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Full Length Research Paper

Microbiological, physico-chemical and sensory quality aspects of yoghurt enriched with *Rosmarinus officinalis* oil

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Two types of yoghurt with *Rosmarinus officinalis* oil was prepared and stored up to 21 days. The extract plant was added at the rate of 0.14, 0.21, 0.29, and 0.36 g/L. Yoghurt samples were analyzed for some physical, chemical, microbiological and sensory some characteristics. The total and fecal coliform count, *Staphylococcus aureus* count, *Salmonella* count, yeast and mold counts were determined in yoghurt samples at two, seven and 21 days of storage period. There were significantly differences between the control and enriched yogurts in the dry matter, lactose, ash and protein contents. No germ detected in all samples during the storage period. Sensory analysis indicated that the highest flavour, taste and texture, of the two yoghurt types added was with 0.14g/L of essential oil among the yoghurts samples.

Key words: Yoghurt, *Rosmarinus officinalis* oil, physical, chemical, microbiological and sensory properties.

INTRODUCTION

The consumption of milk and dairy products is common in the world. Of these, yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Bourlioux and Pochart, 1988), and is a popular product throughout the world. The highest production or consumption of yogurt is in Mediterranean, Asian countries and in central Europe (Sahana et al., 2008). Low calorie skimmed or half-skimmed yogurts have won popularity during the last decade (Penna et al., 2007). Yogurt has been known for its nutraceutical, therapeutic, and probiotic effects such as digestion enhancement, immune system boosting, anticarcinogenic activity and reduction of serum cholesterol (Penna et al., 2007; Najafi et al., 2008). The increase in the per capita annual consumption of yoghurt in the majority of the countries has been attributed to both the ever-increasing availability of fruit or flavored yoghurt, and to the diversity of presentations of the product (Tamime and Robinson, 1999). A variety of different flavoring ingredient (fruits, natural flavors or synthetic flavors) are currently added to yoghurt (Tamime and Robinson, 1999).

Spices are common food adjuncts, which have been used as flavoring, seasoning and coloring agents, and sometimes as preservatives throughout the world for thousands of years (Shan et al., 2005). The *Labiatae* family is one of the most employed medicinal plants as a worldwide source of spices and also as a consolidated source of extracts with strong antibacterial and antioxidant properties (Kaya et al., 2008). Within this family, the genus *Rosmarinus* consists of three species of aromatic shrubs native to the Mediterranean region, and *Rosmarinus officinalis* L is the best known and widespread species (Upson, 2000; Harley et al., 2004; Segarra-Moragues and Gleiser, 2009). It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing of a wide range of diseases such as diabetes mellitus, respiratory disorders, stomach problems and inflammatory diseases (Bakirel et al., 2008). Its dried leaves are also used to prepare soups and sauces. With due attention to their antioxidant, antibacterial and antifungal effects and that they give flavor to meat, fish and chicken they are used to keep the quality of fats

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and meats (Moghtader et al., 2011).

The purpose of this study was to evaluate the efficacy of the addition of *R. officinalis* extract on physical, chemical, sensory and microbiological properties of yogurt.

MATERIALS AND METHODS

Plant material and essential oil extraction

Fresh leaves of *R. officinalis* were harvested in Mascara University campus (North West of Algeria) during the first week of May 2011, and then identified according to the Flora of Algeria (Quezel and Santa, 1963). The oils were obtained from the fresh leaves by hydrodistillation for 3 h using a Clevenger-type apparatus. Briefly, the samples were completely immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour (until no more essential oil was obtained). The vapours were allowed to condense and the oil was then separated from the aqueous phase (Shafaghat et al., 2012). They were then kept in dark glass bottles at 4°C to prevent the negative effect of light, especially direct sunlight.

Yoghurt production

Two variations of yoghurts were manufactured at the Tizi Dairy laboratory, Mascara, Algeria: steamed yoghurt (SY) and fruited stirred yoghurt (FSY). Pasteurized milk was used for yoghurt production and milk powder (60 g/L) and sugar (100 g/L) were added. The mix was heated to 95°C for 2 min, homogenized and then rapidly cooled to 45°C. Commercial frozen yoghurt starter culture was reactivated by inoculation in sterilized reconstituted milk and then added to the mix, which distributed into five equal batches. One batch with no additive was taken as control. The other four batches were treated with *R. officinalis* extract at the following concentrations (per liter of milk): 0.14, 0.21, 0.29 and 0.36 g/L.

The mixture was agitated for 15 min, steamed at 45°C for 2 to 3 h, cooled and stored at 4 to 6°C. For the fruited stirred yoghurt, after the agitation (15 min) of the mixture (pasteurized milk, milk powder, and sugar) and incubation at 45°C for 2 to 3 h, essential oils and fruit preparation were added, subsequently stirred, cooled and stored in the refrigerator. At time intervals of 2, 7 and 21 days, yoghurt samples were subjected to microbiological, physicochemical and sensorial analysis.

The compositional analysis

The physicochemical characteristics of the samples were evaluated as follows:

1) The pH of yoghurt was measured using a digital pH-meter.
2) Titratable acidity, °D (Dornic degree), was measured by titrating 10 g of sample with 0.1 N NaOH using phenolphthalein as indicator.
3) Dry matter content was calculated after evaporation of the water present in the samples placed in an oven (102°C) for 48 h (until constant mass was obtained).
4) The total fat and lactose contents of the milk and samples were determined by the Gerber and Bertrand methods (O'Connor, 1995; Wehr and Frank., 2004).
5) The Formaldehyde titration method was used to determine the total protein content of yoghurt (Ceirwyn, 1999).
6) Total ash content was determined by incineration at 550°C for 3 h.

Microbiological methods

Total viable count and coliform bacteria

For microbial analysis, desoxycholate lactose agar and violet red bile lactose agar (VRBL) were used to determine total and fecal coliforms counts, respectively, using the pour-plate and overlay technique. Incubations were for 24 to 48 h at 37 and 44°C.

Detection of Staphylococcus aureus

Staphylococcus aureus counts were determined on Giolitti Cantonii and Chapman agar. The plates were incubated for 24 to 48 h at 37°C.

Detection of Salmonella

Dilutions were plated on *Salmonella shigella* (SS) agar and incubated for 24 to 48 h at 37°C, after enrichment in SFB medium for 24 to 48 h at 37°C.

Total yeast and molds

Total yeast and molds was determined on potato dextrose agar (PDA) to which antibacterial agents (oxytetracycline) was incorporated and incubated for three to five days at 25°C.

Sensory evaluation

The flavor, taste and texture of all yogurt samples were evaluated sensorial by Non-trained panel of ten students of the Mascara University, Mascara, Algeria using a five-point score system (1, excellent; 2, good; 3, acceptable; 4, bad; 5, insupportable). The sensory profiles were conducted on coded samples served in plastic cups and freeze stored after 21 days of storage.

RESULTS AND DISCUSSION

The physical-chemical characterization

The effect of *R. officinalis* extract on the physicochemical characteristics of yoghurt is detailed in Table 1. The pH changes of the yoghurt samples treated with plant extracts during 21 days of storage are shown in Table 1. The initial pH values were 4.66 for steamed yoghurt and 4.67 for fruited stirred yoghurt. Significant differences between the control sample and all treatments were observed at day 21. The pH values of all treatments were 4.61 for steamed yoghurt and 4.70 for fruited stirred yoghurt, whereas that of the control ranged from 4.66 to 4.08 for steamed yoghurt and 4.67 to 3.80 for fruited stirred yoghurt after 21 days of storage. The pH values were stable in the yoghurt samples treated with *R. officinalis* essential oil throughout the storage period, whereas the pH of the control sample decreased significantly. The reduction in pH can be due to the breakdown of lactose into lactic acid (Hassan and Amjad, 2010). The results are in line with the observation of Shan et al. (2011) in the cheese enriched with five spice and herb extracts. Shan et al. (2011) reported that the

Table 1. Physico-chemical changes of Yoghurt samples during storage.

Parameter		Storage period (days)					
		2		7		21	
		SY	FSY	SY	FSY	SY	FSY
pH	C	4.66	4.67	4.50	4.40	4.08	3.80
	C_1	4.61	4.70	4.61	4.70	4.61	4.70
	C_2	4.61	4.70	4.61	4.70	4.61	4.70
	C_3	4.61	4.70	4.61	4.70	4.61	4.70
	C_4	4.61	4.70	4.61	4.70	4.61	4.70
Titratable acidity (°D)	C	99	95	109	101	129	124
	C_1	102	90	102	90	102	90
	C_2	102	90	102	90	102	90
	C_3	102	90	102	90	102	90
	C_4	102	90	102	90	102	90
Dry matter (%)	C	26.4	24	21.06	21	14	16.95
	C_1	14.9	15. 4	14.94	15. 44	14.97	15. 48
	C_2	15	16	15.03	16.09	15.09	16.11
	C_3	15	16.2	15.12	16.27	15.15	16.31
	C_4	15.02	16.3	15.22	16.42	15.27	16.46
Ash (%)	C	0.92	0.92	0.84	0.89	0.70	0.70
	C_1	0.93	0.92	0.92	0.91	0.92	0.91
	C_2	0.95	0.92	0.95	0.92	0.95	0.92
	C_3	0.94	0.92	0.94	0.92	0.94	0.92
	C_4	0.95	0.92	0;95	0.92	0.95	0.92
Proteins (%)	C	4.4	4.6	4.00	4.06	3.6	3.9
	C_1	4.54	5.32	4.50	5.30	4.45	5.30
	C_2	4.60	5.51	4.57	5.51	4.57	5.50
	C_3	4.64	5.54	4.63	5.54	4.63	5.54
	C_4	4.67	5.54	4.67	5.54	4.67	5.54
Lactose (%)	C	15	13.5	13.6	11	11.34	9.89
	C_1	14.70	14.07	14.68	14.00	14.68	14.00
	C_2	14.70	14.10	14.70	14.06	14.70	14.06
	C_3	14.70	14.10	14.70	14.10	14.70	14.10
	C_4	14.70	14.10	14.70	14.10	14.70	14.10
Fat (%)	C	2.6	2	2.6	2	2.6	2
	C_1	2.7	1.9	2.7	1.9	2.7	1.9
	C_2	2.7	1.9	2.7	1.9	2.7	1.9
	C_3	2.7	1.9	2.7	1.9	2.7	1.9
	C_4	2.7	1.9	2.7	1.9	2.7	1.9

SY, Steamed yoghurt; FSY, fruit stirred yoghurt; C, concentration of the oil add to yoghurt; C, 0 g/L; C1, 0.14g/L; C2, 0.21 g/ L; C3, 0.29 g/ L; C4, 0.36 g/ L.

natural extracts, such as *R. officinalis* oil as mentioned by Moreno et al. (2006), contained high levels of phenolic compounds that contributed to the maintenance of lower pH in cheese (a dairy product like yoghurt). The titratable acidity (TA) of the control increased with the storage period. The values changed from 99 to 129 for steamed yoghurt control and from 95 to 124 for fruited stirred yoghurt control but are constant for the rest of the samples of the enriched yoghurt. During the two days of storage, the titratable acidity increased from 99 to 102 °D

for steamed yoghurt but decreased from 95 to 90 for fruited stirred yoghurt. These results are in agreement with that obtained by Abbas and Osman (1998) and Al.Otaibi and El.Demerdash (2008), who reported that the TA increased gradually during storage period. Increase in acidity was mainly due to increase in the number of lactic acid bacteria which converted lactose into lactic acid (Abdalla and Abdel Nabi Ahmed, 2010). Dry matter (DM) contents of samples ranged between 14 and 26.4%. With the exception of controls (DM decrease with storage period), dry matter contents of enriched yoghurt increased with the amount of essential oil added and storage period. The content of fruited stirred type yoghurts was slightly higher than that of steamed type yoghurts. These results are similar with the findings of Tamime (1978a; b), Tamime and Robinson (1985), Mehaia and El Khadragy (1999), Ismail et al. (2006), Al Otaibi et El Demerdash, (2008) and Abd-El Fattah et al. (2010). The addition of essential oil caused an increase in ash content of steamed yoghurt, during the first two days of storage from 0.92 to 0.95%. The ash values of the control treatment decreased gradually during storage period, but no changes were reported in the enriched-yoghurt. Keke et al. (2009) reported an increase in ash content of cheeses treated with *Sorghum vulgaris* and *Pimenta racemosa.* The protein content of yoghurts decreased during storage from 4.4 to 3.6% for steamed type and from 4.6 to 3.9% for fruited stirred type yoghurt. The decrease in protein content during storage might be due to protein degradation leading to formation of soluble compounds (Abdalla et al., 1993). In the same time, it increased with the amount of essential oil added during the first period of storage from 4.4 to 4.67% for steamed yoghurt and from 4.6 to 5.54% for fruited stirred type yoghurt. These findings are in accordance with the results of Gündoğdu et al. (2009) who observed that the protein contents of yoghurts changed varied between 4.13 and 4.19% and decreased during the storage period. The lactose contents of the steamed yoghurt decreased from 15 to 11.34% and from 14.5 to 9.89% for the fruited stirred type during 21 days of storage. With the amount of essential oil added, the lactose contents of the two yoghurt types decreased during the first two days of storage, but after this period a slight change was reported in the enriched-yoghurt. The decrease of lactose during storage could be attributed to the activity of microorganisms (Omer and Eltinay, 2009). Slightly effect on fat content was observed when the essential oil was added to the two yoghurt types. The values increased from 2.6 to 2.7% for the steamed yoghurt, but decrease from 2.0 to 1.9 for fruited stirred type. These results are similar with the findings of Tornambé et al. (2008).

Microbiological quality

On the microbiological level of the two types of yoghurt (Table 2), one notes complete absence of the total and fecal coliforms, *Staphylococcus aureus*, *Salmonellas* yeast and mould in the two categories of yoghurt.

In accordance with Canadian (AAC,2012), French (CuQ, 2010) and Algerian (OJAR, 1998) criteria, the enriched yoghurts have satisfactory hygienic good quality and do not involve any risk of toxi-infection on the level of the consumer.

This complete abolition of the germs is at the origin of monoterpenes such as α-pinene, β-pinene, myrcene 1,8-cineole and borneol major components of *R. officinalis* which possess strong antibacterial and antimicrobial activities (Deba et al., 2008; Okoh et al., 2010; Sokmen et al., 2003) on micro-organisms osmophiles responsible for the deterioration of the marketable quality of the food products.

Sensory characteristics

The flavor scores show that samples with 0.29 and 0.36 g/L essential oil took the lowest percentage compared to 0.14 and 0.21 g/L extract plant added to the two yoghurt types, indicating that the increase in the mass fraction of essential oil had a negative effect. The highest flavor percentage (80%) was obtained in the samples with 0.14 g/L extract plant added to fruited stirred yoghurt. Table 3 shows that samples C_1, C_2 and C_3 of steamed type yoghurt and C, C_1 of fruited stirred type yoghurt had sweetness with clean acid taste undertone, in contrast with samples C and C_4 of steamed yoghurt and C_2, C_3 and C_4 of fruited stirred yoghurt which have unacceptable or undesirable taste.

From the results of the jury, all yoghurt samples received significantly higher texture percentage. The steamed yoghurt control received the lowest percentage among the samples, while the rest are considered excellent with a percentage ranged between 80 to 100%. Essential oil mass fraction did not significantly change the texture percentage of the samples.

Conclusion

There were significant differences in physical, chemical microbiological and sensory properties of *R. officinalis* essential oil added to yogurts compared to the control. Extract plant addition decreased pH (slightly), dry matter and lactose values; increased titratable acidity (slightly), ash, proteins and fat (slightly) values. Generally, in respect to controls, storage time had no effect on physico-chemical properties. The enriched yoghurt presented a satisfactory hygienic quality, by the absence of any pathogenic germs. Panelists gave the highest flavour, taste and texture, to the two yoghurt types with 0.14g/L of essential oil among the yoghurts samples. On the basis of the findings, it can be concluded that addition of *R. officinalis* essential oil enhanced the qualities of yogurt.

Table 2. Microbiological quality of Yoghurt samples during storage.

Parameter	Germ	2nd day		7th day		21st day	
		FSY	SY	FSY	SY	FSY	SY
C_1	T.C	Abs	Abs	Abs	Abs	Abs	Abs
	F.C	Abs	Abs	Abs	Abs	Abs	Abs
	S.aureus	Abs	Abs	Abs	Abs	Abs	Abs
	Salmonella	Abs	Abs	Abs	Abs	Abs	Abs
	yeast and mould	Abs	Abs	Abs	Abs	Abs	Abs
C_2	T.C	Abs	Abs	Abs	Abs	Abs	Abs
	F.C	Abs	Abs	Abs	Abs	Abs	Abs
	S.aureus	Abs	Abs	Abs	Abs	Abs	Abs
	Salmonella	Abs	Abs	Abs	Abs	Abs	Abs
	yeast and mould	Abs	Abs	Abs	Abs	Abs	Abs
C_3	T.C	Abs	Abs	Abs	Abs	Abs	Abs
	F.C	Abs	Abs	Abs	Abs	Abs	Abs
	S.aureus	Abs	Abs	Abs	Abs	Abs	Abs
	Salmonella	Abs	Abs	Abs	Abs	Abs	Abs
	yeast and mould	Abs	Abs	Abs	Abs	Abs	Abs
C_4	T.C	Abs	Abs	Abs	Abs	Abs	Abs
	F.C	Abs	Abs	Abs	Abs	Abs	Abs
	S.aureus	Abs	Abs	Abs	Abs	Abs	Abs
	Salmonella	Abs	Abs	Abs	Abs	Abs	Abs
	yeast and mould	Abs	Abs	Abs	Abs	Abs	Abs

SY, Steamed yoghurt; FSY, fruit stirred yoghurt; C, concentration of the oil add to yoghurt; C, 0 g/L; C1, 0.14g/L; C2, 0.21 g/ L; C3, 0.29 g/ L; C4, 0.36 g/ L; T.C, total coliform; F.C, fecal coliform; Abs, absence.

Table 3. Sensory qualities of Yoghurt samples after 21 days of storage.

Parameter			Excellent (%)	Good (%)	Acceptable (%)	Bad (%)	Insupportable (%)
Flavor	SY	C	40	30	30	-	-
		C_1	70	10	10	-	10
		C_2	20	30	40	10	-
		C_3	-	-	-	-	100
		C_4	-	-	-	10	90
	FSY	C	50	20	30	-	-
		C_1	80	10	10	-	-
		C_2	-	-	30	70	-
		C_3	-	-	-	-	100
		C_4	-	-	-	-	100
Taste	SY	C	-	40	40	10	10
		C_1	70	10	10	-	10
		C_2	20	30	40	10	-
		C_3	50	30	10	-	10
		C_4	10	10	10	-	70
	FSY	C	50	20	30	-	-
		C_1	80	10	10	-	-
		C_2	-	-	-	-	100
		C_3	-	-	-	-	100

Table 3. Contd.

		C_4	-	-	-	-	100
Texture	SY	C	40	20	40	-	-
		C_1	100	-	-	-	-
		C_2	100	-	-	-	-
		C_3	100	-	-	-	-
		C_4	90	10	-	-	-
	FSY	C	-	20	80	-	-
		C_1	100	-	-	-	-
		C_2	80	20	-	-	-
		C_3	90	10	-	-	-
		C_4	100	-	-	-	-

SY, Steamed yoghurt; FSY, fruit stirred yoghurt; C, concentration of the oil add to yoghurt; C, 0 g/L; C1, 0.14g/L; C2, 0.21 g/ L; C3, 0.29 g/ L; C4, 0.36 g/ L.

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