2	8.1	10.4

Effect of pH, temperature and preservative on the stability of spray dried beetroot colour powders:

One gram of developed spray dried beetroot colour powders were dissolved separately in 100 ml of distilled water. Stability of colour at different pH (4-9) was measured by mixing 2 ml of aliquot of spray dried beetroot colour powder in 10 ml of buffer (pH 4-9). These samples were stored in room (R_1) and refrigeration (R_2) temperatures for a period of seven days and absorbance was measured at 24 hours interval. The degradation of colour was studied by exposing the mixture of 2 ml aliquot sample with 10 ml of distilled water and exposed at 75, 100, 125and 150ºC and the degradation was measured for a period of 10 minutes. Effect of sodium benzoate was studied by mixing an aqueous solution of sodium benzoate (concentration 200 ppm - 1000 ppm) with 2ml aliquot samples. These samples were stored at room (R_1) and refrigerator (R_2) temperatures for a period of seven days and absorbance was measured at 24 hours interval (Rekha *et al.,* 2007).

Application of spray dried beetroot colour powders in confectionary products:

The developed spray dried beetroot colour powders were added at a concentration of 0.04g (B_1), 0.02g (B_2) and 0.01g (B_3) in selected confectionary products like jujubes, lollipop and icing and the colour value (L* a* b*) was evaluated and compared with the product developed with synthetic food colour (carmoesin).

Effect of colour intensity (L* a* b*):

The spray dried beetroot colour powder was packed in aluminium pouch (P_1), brown pet bottle (P_2) and brown glass bottle (P_3) and stored in room (R_1) and refrigeration (R_2) temperatures for 3 months. The colour intensity (L* a* b*) of the packed samples were estimated at monthly intervals by using Hunter Colour Lab System, which consists of a rectangular coordinates system for the definition of colour in term of luminosity (L*), red versus green (a*) and yellow versus blue (b*). The colour intensity (L* a* b*) were carried out by the direct reading of the reflectance of the coordinates L* a* and b* using 45° / 0° optical geometry. As a standard, the standard illuminate A incandescent light was used by the method described by Kakali Roy *et al*. (2004).

Cost analysis:

Cost analysis of spray dried beetroot colour powders were computed taking into account the fixed cost, variable cost, interest, depreciation and products profit.

Statistical analysis:

The data obtained were subjected to statistical analysis to find out the impact of packaging materials and storage periods on the quality of the prepared natural colour powder. Factorial Completely Randomised Design (FCRD) was applied for the analysis of the study as described by Rangaswamy, (1995).

3.Results and discussion

The experimental results revealed that, the maximum absorbance value *(0.950)* was found in 3 percent acetone extraction followed by water extraction (0.825) and the minimum absorbance was found in ethanol extraction (0.465) based on the optical density at 476 nm in spectronic 20 colorimeter (Table 3).

Thus beetroot colour was extracted using 3 per cent acetone and 100 per cent water.

Effect of drying temperature, feed rate and maltodextrin (MD) on spray dried beetroot colour powders:

The beetroot colour powder recovery on spray drying is presented in Table 4. The highest powder recovery (14.6 g / 100 ml) was found at 200ºC, 25 ml / min feed rate with incorporating 20 % maltodextrin (MD) and increase in feed rate (from 25 ml to 30 ml / min) and decreasing the level of maltodextrin (from 20 % to 10 %) affected the powder recovery during spray drying. Statistical analysis of the data obtained for the

powder recovery of beetroot extract by spray drying showed highly significant difference between drying temperature, feed rate and addition of maltodextrin.

Hassan *et al*. (1990) found that the inlet air temperature of 198.5ºC was best for development of roselle extract (*Hibiscus sabdariffa L.*) powder by spray drying

Table 3. Extraction of colour from beetroot by solvents

Samples	Solvents	Absorbance at 476 nm	Transparency in percentage
Beetroot	Water extraction (100 %)	0.825	14.97
	Ethanol extraction		
	Ethanol 1 %	0.465	34.20
	Ethanol 2 %	0.563	27.34
	Ethanol 3 %	0.493	32.13
	Ethanol 4 %	0.692	12.02
	Ethanol 5 %	0.704	19.73
	Acetone extraction		
	Acetone 1 %	0.537	29.03
	Acetone 2 %	0.640	22.88
	Acetone 3 %	0.950	11.21
	Acetone 4 %	0.524	29.91
	Acetone 5 %	0.671	21.06

Table 4. Effect of drying temperature, feed rate and maltodextrin on spray dried beetroot powder

Drying Temperature (ºC) (T)	Feed rate (ml /min) (F)	Powder recovery (g / 100 ml)		
		Maltodextrin (%) (MD)		
		10	15	20
180	25	6.4	8.6	12.5
	30	6.1	7.9	10.3
200	25	7.7	10.1	14.6
	30	6.7	8.2	12.1
220	25	7.5	9.5	13.7
	30	6.2	8.1	10.4

Effect of pH on the stability of spray dried beetroot colour powders during storage:

	SED	CD(0.05)	CD (0.01)
T	0.03333	0.06761	0.09066**
F	0.02722	0.05520	0.07402**
M	0.03333	0.06761	0.09066**
TF	0.04714	0.09562	0.12821**
FM	0.04714	0.09562	0.12821**
TM	0.05774	0.11711	0.15702**
TFM	0.08165	0.16561	0.22206**

The steady degradation of colours were observed at varying ionic strength (pH 4, pH 7 & pH 9) throughout the storage period of one week at room (R_1) and refrigeration (R_2) temperatures (Table 5). The initial absorbance values of B_1, B_2 and B_3 were 0.64, 0.70 and 0.72, respectively at pH 4, which were decreased to 0.38, 0.54 and 0.60, respectively at room (R_1) temperature. Whereas, in refrigeration (R_2) temperature, the absorbance values were decreased to 0.53, 0.58 and 0.64, respectively at the end of the storage period of one week. Hence, the colour stability at pH 4 was higher compared to pH 7 and pH 9 in all treatments during storage in room (R_1) and refrigeration (R_2) temperatures. The samples at pH 7 showed a satisfactory stability in all samples during storage at both room (R_1) and refrigeration (R_2) temperatures when compared with samples at pH 9 and the stability of the colour was better in refrigeration (R_2) temperature than room (R_1) temperature in all treatments. The study revealed that the treatment had best colour value. It may be due to action of citric acid, which may be acted as a stabilising agent.

Table 5.Effect of pH on the stability of spray dried beetroot colour powders during storage

Beetroot powders (T)	Storage period in days (S)	Optical density values at 476nm					
		pH4		pH7		pH9	
		R_1	R_2	R_1	R_2	R_1	R_2
B_1	Initial	0.64	0.64	0.58	0.58	0.32	0.32
	1	0.62	0.62	0.56	0.58	0.28	0.30
	2	0.60	0.62	0.52	0.54	0.23	0.26
	3	0.51	0.60	0.43	0.52	0.20	0.24
	4	0.48	0.58	0.37	0.48	0.17	0.21
	5	0.45	0.56	0.31	0.45	0.15	0.20
	6	0.42	0.54	0.28	0.42	0.11	0.19
	7	0.38	0.53	0.22	0.40	0.05	0.17
B_2	Initial	0.70	0.70	0.60	0.60	0.40	0.40
	1	0.68	0.70	0.58	0.58	0.24	0.36
	2	0.66	0.68	0.56	0.54	0.20	0.28
	3	0.60	0.65	0.48	0.50	0.17	0.26
	4	0.58	0.64	0.45	0.48	0.15	0.25
	5	0.56	0.61	0.43	0.46	0.13	0.24
	6	0.55	0.60	0.40	0.45	0.10	0.23
	7	0.54	0.58	0.37	0.42	0.07	0.21
B_3	Initial	0.72	0.72	0.66	0.66	0.47	0.47
	1	0.70	0.72	0.65	0.66	0.42	0.46
	2	0.68	0.70	0.63	0.65	0.38	0.44
	3	0.67	0.69	0.61	0.62	0.33	0.40
	4	0.65	0.68	0.59	0.60	0.30	0.38
	5	0.63	0.67	0.57	0.58	0.28	0.36
	6	0.62	0.65	0.54	0.57	0.26	0.35
	7	0.60	0.64	0.53	0.55	0.24	0.33

	SED	CD(0.05)	CD (0.01)
T	0.00118	0.00233	0.00307**
S	0.00193	0.00380	0.00501**
P	0.00118	0.00233	0.00307**
R	0.00097	0.00190	0.00250NS
TS	0.00334	0.00658	0.00867**
TP	0.00205	0.00403	0.00531**
TR	0.00167	0.00329	0.00434**
SP	0.00334	0.00658	0.00867**
SR	0.00273	0.00538	0.00708**
PR	0.00167	0.00329	0.00434**
TSP	0.00579	0.01140	0.01502**
TSR	0.00473	0.00931	0.01227**
TPR	0.00290	0.00570	0.00751**
SPR	0.00473	0.00931	0.01227**
TSPR	0.00819	0.01613	0.02125**

Highly significant difference was observed between treatments, storage period and pH, whereas the storage temperature revealed no significant difference. The interaction between treatments, period of storage and pH showed highly significant changes for the colour stability of the beetroot powders.

Yizhong Cai *et al*. (2001) reported that the betaxanthin (*Celosia argentea)* was more stable at pH 5.5 than pH 7.0. Wang *et al*. (2006) reported that the initial absorbance value of betacyanin was 0.2, which was decreased to below 0.1, when the pigment was stored in pH < 7. Pattnaik *et al.* [2] reported that the betalain was fairly stable at pH range between 3.5 to 7.0 and had maximum stability in the range of pH 4.0 to 5.0. Similar trend was observed in the present study also.

Effect of preservative on the stability of spray dried beetroot colour powders during storage:

The minimum colour degradation was observed in 200 ppm of sodium benzoate in all treatments of the samples during storage in room (R_1) and refrigeration (R_2) temperatures (Table 6) and the colour degradation was increased with increase in the concentration of sodium benzoate (200 ppm to 1000 ppm).

Hence the experimental results revealed that addition of sodium benzoate had an adverse effect on the stability of beetroot colours during storage. Statistical analysis revealed that there was a significant difference between treatments, concentration of preservative and storage periods.

Rekha *et al*. (2007) found that the stability of the betalain pigment was above 80 per cent at 200 ppm of sodium benzoate and it was gradually decreased as the concentration of preservative increased. Similar trend was observed in the present study.

Table 6: Effect of preservative on the stability of beetroot powder during storage

Beet root powder (T)	Storage period in days (S)	Optical density values at 476nm									
		PR_1 (200ppm)		PR_2 (400ppm)		PR_3 (600ppm)		PR_4 (800ppm)		PR_5 (1000ppm)	
		R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
	Initial	0.49	0.49	0.47	0.47	0.45	0.45	0.42	0.42	0.40	0.40
	1	0.47	0.48	0.45	0.46	0.37	0.41	0.35	0.39	0.28	0.30
	2	0.42	0.46	0.39	0.42	0.32	0.37	0.26	0.34	0.25	0.25
B_1	3	0.38	0.39	0.36	0.38	0.27	0.32	0.15	0.31	0.12	0.27
	4	0.34	0.36	0.31	0.29	0.24	0.26	0.11	0.27	0.07	0.21
	5	0.27	0.29	0.23	0.25	0.18	0.23	0.08	0.22	0.06	0.16
	6	0.25	0.26	0.18	0.21	0.16	0.19	0.06	0.17	0.04	0.12
	7	0.23	0.25	0.17	0.19	0.14	0.17	0.05	0.15	0.04	0.08
	Initial	0.66	0.66	0.63	0.63	0.56	0.56	0.54	0.54	0.52	0.52
	1	0.54	0.52	0.52	0.52	0.47	0.49	0.45	0.46	0.40	0.43
	2	0.49	0.50	0.46	0.49	0.39	0.42	0.36	0.48	0.29	0.22
B_2	3	0.41	0.44	0.38	0.42	0.36	0.40	0.27	0.31	0.18	0.19
	4	0.37	0.39	0.25	0.34	0.22	0.37	0.18	0.27	0.09	0.15
	5	0.30	0.35	0.21	0.30	0.19	0.22	0.17	0.22	0.06	0.12
	6	0.27	0.31	0.17	0.28	0.06	0.21	0.05	0.16	0.04	0.10
	7	0.21	0.23	0.13	0.21	0.05	0.19	0.04	0.11	0.03	0.09
	Initial	0.89	0.89	0.75	0.75	0.68	0.68	0.64	0.64	0.56	0.56
	1	0.86	0.89	0.72	0.75	0.66	0.68	0.60	0.63	0.50	0.54
	2	0.84	0.87	0.69	0.74	0.60	0.66	0.57	0.60	0.47	0.50
B_3	3	0.80	0.85	0.67	0.72	0.58	0.64	0.54	0.59	0.42	0.48
	4	0.78	0.81	0.65	0.70	0.54	0.60	0.50	0.57	0.38	0.43
	5	0.76	0.79	0.64	0.68	0.50	0.58	0.47	0.55	0.36	0.39
	6	0.74	0.78	0.62	0.66	0.48	0.56	0.44	0.53	0.30	0.37
	7	0.73	0.76	0.60	0.64	0.46	0.52	0.40	0.49	0.24	0.28

	SED	CD (0.05)	CD (0.01)
T	0.00091	0.00180	0.00237 **
S	0.00149	0.00294	0.00386 **
P	0.00118	0.00232	0.00305 **
R	0.00075	0.00147	0.00193 NS
TS	0.00259	0.00508	0.00669 **
TP	0.00205	0.00402	0.00529 **
TR	0.00129	0.00254	0.00335 NS
SP	0.00334	0.00656	0.00864 **
SR	0.00211	0.00415	0.00546 **
PR	0.00167	0.00328	0.00432 **
TSP	0.00579	0.01137	0.01496 **
TSR	0.00366	0.00719	0.00946 **
TPR	0.00289	0.00568	0.00748 **
SPR	0.00472	0.00928	0.01222 **
TSPR	0.00818	0.01608	0.02116 **

Effect of temperature on the stability of spray dried beetroot colour powders:

The experimental results (Fig 1) revealed that temperature had a great influence on the stability of beetroot colour during storage. The colour degradation rate was higher at elevated temperature in all samples. The beetroot colour was not stable at high temperature (above 125°C). Among the different treatments, beetroot colour powder extracted with 3 per cent acetone and 0.1 per cent citric acid (B_3) showed maximum retention of colour. Highly significant difference was observed between treatments, temperature and storage periods. Kanner *et al.* (1976) reported that the degradation of carotenoid pigments was high at high temperature during the storage period of 90 days.

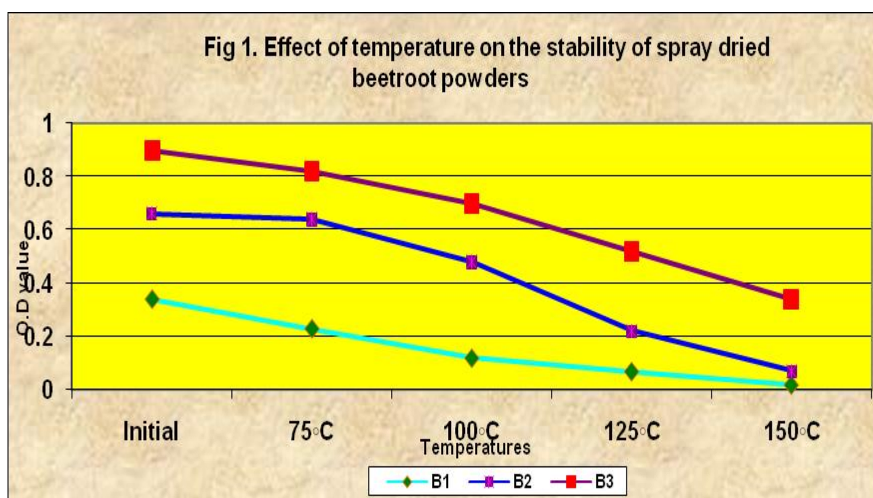

Figure 1 Effect of temperature on the stability of spray dried beetroot powders

Application of spray dried beetroot colour powders in confectionary products (jujubes, lollipop and icing with synthetic food colour (carmoesin) :

The L* a* b* value of the products coloured with carmoesin was 34.09, 22.28, 8.14 for jujubes, 26.38, 16.06, 2.35 for lollipop and 24.11, 15.54, 5.76 for icing , respectively. The jujubes, lollipop and icing with carmoesin revealed better colour stability compared to products containing beetroot powders (B_1, B_2 and B_3). At the same time the jujubes, lollipop and icing with B_3 had sufficient L* a* b* value compared to B_1 and B_2. The experimental results indicated that B_3 had best colour strength next to carmoesin than B_1 and B_2 during storage (Table 7)

Hanan *et al.* (2011) reported that the filling cream and glazing jelly containing extracted carotenoids (Tomato skin) had a high sensory attributes compared to synthetic food colour (Sun set yellow).

Alia *et al.* (2011) reported that the beef sausage containing 1 % and 3 % red beet powder had highest sensory scores.

Kiattisak *et al.* (2004) reported that the drink containing carmoesin or SAN RED RC had best colour stability and was acceptable for over 84 days. But the drinks containing roselle anthocyanin powder was least stable in colour and acceptable for over 56 days.

Table 7. Changes in the colour intensity (L* a* b*) of jujubes, lollipop and icing with synthetic food colour (carmoesin) and spray dried beetroot colour powders

Products	Synthetic food colour (carmoesin)			Beetroot extract powders								
				B_1			B_2			B_3		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Jujubes	34.09	22.28	8.14	44.95	13.52	1.92	45.17	15.72	2.96	46.16	17.07	4.21
Lollipop	26.38	16.06	2.35	20.16	4.01	7.81	20.51	5.56	7.85	22.14	5.95	7.93
Icing	24.11	15.54	5.76	19.39	2.95	7.82	91.54	3.76	7.87	21.74	5.12	7.93

Table 8. Changes in the colour intensity (L* a* b*) of spray dried beetroot powders during storage

Beetroot powders		Optical density values at 476nm					
		P_1		P_2		P_3	
		R_1	R_2	R_1	R_2	R_1	R_2
		a*	a*	a*	a*	a*	a*
B_1	Initial	25.47	25.47	25.47	25.47	25.47	25.47
	30	22.26	22.98	23.17	23.51	23.57	23.62
	60	20.52	21.07	22.75	22.82	22.84	22.95
	90	17.19	19.65	21.54	21.75	21.72	21.86
B_2	Initial	43.77	43.77	43.77	43.77	43.77	43.77
	30	35.16	37.17	36.75	38.01	36.84	37.91
	60	31.32	34.15	34.18	36.74	34.75	34.87
	90	28.93	30.16	33.26	35.31	33.81	34.65
B_3	Initial	44.48	44.48	44.48	44.48	44.48	44.48
	30	39.02	39.31	39.45	39.71	39.81	39.85
	60	37.64	37.84	38.01	38.51	38.54	38.74
	90	35.17	35.77	35.93	36.02	36.64	36.77

Effect of colour value (l* a* b*) on spray dried beetroot colour powders during storage:
Colour value (L* a* b*):

The experiment results indicated that the beetroot powder B_3 had highest a* (44.48) value in P_1, P_2 and P_3 packaging at both room (R_1) and refrigeration (R_2) temperatures (a* value which indicates redness of powder). The treatment B_3 had good colour stability compared to B_1 and B_2 during storage room (R_1) and refrigeration (R_2) temperatures which may be due to effect of added 0.1 per cent citric acid (stabilising agent) and also the brown glass bottles (P_3) had best colour retention during storage (Table 8).

Henriette *et al.* (2007) observed 30 per cent degradation of betacyanin in dark HDPE jars and the degradation rate was

increased to 57 per cent in translucent jars after six months of storage. Balaswamy *et al*. (2005) reported that the colour intimate to the dye powder was minimized during storage at refrigeration temperature compared than room temperature. The same trend was observed in the present investigation.

Cost analysis:

The production cost of spray dried beetroot colour powder was comparatively lesser than the market price. The cost of production of spray dried beetroot colour powder for 10 kg was worked out taking into account the profit, interest and depreciation. The total production of spray dried beetroot powder cost was Rs. 50 / 100g.

4.Conclusion

Among the solvents, acetone was found to be suitable for colour extraction from beetroot at the concentration of 3 per cent. The treatment B_3 had better colour intensity and stability than B_2 and B_1, which may be due to effect of added 0.1 per cent citric acid (stabilising agent) and reduces the colour losses in beetroot powders during spray drying and subsequent storage. Powder recovery percentage was found to be highest at 200ºC drying air temperature, 25 ml / min feed rate with incorporation of 20 per cent maltodextrin in spray drying. While the colour stability was better at pH4, the addition of preservative (Sodium Benzoate) and an elevated processing temperature had a detrimental effect on stability of colour during storage. The brown glass bottle (P_3) had better colour retention than brown pet bottle (P_2) and aluminium pouch (P_1) during storage at room (R_1) and refrigeration (R_2) temperatures. Jujubes, lollipop and icing prepared with beetroot colour powder B_3 had best colour strength next to carmoesin than B_1 and B_2 during storage and also found to posses highly acceptable quality attributes. The cost of the spray dried beetroot colour powder was Rs.50 / 100 g.

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Follow this order when typing manuscripts: Title, Authors, Affiliations, Abstract, Keywords, Main text, Acknowledgements, References, Figure Captions and then Tables. Do not import the Figures or Tables into your text.

Page setup:

- size A4
- top 3 cm
- bottom 1.5 cm
- left 2.5 cm
- right 2.5 cm

Title: Concise and informative, TNR, 14, bold, capital letters, center.

Author names: TNR, 12, bold, center (Ex. **Stănescu Maria**[1*]).

Affiliation: Indicate all affiliations with a lower-case superscript letter immediately after the author's name and provide the e-mail address of corresponding author, TNR, 10, italic, center. The corresponding author should be identified with an asterisk and should provide on a separate page also telephone and fax numbers (with country and area code) and the complete postal address.

Abstract: TNR, 10, the abstract should state briefly the purpose of the research, the principal results and major conclusions, it should contain 200-250 words.

Keywords: TNR, 10, italic, please provide 4-5 keywords that are not presented in the title.

Main text must be typewritten, one-spaced, TNR with a font size of 12 and should follow the arrangement described below:

- *Introduction* must give essential background but no subheadings; objectives must be clearly stated;
- *Materials and methods* with sufficient full experimental detail (where possible by reference) to permit repetition;
- *Results and discussion* should be presented concisely using well-designed tables and/or figures; the same data may not be used in both; appropriate statistical data should be given.

- *Conclusions* should be concise;
- *References* in the text should be identified by numbers in square brackets. References list should be arranged according to their appearance in the text. Always use the standard abbreviation of a journal's name according to ISI Journal Abbreviations Index.

Examples:

Reference to a journal publication:

Karacam, H., & Boran, M. (1996). Quality changes in frozen whole and gutted anchovies during storage at -18° C. *Int J Food Sci Tech*, *31*, 527–531.

The name of journals will be written according to ISI Journal Abbreviation Index.

Reference to a book:

Strunk, W., & White, E. B. (1979). *The elements of style.* (3rd ed.). New York: Macmillan, (Chapter 4).

Reference to a chapter in an edited book:

Lundberg, W.O. (1997). General deterioration reactions. In M. E. Stansby (Ed.)*, Fish oils: Their chemistry, technology, stability, nutritional properties and uses*. (pp. 141–147), Westport, Conn: Avi Publishing Co..

- *Figures and Tables* should be on separate pages after the reference list, and not be incorporated into the main text.

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