



Effect of volatile oils from *Petroselinum crispum* and *Foeniculum vulgare* on the quality and shelf-life of steamed yoghurts

Ahmed HARIRI ^{1*} and Naouel OUIS ²

^{1*} Bioconversion Laboratory, Microbiology Engineering and Health Safety, University Mustapha STAMBOULI of Mascara (UN 2901), BP. 763, Sidi Said, Mascara, 29000, Algeria.

² Laboratory of Physical Chemistry of Macromolecules and Biological Interfaces, University Mustapha STAMBOULI of Mascara (UN 2901), BP. 763, Sidi Said, Mascara, 29000, Algeria.

Corresponding author: Tel.: +213 661721190; E-Mail: haririahmed@yahoo.fr

Received: 01/12/2019

/Accepted: 16/06/2020

Abstract: The main objective of this work was to investigate the effect of the parsley and fennel essential oils addition on the qualities of the steamed yoghurts stored during four weeks. The aerial plants were subjected to the hydrodistillation for 3h using a Clevenger-type apparatus. Three yoghurts were prepared: Control, yoghurt added with 5µL of parsley essential oil, and yoghurt treated with 5 µL of fennel essential oil. The addition of 5 µL of parsley and fennel essential oils causes certain stability after 29 days of storage of the acidity respectively (95, 83 °D), dry matter (9.2, 12%), sugar (94, 118 g/L), protein (4, 4.2 g/L), and ash contents (1.02, 1.14%) compared to the control (118 °D acidity, 4% dry matter, 42 g/L sugar, 1.05 g/L protein and 0.41% for ash content). A sharp decreases after 29 days of storage of the level of fat content from 14.2% for control yoghurt to respectively 5 and 0.1% for yoghurts treated by essential oil of parsley and fennel. Results indicated complete absence of the microorganisms in all treated yoghurts and sensory evaluation indicated that the highest acidity, taste and texture were obtained with yoghurts treated with essential oils. This study found that yoghurts treated with essential oils seem preserve the nutritional, microbiological and sensory parameters during 29 days of storage.

Keywords: Essential oils; fennel; parsley, quality; yoghurt.

I. Introduction

Actually, the use of aromatic and medicinal plants in herbal medicine has been developed intensively by exploiting different herbs, fruits and legumes. Several studies were focused to naturally bioactive compounds that can preserve human health from oxidative stress damage caused by reactive oxygen species. The imbalance between reactive oxygen species and antioxidant defense system may lead to chemical modification of biologically relevant macromolecules (DNA, carbohydrates, proteins or lipids). These patho-biochemical mechanisms cause the development of different diseases [1]. Parsley *Petroselinum crispum* Hoffm and fennel *Foeniculum vulgare* L. belonging to the *Apiaceae* family are considered as aromatic and medicinal plants used often in traditional medicine for their vermifuge, emmenagogue diuretic, and purgative properties [2-5]. Parsley is known for its antidiabetic, antimicrobial, antihypertensive, anticoagulant, antihepatotoxic, membrane protective, antihyperlipidaemic, and antioxidant effects [6-8]. Fennel is known for a long time as a medicinal plant used for the treatment of several stomach affections and obesity. Presently, the ripe fruit is still widely used in Arabian folk medicine systems as a diuretic, stimulant, appetizer, digestive and infantile febrifuge [9]. This herb is traditionally used as treatment for colic, wind, irritable

bowel, kidneys, spleen, liver, suppressing appetite, breast enlargement, lungs, promoting menstruation, improving digestive system, milk flow and increasing urine flow [10]. This medicinal plant may be beneficial to humans as they have several phytoconstituents to cure diseases [11]. By its biological activities, the essential oil presents great interests in food process. It's having been considered as natural preservatives, and can be used as additional methods of controlling pathogens in food. Previous researches demonstrated the beneficial antimicrobial effect of the essential oils obtained from the two studied plants against pathogenic bacteria, yeasts and moulds [12, 13] and the minimal effect of these oils on useful bacteria such as lactic ferments [14]. However, the food application of these natural products was poorly investigated. Because of the wide use of these plants, it was of interest to evaluate the activity of its essential oils on the food process. Yoghurt a fermented milk product, has gained great popularity throughout the world for its sensorial, nutritional, and health promoting properties [15]. These results have led us to exploit the biological properties of these oils in a product such as steamed yogurt that we seek to eliminate pathogenic germs and preserve lactic ferments. Yoghurts enriched with essential oils provides novelty in the dairy foods market and help consumers ingest nutritional foods that have added health benefits. The aim of this study was to investigate the effects of the essential oils addition of parsley and fennel on the quality of steamed yoghurts during four weeks of storage.

II. Experimental Section

All chemicals and solvents were purchased from Sigma Aldrich (Munich, Germany), unless otherwise specified.

II.1. Plant material and essential oil extraction

The aerial part of *Petroselinum crispum* Hoffm and *Foeniculum vulgare* were harvested in the month of May 2016 from the region of Mascara in the Northwest of Algeria, and then identified by botanist at SNV faculty, University of Mascara. 100g of each plant in 500 mL of distilled water were subjected to the hydrodistillation for 3 hours using a Clevenger-type apparatus (ST15 OSA, Staffordshire, UK) until total recovery of oil. The extracted essential oil was dried over anhydrous sodium sulfate [16]. In order to preserve its original quality, the oil was stored at 4 °C until tested in an opaque glass bottle sealed to protect it from air and light (main agents of degradation).

II.2. Manufacture of steamed yoghurt

Commercial frozen yoghurt starter (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) graciously supplied by the GIPLait unit of Tizi, (Mascara, in West of Algeria) was reactivated by inoculation in sterilized milk. The day prior to yoghurt manufacture, one liter of partially skimmed milk was sterilized at 100 °C for 5 minutes and then the temperature was decreased to 45 °C. The starter culture was then added at 1:1 ratio and the baking was carried out at 45 °C until titratable acidity (TA) of 90 °D to 100 °D, the fermentation was stopped by cooling to 4 °C. Three steamed yoghurts were prepared: Control (CY), yoghurt added with 5 µL of parsley essential oil (PY), and yoghurt added with 5 µL of fennel essential oil (FY). The choice of these concentrations was based on preliminary studies showing that, beyond this concentration, these essential oils may have a negative effect on the activity and viability of lactic acid bacteria. The control mix (CY) was prepared by adding to one liter of partially skimmed pasteurized milk, 54 grams of the skimmed milk powder and 84 grams of crystallized sucrose. The mix was heated to 95 °C for 2 min, homogenized with a mixer Ultra-Turrax T25 (IKA-Werke GmbH, Germany) and then rapidly cooled to 45 °C. Starter culture was then added to each mix at 2% and then agitated after adding 5 µL of the essential oil of parsley or fennel. The inoculated yoghurt mixes were incubated at 45 °C for 2 to 3 hours until the TA of 70-90 °D and stopped by cooling to 4 °C.

II.3. Proximate biochemical analysis

All the yoghurt samples were stocked at 4 °C and then analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist [17] on the first day of manufacture and after each week of storage. The pH of yoghurt was measured using a digital pH meter apparatus (Mettler Toledo. MP220) and TA was determined by manual titration of suitable quantity 10g with 0.1 N NaOH using phenolphthalein as indicator. The volume of NaOH required to

neutralize the yoghurt was recorded and used to calculate the content of titratable acids [17]. The dry matter content was calculated after evaporation of the water present in the samples placed in an oven 105 °C for 24 hours (SPAG, Massy, France) until constant mass was obtained. The total fat content of the milk and yoghurts was determined by the Gerber method [18]. The Formaldehyde titration method was used to determine the total protein content of yoghurt [19]. Total sugar was determined colorimetrically at 480 nm by Dubois method [20]. Standards were prepared with glucose solutions at different concentrations. The ash content was determined according to the AOAC official method by incineration five gram of sample in a muffle furnace (Nabertherm, Germany) at 600 °C for 3 hours [17].

II.4. Microbiological analysis

These analyzes were carried out on the raw material and on the yoghurts throughout the period of storage. The total and fecal coliforms were counted, respectively in desoxycholate lactose agar and violet red bile lactose agar (VRBL) after 24 to 48 hours at 37 °C for total coliforms and at 44 °C for faecal coliforms [21]. Fecal streptococci were counted in Roche presumptive medium contains sodium azohydrate and Litsky confirmation medium contains sodium azohydrate and purple ethyl [21]. *Staphylococcus aureus* on Giolitti Cantonii and Chapman agar after incubation for 24 to 48 hours at 37 °C [22]. Mean *Salmonella* in Salmonella agar and incubated for 24 to 48 hours at 37 °C, after enrichment in Selenite-F Broth (SFB) medium. Search sulfite-reducing *Clostridium* can be done by counting the sporulated forms which develop in media Meat Liver VF containing sodium sulphite and iron alum after incubation for 48 h at 37 °C. The yeasts and moulds were quantified on potato dextrose agar (PDA) supplemented with oxytetracycline after 5 days at 25°C [23]. All colonies were counted on the plates containing less than 50 colonies and multiplied by dilution factor. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were counted, respectively in M17 and MRS mediums after 48 hours at 37 °C for *Streptococcus thermophilus* and at 44 °C at 72 hours for *Lactobacillus bulgaricus*.

II.5. Sensory evaluation

To determine the acceptability of steamed yoghurts stored after 21 days [24], all samples were evaluated for sensory characteristics (taste, texture and acidity) by 10 panelists (students and technicians from University of Mascara, Algeria); using a point scale 5: good, 3: acceptable, 1: bad. The selected trained participated in the evaluation and they were asked to fill in a questionnaire which included the following questions for the taste, texture and acidity. Panelist were informed and agreed to taste the sample before the tests, and they were informed of the type of product being tested and asked about their yoghurt consumption habits. Tap water was provided between samples to cleanse the palate.

II.6. Data analysis

All analyses were done in triplicate and the data were statistically evaluated by analysis of variance ANOVA by applied the level of significance ($P \leq 0.05$) using Excel 2010 and SPSS statistics Software 8.1.

III. Results and Discussion

III.1. Proximate composition

As shown in table 1, it seems that the raw materials used for the manufacture of steamed yoghurts (milk powder, pasteurized milk and sugar) present a good physicochemical, microbiological and hygienic quality. The TA was measured during the incubation at 45 °C and during the storage period of the yoghurts. For CY, the TA increase during the incubation period and reaches the desired value 77 °D after 2.5 hours but for FY and PY, the incubation lasted 5 hours to reach an acidity of 70 °D and 4 hours to reach acidity of 74 °D respectively (Figure 1). During the storage time, the TA of the CY gradually increases to 118 °D after 29 days (making the product very acidic), while the addition of essential oils of fennel and parsley to the yoghurts causes a certain stability of the acidity of 83 and 95 °D respectively after 29 days of storage (Table 2).

Table 1. Characteristics of the raw materials used for the preparation of steamed yoghurt. (/ No determined; Abs: absence; values represent Mean $\pm$ SD; n=3; Confidence level $P \leq 0.05$)

Raw Materials	Milk Powder	Pasteurized Milk	Sugar
pH	6.55 $\pm$ 0.1	6.52 $\pm$ 0.2	/
Titrateable acidity (TA) °D	14.5 $\pm$ 0.3	17 $\pm$ 0.1	/
Dry Mater content (DM) %	96 $\pm$ 0.4	2.95 $\pm$ 0.05	/
Total fat content %	1.22 $\pm$ 0.2	15 $\pm$ 0.4	/
Total proteins content g/L	27 $\pm$ 0.2	/	/
Density	/	1.030	/
Total coliforms	1 $\pm$ 0.1	Abs	Abs
Fecal coliforms	Abs	Abs	Abs
<i>Staphylococcus aureus</i>	Abs	Abs	Abs
Fecal streptococci	Abs	Abs	Abs
Sulfite-reducing <i>Clostridium</i>	Abs	Abs	Abs
<i>Salmonella</i>	Abs	Abs	Abs
Yeasts and moulds	12 $\pm$ 0.1	Abs	1 $\pm$ 0.1

Table 2. Results of the physicochemical and biochemical characterization of the steamed yoghurts (values represent Mean $\pm$ SD; n=3; Confidence level $P \leq 0.05$)

Parameters		Days of storage				
		1	7	15	21	29
pH	CY	4.78 $\pm$ 0.2	4.5 $\pm$ 0.1	4.33 $\pm$ 0.1	4 $\pm$ 0.1	3.78 $\pm$ 0.2
	PY	5.18 $\pm$ 0.2	4.95 $\pm$ 0.1	4.73 $\pm$ 0.2	4.5 $\pm$ 0.1	4.2 $\pm$ 0.1
	FY	5.34 $\pm$ 0.2	5.2 $\pm$ 0.1	4.99 $\pm$ 0.1	4.85 $\pm$ 0.1	4.76 $\pm$ 0.1
Titrateable acidity (°D)	CY	78 $\pm$ 0.3	85 $\pm$ 0.2	92 $\pm$ 0.2	100 $\pm$ 0.4	118 $\pm$ 0.3
	PY	74 $\pm$ 0.2	79 $\pm$ 0.1	83 $\pm$ 0.3	90 $\pm$ 0.3	95 $\pm$ 0.3
	FY	70 $\pm$ 0.3	73 $\pm$ 0.3	75 $\pm$ 0.2	79 $\pm$ 0.1	83 $\pm$ 0.1
Total proteins g/L	CY	4.4 $\pm$ 0.2	4.29 $\pm$ 0.2	4.15 $\pm$ 0.1	3.6 $\pm$ 0.1	1.05 $\pm$ 0.1
	PY	4.78 $\pm$ 0.1	4.55 $\pm$ 0.1	4.4 $\pm$ 0.2	4.25 $\pm$ 0.1	4 $\pm$ 0.1
	FY	5 $\pm$ 0.1	4.8 $\pm$ 0.2	4.5 $\pm$ 0.2	4.43 $\pm$ 0.1	4.2 $\pm$ 0.1
Dry mater %	CY	17 $\pm$ 0.5	15 $\pm$ 0.4	12 $\pm$ 0.4	7 $\pm$ 0.5	4 $\pm$ 0.3
	PY	19 $\pm$ 0.5	16 $\pm$ 0.5	13 $\pm$ 0.5	11.5 $\pm$ 0.4	9.2 $\pm$ 0.4
	FY	20 $\pm$ 0.3	18 $\pm$ 0.4	16.3 $\pm$ 0.4	14.5 $\pm$ 0.2	12 $\pm$ 0.1
Ash %	CY	1.2 $\pm$ 0.2	0.9 $\pm$ 0.2	0.77 $\pm$ 0.1	0.62 $\pm$ 0.1	0.41 $\pm$ 0.2
	PY	1.28 $\pm$ 0.1	1.22 $\pm$ 0.1	1.17 $\pm$ 0.2	1.1 $\pm$ 0.1	1.02 $\pm$ 0.1
	FY	1.3 $\pm$ 0.1	1.26 $\pm$ 0.1	1.21 $\pm$ 0.1	1.19 $\pm$ 0.2	1.14 $\pm$ 0.2
Sugar g/L	CY	130 $\pm$ 0.1	109 $\pm$ 0.2	88 $\pm$ 0.4	65 $\pm$ 0.3	42 $\pm$ 0.1
	PY	136 $\pm$ 0.3	126 $\pm$ 0.2	115 $\pm$ 0.2	104 $\pm$ 0.1	94 $\pm$ 0.1
	FY	140 $\pm$ 0.2	135 $\pm$ 0.2	129 $\pm$ 0.3	123 $\pm$ 0.3	118 $\pm$ 0.3
Total Fat Content %	CY	15 $\pm$ 0.3	14.8 $\pm$ 0.2	14.5 $\pm$ 0.3	14.2 $\pm$ 0.2	14.2 $\pm$ 0.1
	PY	14.3 $\pm$ 0.1	13 $\pm$ 0.2	12 $\pm$ 0.2	10 $\pm$ 0.1	5 $\pm$ 0.1
	FY	13 $\pm$ 0.1	10 $\pm$ 0.1	5 $\pm$ 0.2	1 $\pm$ 0.1	0.1 $\pm$ 0.3

The pH of the CY decrease during the storage period due to the acidification of the product by lactic acid. The low acidity produced by the ferments in treated yoghurts (PY and FY) explain the high pH values compared to the control (CY). The decrease in the dry mater was proportional to the duration of the storage due to the use of these nutrients by the ferments and microorganisms. After 29 days of storage, the dry matter content was 4% for CY, 12% for FY and 9.2% for PY. During the storage period, the protein level decreases progressively from 4.4% on the first day to 3.6% during the third week and rapidly during the fourth week 1.05%.

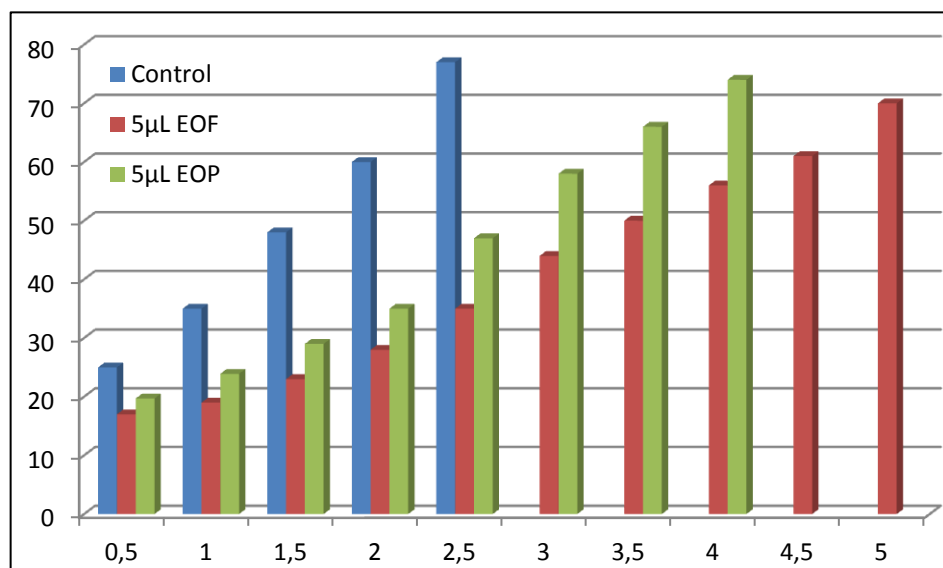


Figure 1: Evolution of the titratable acidity during incubation of the steamed yoghurts (CY, PY, FY).

Yoghurt with essential oils FY and PY preserves their nutritional qualities even after 29 days of storage (protein content was 4.2% and 4% respectively). The ash value of the control decreased gradually during storage period and reaches a value of 0.4% after 29 days of storage, whereas the treated yoghurts showed a very slight decrease with a mineral salt level after 29 days of storage in order of 1.14% for the FY and 1.02% for the PY. The sugar contents of the CY decreased from 130 g/L to 42 g/L after 29 days of storage. This decrease in the amount of sugar alters the nutritional quality of the steamed control yoghurt. Yoghurts with essential oils preserve their nutritional quality where the amount of residual sugar after 29 days of storage was 118 g/L for FY and 94 g/L for PY. During the storage time, a very slight variation of the fat content of the CY (14.2 g/L after 29 days of storage) was observed, in contrast to the FY remains as traces on the last day of the storage. For the PY, the fat content at the last day of storage remains low compared to the control (5 g/L).

III.2. Microbiological analysis

Results of microbiological analysis indicated complete absence of the total and fecal coliforms, *Staphylococcus aureus*, faecal streptococci and *Salmonella* in all types of yoghurt and throughout the storage period. This absence can be explained by the respect of hygienic conditions, the effectiveness of heat treatments and the acidifying activity of the lactic ferments which inhibits the proliferation of these germs. Concerning yeasts and moulds, these microorganisms appear in the first week of storage (2 yeasts/mL), then this number increases to 5 yeasts/mL during the last week of storage for CY. Treated yoghurts (PY and FY) were marked by a total absence of these microorganisms throughout the storage period. The treated yoghurts have satisfactory hygienic good quality and do not involve any risk of toxi-infection on the level of the consumer.

III.3. Count of lactic starters (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*)

Table 3 shows that the logarithm of the number of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* remains stable for CY around 8 and then decreases rapidly during the last week of storage up to 6. This decrease can be explained by the inhibition of growth of these lactic acid bacteria by its own acidity. For treated yoghurts, the amount of two lactic acid bacteria was stable at a logarithmic value of 7 throughout the storage period. The ratio *Lb. bulgaricus* / *St. thermophilus* was 1.20 during the first three weeks of storage for CY, and then increases to 1.9 after 29 days of storage. The increase of this ratio was due to the inhibition of the growth of *Streptococcus thermophilus* by the high acidity produced and its replacement by *Lactobacillus bulgaricus* which produces and supports more acidity. This high acidity impairs the organoleptic and nutritional quality of CY during the last week of storage, making it unsafe for human consumption. For yoghurts supplemented with 5 µL of fennel essential oil, this ratio was low from the first day of manufacture (1.1) and then increases slightly during storage to a ratio of 1.15 on the 29th day of storage, making the yoghurt always fit for

consumption. Yoghurt enriched with 5 μ L of parsley essential oil was labeled with a report *Lb. bulgaricus* / *St. thermophilus* ratio of 1.33 on the first day of manufacture and then decreases slightly to 1.26 at the end of the storage period making yogurt always fit for human consumption.

Table 3. Logarithmic count of the number of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and ratio *Lb. bulgaricus* / *St. thermophilus* during the storage of the steamed yoghurts. (Values represent Mean $\pm$ SD; n=3; Confidence level $P \leq 0.05$)

Parameters		Days of storage				
		1	7	15	21	29
<i>St. thermophilus</i>	CY	8.176 $\pm$ 0.01	8.178 $\pm$ 0.02	8.181 $\pm$ 0.01	8.183 $\pm$ 0.01	6.00 $\pm$ 0.02
	PY	7.079 $\pm$ 0.02	7.103 $\pm$ 0.01	7.123 $\pm$ 0.01	7.143 $\pm$ 0.01	7.158 $\pm$ 0.01
	FY	7.0 $\pm$ 0.02	7.008 $\pm$ 0.01	7.021 $\pm$ 0.02	7.029 $\pm$ 0.02	7.041 $\pm$ 0.01
<i>Lb. bulgaricus</i>	CY	8.257 $\pm$ 0.03	8.260 $\pm$ 0.02	8.263 $\pm$ 0.02	8.265 $\pm$ 0.02	6.278 $\pm$ 0.03
	PY	7.204 $\pm$ 0.02	7.220 $\pm$ 0.01	7.235 $\pm$ 0.03	7.248 $\pm$ 0.01	7.258 $\pm$ 0.01
	FY	7.041 $\pm$ 0.02	7.056 $\pm$ 0.03	7.073 $\pm$ 0.02	7.087 $\pm$ 0.01	7.103 $\pm$ 0.01
Ratio: <i>Lb/St.</i>	CY	1.206 $\pm$ 0.02	1.206 $\pm$ 0.02	1.206 $\pm$ 0.01	1.208 $\pm$ 0.01	1.900 $\pm$ 0.1
	PY	1.333 $\pm$ 0.01	1.307 $\pm$ 0.02	1.293 $\pm$ 0.01	1.273 $\pm$ 0.02	1.260 $\pm$ 0.02
	FY	1.100 $\pm$ 0.01	1.117 $\pm$ 0.02	1.128 $\pm$ 0.02	1.143 $\pm$ 0.01	1.154 $\pm$ 0.01

III.4. Sensory evaluation

Sensory evaluation was carried out on yoghurts kept for 3 weeks because yoghurt control was unfit for consumption during the fourth week of storage (Table 4). The sensory tests were carried out on the acidity, taste and texture of yoghurts. According to the results, the FY was best appreciated by the members of the panelist and has the best acidity 80%, followed by the CY then the PY. Unanimously, the members of the jury appointed the FY as the yoghurt with the best taste 100%, followed by the CY then the PY. The panelist did not appreciate the acidity and the taste of the yoghurt with essential oil of parsley. The taste of the yoghurt comes from the aromas produced by the lactic ferments when storing mainly diacetyl and butyric acid. According to the panel members, the steady texture of FY was best appreciated 80%, followed by CY and PY. The study found that steamed yoghurt enriched with essential oil of fennel was the best appreciated than parsley.

Table 4. Sensory evaluation of the steamed yoghurts (values represent Mean $\pm$ SD; n=3; Confidence level $P \leq 0.05$).

Sensory characteristics	Steamed yoghurts	Good	Acceptable	Bad
Acidity	CY	/	60	40
	FY	80	20	/
	PY	/	40	60
Taste	CY	30	70	/
	FY	100	/	/
	PY	/	/	100
Texture	CY	/	80	20
	FY	80	20	/
	PY	/	60	40

The TA of CY increases during the incubation period due to the utilization of residual sugar by viable lactic starters and production of lactic acid [25]. Proteolytic enzymes secreted by microorganisms degrade yoghurt proteins into free amino acids and contributes to increase of acidity [26]. It seems that the addition of 5 μ L of essential oils has an influence on the activity and viability of the lactic starter which causes an increase in incubation time. This effect is more pronounced for yoghurt treated by essential oil of fennel. The addition of these oils was difficult after incubation because of the firm texture which develops as a result of the activity of the ferments making it difficult to distribute these oils homogeneously inside the yoghurt. During the storage time, the TA of the CY

gradually increases while the addition of two essential oils to the yoghurts causes a certain stability of the acidity. These results confirm the results obtained by Bachir Raho and Benattouche [27] and the finding of Kucukoner and Tarakci [28], who found that the TA values increase with the storage time. According to Hess et al. [29], the pH of the yoghurt should be in the range of 4 to 5. Our results are in agreement with the findings of Bachir Raho and Benattouche [27], and Singh et al. [30], who reported a decrease in pH of yoghurt during the storage period. The values obtained show that the CY was very acidic after 29 days of storage 3.78 compared to FY and PY which display pH values of 4.76 and 4.2 respectively after 29 days of storage. The dry matter was represented mainly by the sugar, proteins, mineral salts, fats and vitamins of the yoghurt. The addition of the essential oils to the yoghurt seems to preserve the dry matter and thus its nutritional value. These results are similar with the findings of several authors [31, 32]. Proteins are nitrogenous substances used by lactic starters and microorganisms for their growth and multiplication. Fermentation of milk by lactic acid bacteria enhances its nutritional value through improved bioavailability and bio-disponibility of nutrients and production of energy, bioactive peptides and substances which have biological actions [33]. They provide numerous peptides with bioactive properties, form lactic acid and flavor compounds during fermentation and storage [34]. Exopolysaccharides (EPS) producing lactic acid bacteria are naturally produced during the fermentation process. Thus lactic starters can then perform formation of the protein network, which is responsible for yoghurt texture and addition of functionality through the capacity of EPS to improve serum retention and modulate viscosity [35]. The total protein level obtained was higher than 3.2 g/100 g reported by Buttriss [36] and 3.7 g/100 g reported by Rubico et al. [37]. During the storage time, the protein level decreases progressively due to the intense development of lactic ferments and degradation leading to formation of soluble compounds. These findings are in accordance with the results of Gündoğdu et al. [15] who observed that the protein contents of yoghurts varied between 4.13 and 4.19% and decreased during the storage period. However, in treated yoghurts, proteins were significantly preserved since bacterial growth was blocked by the use of these oils. These results are in agreement with those cited by Bachir Raho and Benattouche [27] who studied the effect of the incorporation of *Lavandula* and *Chamaemelum* essential oils in steamed yoghurt. These authors reported that the addition of those essential oils had a marked effect on inhibiting bacterial growth. The ash level obtained was higher to 0.27 g/100 g reported by Isanga and Zhang [38] and in line with the finding of Bachir Raho and Benattouche [27]. Sucrose used in the manufacture of yoghurt and the milk sugar (lactose) were used by the lactic ferments and microorganisms during the storage period [39]. The fat content obtained are non agreement with the finding of Bachir Raho and Benattouche [27] who reported that the addition of essential oil of *Rosmarinus officinalis* cause no change in the total fat level. Fermentation has a strong effect, resulting in a decrease of fat in yoghurts. After fermentation, the fat content of treated yoghurts was decreased. The results are in line with other observations [40]. However the fat levels in the different yoghurts with essential oils were lower than the control. Other researchers Sunny-Roberts et al., [41] also reported a similar trend in fermented groundnut milk. They attributed the reduction in fat level to utilization by starter's culture to yield energy. This may also be attributed to the process of homogenization. The extent of lipolysis in homogenized milk is much greater than in non homogenized milk, due, to the destruction of the protective layer of the fat globule membrane [42]. The reduction in the fat content may be an advantage to the keeping quality of yoghurt as chances of rancidity would be greatly reduced.

Actually the dairy products are a great medium for the growth of many spoilage and pathogenic microorganisms. Our microbiological results of all yoghurts are in line with the finding of several authors [27, 43].

Yoghurt is characterized as a fermented milk product with refreshing flavor, a slight sour taste and a smooth viscous gel [44]. These sensory properties offer quality control criteria, and therefore, yoghurt should be evaluated for acidity, taste, and texture. Yoghurt flavor (acidity) is influenced by the presence of lactic acid and other flavoring compounds produced by culture starter during the fermentation process. The two essential oils added did not inhibit the standard yoghurt cultures or overtly contribute to acid production from conversion of lactose to lactic acid. This finding is important because the addition of some oils can spoil milk and yoghurt and results in unpleasant flavor and odor. The texture of yoghurt is affected by the specific rate of acid production during the fermentation process, as well as the fat content and presence of stabilizing agents such as gelatin, milk solids, and

sugar. Heating the mix denatures whey proteins, increases the water-holding capacity of milk protein, and reduces syneresis in yoghurt [45].

IV. Conclusion

Yoghurts enriched with essential oils provides novelty in the dairy foods market and help consumers ingest nutritional foods that have added health benefits. The addition of essential oils of fennel and parsley to the steamed yoghurts preserve its nutritional qualities even after 29 days of storage and causes a certain stability of the physicochemical and biochemical characteristics (acidity, dry matter content, protein level, ash content and sugar content) compared to the control yoghurt marked by decrease these parameters after 21 days of storage. This decrease alters the nutritional quality of the steamed yoghurt. During the storage time, a very slight variation of the fat content of the control was observed, in contrast to the treated yoghurts remains as traces on the last day of the storage. The treated yoghurts have satisfactory hygienic good quality. Yoghurt containing fennel essential oil was successfully manufactured and found by sensory evaluation to be comparable in acidity, taste, and texture, to the standard yoghurt and has sensory attributes suitable for human consumption. This study found that steamed treated yoghurts were best appreciated than control yoghurt. As perspectives, it seems important to determine the active principles of the two essential oils involved in the inhibition of the development of pathogenic germs and to deepen studies to understand the reasons for the decrease in the total fat content in yogurts treated with these oils. An application of these oils in the preservation of other food products seems to be a good alternative to the addition of chemical preservatives.

V. References

- [1] Troszynska, A.; Estrella, I.; Luisa Lopez-Amores, M.; Hernandez, T. Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. *LWT-Food Sciences and Technology*, (2002), 35(2): 158-164. <https://doi.org/10.1006/fstl.2001.0831>
- [2] Charles, D.J. Parsley. In Peter, K.V. Handbook of herbs and spices 2nd Ed, Cambridge, Woodhead Publishing Limited, (2012): 430-451.
- [3] Rather, M.A.; Dar, B.A.; Sofi, S.N.; Bhat, B.A.; Qurishi, M.A. *Foeniculum vulgare*: a comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arabian Journal of Chemistry*, (2012). <http://dx.doi.org/10.1016/j.arabjc.2012.04.011>.
- [4] Valsikova, M.; Mezeyova, I.; Rehus, M.; Slosar, M. Changes of vitamin C content in celery and parsley herb after processing. *Potravinarstvo*, (2016), 10(1): 637-642. <https://doi.org/10.5219/687>
- [5] Rahimic, A.; Komlen, V.; Govedarica-Lucic, A.; Juric, AS. The influence of variety and fertilization on yield and content of vitamin C in the root of parsley (*Petroselinum* spp.). *Acta Agriculturae Serbica*, (2018), XXIII(45): 77-84. <https://doi.org/10.5937/AASer1845077R>
- [6] Fejes, S.; Blázovics, A.; Lemberkovics, E.; Petri, G.; SZ"oke, E.; Kéry, A. Free radical scavenging and membrane protective effects of methanol extracts from *Anthriscus cerefolium* L. (hoffm) and *Petroselinum crispum* (Mill) nym. Ex A. W. Hill. *Phytotherapy Research*, (2000), 14(5): 362-365.
- [7] Soliman, HA.; Eltablawy, NA.; Hamed, MS. The ameliorative effect of *Petroselinum crispum* (parsley) on some diabetes complications. *Journal of Medicinal Plants Studies*, (2015), 3(4): 92-100.
- [8] Akbar, S. Fennel (*Foeniculum vulgare* Mill.): A common spice with Unique Medicinal Properties. *Annals of Complementary and Alternative Medicine*, (2018), 1(1): 1-9.
- [9] Beaux, D. ; Fleurentin, J.; Mortier, F. Diuretic action of hydroalcohol extracts of *Foeniculum vulgare* var dulce (D.C.) roots in rats. *Phytotherapy Research*, (1997), 11: 320-322.
- [10] Delaram, M.; Kheiri, S.; Hodjati, M.R. Comparing the effects of echinop hora-platyloba, fennel and placebo on pre-menstrual syndrome. *Journal of Reproduction and Infertility*, (2011), 12 (3): 221-226.
- [11] Kooti, W.; Ghasemiboroon, M.; Asadi-Samani, M.; Ahangarpour, A.; Abadi, A.; Noori, M. The effects of hydro-alcoholic extract of celery on lipid profile of rats fed a high fat diet. *Advances in Environmental Biology*, (2014), 8(9): 325-330.
- [12] Ouis, N.; Hariri, A.; El abed, D. Phytochemical analysis and antimicrobial bioactivity of the Algerian parsley essential oil (*Petroselinum crispum*). *African Journal of Microbiology Research*, (2014), 8(11): 1157-1169. <https://dx.doi.org/10.5897/AJMR12.1021>
- [13] Ouis, N.; Hariri, A.; El abed, D. Composition chimique de l'huile essentielle du Fenouil (*Foeniculum vulgare*) de la région de Mascara. *PhytoChem & BioSub Journal*, (2014), 8(3): 198-203.

- [14] Ouis, N.; Hariri, A.; El abed, D. Effect of the essential oils from parsley and fennel seeds on the growth of *Lactobacillus casei* subsp. *rhannosus*. *Journal of Biotechnology and Biomaterial*, (2012), 2(3): 1-5. <http://dx.doi.org/10.4172/2155-952X.1000130>
- [15] Gündoğdu, E.; Çakmakçı, S.; Dağdemir, E. The effect of garlic (*Allium sativum* L.) on some quality properties and shelf-life of set and stirred yoghurt. *Turkish Journal of Veterinary and Animal Sciences*, (2009), 33: 27-35.
- [16] Benomari, F.Z.; Andreu, V.; Kotarba, J.; Dib, MEA.; Bertrand, C.; Muselli, A.; Costa, J.; Djabou, N. Essential oils from Algerian species of *Mentha* as new bio-control agents against phytopathogen strains. *Environmental Science and Pollution Research*, (2018), 25(30): 29889-29900. <https://doi.org/10.1007/s11356-017-9991-4>
- [17] AOAC (2007). Official methods of Analysis of AOAC international. (2007). Gaithersburg. Maryland.
- [18] Wehr, H.M.; Frank, J.F. Standard Methods for the examination of dairy products. 17th ed., 327-404, *American Public Health Association, Baltimore, U.S.A.* (2004): 363-527.
- [19] Ceirwyn, S.J. Analytical chemistry of foods. Edition Chapman and Hall, New York, (1999): 90.
- [20] Dubois, M.; Gilles, KA.; Hamilton, JK.; Rebers, PA.; Smith, F. Colorimetric method for Determination of sugars and related substances. *Analytical Chemistry*, (1956), 38(3): 350-356.
- [21] Tribst, AA.; Sant'Ana, S.; de Massaguer, PR. Microbiological quality and safety of fruit juices-past, present and future perspectives. *Critical Reviews in Microbiology*, (2009), 35(4): 310-339. <https://doi.org/10.3109/10408410903241428>
- [22] Anon. Approved Methods of Analysis. 10th Ed. The American Association of Cereal Chemists (AACC). St. Paul, MN. (2000).
- [23] Mossel, D.A.A.; Visser, M.; Mengerink, W.H.J. A comparison of media for the enumeration of moulds and yeasts in foods and beverages. *Laboratory Practice*, (1962), 11: 109-112.
- [24] Metin, M. Analysis Methods of Milk and Dairy products (Sensory, Physical and Chemical Analysis). Ege University Publications, Bornova. (2006).
- [25] Parmjit, S.P.; Shinde, C. Effect of storage on syneresis, pH, *Lactobacillus acidophilus* count, *Bifidobacterium bifidum* count of *Aloe vera* fortified probiotic yoghurt. *Current Research in Dairy Sciences*, (2012), 4: 17-23.
- [26] Widyastuti, Y.; Febrisiantosa, R.A. The role of lactic acid bacteria in milk fermentation. *Food and Nutrition Sciences*, (2014), 5: 435-442.
- [27] Bachir Raho, G.; Benattouche, Z. Evaluation of the quality of steamed yogurt treated by *Lavandula* and *Chamaemelum* species essential oils. *Journal of Medicinal Plants Research*, (2013), 7(42): 3121-3126.
- [28] Kucukoner, E.; Tarakci, Z. Influence of different fruit additives on some properties of stirred yogurt during storage. *Tarim Bilimleri Dergisi*, (2003), 13(2): 97-101.
- [29] Hess, S.J.; Roberts, R.F.; Ziegler, G.R. Rheological properties of nonfat yogurt stabilized using *Lactobacillus delbrueckii* ssp. *bulgaricus* producing exopolysaccharide or using commercial stabilizer systems. *Journal of Dairy Sciences*, (1997), 80: 252-263
- [30] Singh, G.; Singh, K.I.P.; Singh, P. Effect of volatile oil and oleoresin of anise on the shelf life of yogurt. *Journal of Food Process Preservation*, (2011), 35: 778-783.
- [31] Abd-El Fattah, S.M.; Yahia Hassan, A.; Bayoum, H.M.; Eissa, H.A. The use of lemongrass extracts as antimicrobial and food additive potential in yoghurt. *American Journal of Science*, (2010), 6: 582-594.
- [32] Al-Quatibi, M.; El-Demerdash, H. Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils. *African Journal of Microbiological Research*, (2008), 2: 156-161.
- [33] Korhonen, H.; Pihlanto, A. Bioactive peptides: production and functionality. *International Dairy Journal*, (2006), 16: 945-960.
- [34] Tamine, A.Y.; Deeth, H.C. Yoghurt: technology and biochemistry. *Journal of Food Protect*, (1980), 43: 939-977.
- [35] Gentès, M.C.; St-Gelais, D.; Turgeon, S.L. Exopolysaccharide–milk protein interactions in a dairy model system simulating yoghurt conditions. *Dairy Science and Technology*, (2013), 93: 255-271.
- [36] Buttriss, J.L. Food and nutrition: attitudes, beliefs, and knowledge in the United Kingdom. *American Journal of Clinical Nutrition*, (1997), 65(Suppl. 6): 1985S-1995S.
- [37] Rubico, S.M.; Resurreccion, A.V.A.; Frank, J.F.; Beuchat, L.R. Suspension stability, texture, and color of high temperature treated peanut beverage. *Journal of Food Science*, (1987), 52(6): 1676-1679.
- [38] Isanga, J.; Zhang, G. Production and evaluation of some physicochemical parameters of peanut milk Yoghurt. *LWT - Food Science and Technology*, (2009), 42: 1132-1138.
- [39] Omer, R.H.; Eltinay, A.H. Changes in chemical composition of camel's raw milk during Storage. *Pakistan Journal of Nutrition*, (2009), 8, 607-610.
- [40] Rahmawatia, I.S.; Suntornsuk, W. Effects of fermentation and storage on bioactive activities. Molecular and cellular life sciences: Infectious Diseases, Biochemistry and Structural Biology, 2015 Conference, MCLS 2015. *Procedia Chemistry*, (2016), 18: 53 - 62

- [41] Sunny-Roberts, E.O.; Otunola, E.T.; Iwakun, B.T. An evaluation of some quality parameters of laboratory-prepared fermented groundnut milk. *European Food Research and Technology*, (2004), 218: 452–455.
- [42] Tamime, A.Y.; Robinson, R.K. *Yoghurt science and technology*. London: Woodhead Publishing Ltd. (1999).
- [43] Olmedo, R.H.; Nepote, V.; Grosso, N.R. Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. *Food Science and Technology*, (2013), 53: 409-417.
- [44] Bodyfelt, F.W.; Tobias, J.; Trout, G.M. Sensory evaluation of dairy products. New York NY7 Van Nostrand Reinhold, (1988): 22-31.
- [45] Hekmat, S.; McMahon, DJ. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *Journal of Dairy Sciences*, (1992), 75: 1415-20.