academicJournals

Vol. 7(42), pp. 3121-3126, 10 November, 2013
DOI: 10.5897/JMPR12.1037
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Evaluation of the quality of steamed yogurt treated by *Lavandula* and *Chamaemelum* species essential oils

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Accepted 28 October, 2013

Yoghurt is a fermented milk known with considerable development in Algeria and worldwide. The physicochemical, microbial and organoleptic qualities of a milk-derived product manufactured in the milk factory of Tizi in Mascara city North West of Algeria and enriched with two commercial essential oils extracted from *Lavandula* and *Chamaemelum* species were of great interest. Yoghurt samples were analyzed for some physical, chemical, microbiological and sensory characteristics. The total and fecal coliform count, *Staphylococcus aureus* count, *Salmonella*, yeast and mold counts were determined in yoghurt samples at 2, 7 and 21 days interval. The results showed marked differences of the physicochemical parameters between the control and enriched yogurts. The samples tested are free from the fecal coliform, *Salmonella*, *Staphylococcus*, yeasts and molds germs whose research is recommended to define the microbiological quality of this fermented milk. The essential oil did not influence the sensory properties of the yoghurt at the lower concentration (C_1 and C_2). The enriched yoghurt with *Chamaemelum* spp. oil (C_3 and C_4) had presented better texture than those of control.

Key words: Yoghurt, essential oils, quality.

INTRODUCTION

During the last years, protection of food from spoilers and pathogens aroused great interest and was achieved by various physical and chemical methods. Among these numerous and abundant naturally occurring compounds, essential oil extracts have been considered as natural preservatives or food additives, and can be used as additional methods of controlling pathogens (Naidu, 2000). Among these foods, yoghurt a fermented milk product, has gained great popularity throughout the world for its recognized sensorial, nutritional, and health-promoting properties (Gündogdu et al., 2009). *Lavandula* and *Chamaemelum* species belong respectively to the Lamiaceae and Asteraceae families. They grow spontaneously and with abundance in Algeria (Omidbaigi et al., 2004; Mohammedi and Atik, 2011). They are commonly in folk remedies in many human ailments such as hay fever, inflammation, muscle spasms, menstrual disorders, insomnia, ulcers, wounds, gastrointestinal disorders, rheumatic pain, viral diseases and hemorrhoids. These species have some interesting properties and are used in cosmetics and fragrances (Cavanagh and Wilkinson, 2002; Srivastava and Gupta, 2010).

The objectives of this study were to utilize the extracts of *Lavandula* and *Chamaemelum* spp. in developing a yogurt of high acceptability and to determine the effects of essential oils additives on physical, chemical, sensory and microbiological properties of yogurt.

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MATERIALS AND METHODS

Essential oil and yoghurt production

Commercial *Lavandula* and *Chamaemelum* spp. essential oil were produced by Menara Packaging Company of Medicinal Plants and Essential Oils (Bourj Bouarirj, Algeria).

The steamed yogurt was made by mixing the pasteurized milk and 60 g/L of milk powder with 100 g/L of crystallized sugar (sucrose). The mix was pasteurized to 95°C for 2 min, homogenized and then rapidly cooled to 45°C by resting in a temperature room. Commercial frozen yoghurt starter culture was reactivated by inoculation in sterilized reconstituted milk and then added to the mix, then filled into five equal batches. One batch with no additive was taken as control. The other four batches were treated with one of the two commercial extracts (*Lavandula* and *Chamaemelum* spp. oil) at the following concentrations (per liter of milk): 0.14, 0.21, 0.29, and 0.36 g/L. After 15 min agitation, the yogurt formed was steamed at 45°C for 2 to 3 h, cooled and stored at 4 to 6°C.

Physico-chemical and microbial analysis

The pH of yoghurt was determined by using a digital pH-meter and titratable acidity was measured by manual titration of suitable quantity (10 g) of the sample with standardized 0.1 N NaOH using phenolphthalein as indicator. The volume of NaOH required to neutralize the yogurt acid was recorded and used to calculate the content of titratable acids (lactic acid percentage equivalent). Total solids content was determined after evaporation of the water present in the samples placed in an oven at 102°C for 48 h (until constant mass was achieved). The total fat and lactose contents of the samples were determined, respectively by Gerber and Bertrand methods (O'Connor, 1995; Wehr and Frank, 2004). The Formal titration method was used to estimate the total protein content of yoghurt (Ceirwyn, 1999). Crude ash content was measured after incineration for 3 h at 550°C.

The total and fecal coliforms were counted, respectively desoxycholate lactose agar and violet red bile lactose agar (VRBL) after 24 to 48 h at 37 and 44°C, *Staphylococcus aureus* on Giolitti Cantonii and Chapman agar after 24 to 48 h at 37°C, mean *Salmonella* in *Salmonella-Shigella* agar and incubated for 24 to 48 h at 37°C, after enrichment in Selenite-F Broth (SFB) medium for 24 to 48 h at 37°C, and the yeasts and moulds on potato dextrose agar (PDA) supplemented with oxytetracycline after 5 days at 25°C.

Sensory evaluation

All the samples were evaluated for sensory characteristics (flavor, taste and texture) by 10 panelists (students from Mascara University, Mascara, Algeria); using a five point scale (1: excellent, 2: good, 3: acceptable, 4: bad, 5: insupportable). The sensory profiles were conducted on coded samples served in plastic cups, that did not impart any flavor or odor to the products, and freeze stored after 21 days of storage.

RESULTS AND DISCUSSION

Physico-chemical parameters

Yoghurt samples containing different concentrations of commercial essential oils were analyzed after two, seven and twenty-one days of the storage and results are shown in Tables 1 and 2.

The pH values of the samples varied between 4.08 and 4.66 for the yoghurt enriched with *Chamaemelum* spp. extracts and between 4.52 and 4.61 for the yoghurt added by *Lavandula* spp. oils. These results are in line with the findings of Cinbas and Yazici (2008), Singh et al. (2011), Zainoldin and Baba (2009) who reported a decrease in pH of yogurt during storage.

With the amount of essential oil added, the pH of the yoghurts declined slightly during storage period, whereas the pH values were stable in the samples treated with 0.36 g/L of *Chamaemelum* spp. extract and 0.29 and 0.36 g/L of *Lavandula* spp. oil throughout the storage period. These results are in accordance with the findings of Shan et al. (2011) who found no variation in pH of different cheese samples treated with cinnamon stick, oregano, clove, and pomegranate peel extracts during the storage period.

This decrease might be attributed to the utilization of residual carbohydrates by viable microorganisms and production of lactic acid, small amounts of CO_2 and formic acid from lactose (Parmjit et al., 2012). With the exception of the samples treated with 0.36 g/L of *Chamaemelum* spp. extract and 0.29 and 0.36 g/L of *Lavandula* spp. oil, the titratable acidity (TA) of enriched yoghurts increased during the storage period. The TA values of the samples varied between 99 and 129 for the yoghurt enriched with *Chamaemelum* spp. oils and between 102 and 112 for the yoghurt added by *Lavandula* spp. extracts.

These results confirm the results obtained by Kucukoner and Tarakci (2003), who found that the TA values of stirred yogurt supplemented with different fruit additives increase with the time.

The mass fraction of total solids in yoghurts with added *Chamaemelum* spp. oils was lower than that of the yoghurts without extract. The sample with 0.14 g/L contained the least mass fraction of total solids (14.69%), while the yoghurts without oils had the highest protein mass fraction of 26.4%. The mass fraction of total solids in yoghurts with added *Lavandula* spp. extract was higher than that of yoghurts with *Chamaemelum* spp. oils. It ranged between 15 and 15.64% for the first and between 14.69 and 15.3% for the second. The total solids contents of control samples decrease during storage, whereas the mass fractions of the dry matter of the treated yoghurts increase with the time.

The results are in line with the observation of some authors (Cinbas and Yazici, 2008; Hassan and Amjad, 2010; Lutchmedial et al., 2004; Mahmood et al., 2008). The increasing oils rate in yogurts increased ash contents, whereas they decrease during the storage period. The ash values remained stable in samples treated with 0.36 g/L of *Chamaemelum* spp. extract and 0.29 and 0.36 g/L of *Lavandula* spp. oil throughout the storage period at 0.95%.

Table 1. Physio-chemical changes in pH, titratable acidity, total solids and ash of yoghurt samples during 2, 7 and 21 days of storage.

Extract		pH			Titratable acidity (°D)			Total solids (%)			Ash (%)		
		2	7	21	2	7	21	2	7	21	2	7	21
Chamaemelum spp.	C	4.66	4.50	4.08	99	109	129	26.4	21.06	14	0.92	0.84	0.70
	C_1	4.61	4.54	4.48	102	110	115	14.69	14.72	15	0.95	0.89	0.74
	C_2	4.61	4.59	4.57	102	107	108	14.74	14.79	15	0.96	0.87	0.73
	C_3	4.61	4.60	4.60	102	105	105	14.78	14.82	15	0.95	0.90	0.90
	C_4	4.61	4.61	4.61	102	102	102	14.81	14.88	15.3	0.95	0.95	0.95
Lavandula spp.	C_1	4.61	4.57	4.52	102	109	112	15	15.14	15.19	0.94	0.86	0.69
	C_2	4.61	4.59	4.59	102	107	107	15	15.23	15.31	0.95	0.90	0.90
	C_3	4.61	4.61	4.61	102	102	102	15.3	15.42	15.48	0.95	0.95	0.95
	C_4	4.61	4.61	4.61	102	102	102	15.5	15.59	15.64	0.95	0.95	0.95

C: Control, C_1: 0.14 g/L, C_2: 0.21 g/L, C_3: 0.29 g/L, C_4: 0.36 g/L.

Table 2. Physio-chemical changes in proteins, lactose and fat of yoghurt samples during 2, 7 and 21 days of storage.

Extract		Proteins (%)			Lactose (%)			Fat (%)		
		2	7	21	2	7	21	2	7	21
Chamaemelum spp.	C	4.40	4.00	3.6	15	13.6	11.34	2.6	2.6	2.6
	C_1	4.49	4.40	4.09	14.65	14. 65	14.64	3.8	3.8	3.8
	C_2	4.47	4.39	4.06	14.65	14.62	14.57	3.4	3.4	3.4
	C_3	4.46	4.30	3.80	14.65	14.57	14.23	3.1	3.1	3.1
	C_4	4.45	4.20	3.60	14.64	14	13.43	2.9	2.9	2.9
Lavandula spp.	C_1	4.53	4.50	4.50	14.65	14.64	14.64	2.8	2.8	2.8
	C_2	4.49	4.47	4.45	14.65	14.63	14.63	2.6	2.6	2.6
	C_3	4.49	4.40	4.31	14.64	14.58	14.52	2.5	2.5	2.5
	C_4	4.47	4.36	4.27	14.63	14.49	14.40	2.2	2.2	2.2

C: Control, C_1: 0.14 g/L, C_2: 0.21 g/L, C_3: 0.29 g/L, C_4: 0.36 g/L.

The results are in agreement with the findings of Gad et al. (2010), Keke et al. (2009) and Kucukoner and Tarakci (2003). The values for proteins, lactose and fat estimated during storage of yogurt samples are shown in Table 2. The analysis of protein values of the experimental treatments showed a decrease in protein percentage with the amount of essential oil added and a progressed storage period except in a control which present the lowest value.

The decrease in protein content during storage might be due to protein degradation leading to formation of soluble compounds (Abdalla et al., 1993). These findings are in agreement with some results of Kucukoner and Tarakci (2003), Gündoğdu et al. (2009), Lutchmedial et al. (2004), and Tornambé et al. (2008).

The mass fraction of lactose in yoghurts ranged between 11.34 and 15% for the control, 13.43 and 14.65% for the samples supplemented with *Chamaemelum* spp. extracts and between 14.40 and 14.65% for the yoghurt added by *Lavandula* spp. oils. As seen in Table 2, the addition of the two essential oils caused a decrease of lactose values in all samples of yogurts during storage period. Tornambé et al. (2008) reported that addition of 3.0 µl/L of essential oil to milk decreased lactose content from 48.7 to 48.6 g/L. Omer and Eltinay (2009) reported that the decrease in lactose contents during storage is due to the microorganism's activity.

Fat contents ranged from 2.2 to 3.8%. They remained unchanged throughout storage in all treatments, but they decreased with the increasing of the level of essential oils except in a control which present the lowest value as compared to majority of the samples. The fat values of yoghurts with added *Chamaemelum* spp. extract was higher than that of the yoghurts with *Lavandula* spp. oils. These results are similar with the findings of Cinbas and Yazici (2008), Gündoğdu et al. (2009), Keke et al. (2009), Kucukoner and Tarakci (2003), Lutchmedial et al. (2004) and Tarkçi (2010).

Microbial succession during storage of yogurt

Table 3 showed the total and faecal coliforms counts,

Table 3. Effects of *Chamaemelum* spp. and *Lavandula* spp. extracts on microbial counts of yogurts after 2, 7 and 21 days of storage.

Extract		Yogurts enriched with different concentration of essential oils											
		C_1			C_2			C_3			C_4		
		2	7	21	2	7	21	2	7	21	2	7	21
Chamaemelum spp.	T .C	10	11	11	03	05	05	Abs	Abs	Abs	Abs	Abs	Abs
	F .C	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	S. aureus	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Sal	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Y & M	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Lavandula spp.	T .C	07	09	09	03	06	09	Abs	Abs	Abs	Abs	Abs	Abs
	F .C	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	S. aureus	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Sal	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Y & M	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs

C_1: 0.14 g/L, C_2: 0.21 g/L, C_3: 0.29 g/L, C_4:0.36 g/L; T.C: total coliforms; F.C: faecal coliforms; *S. aureus*: *Staphylococcus aureus*; *Sal: Salmonella;* Y&M: yeast and mold

S. aureus counts, *Salmonella* counts, yeast and mould counts of different enriched yoghurt samples. The results revealed the presence of total coliforms in the samples of the yoghurts treated with 0.29 and 0.36 g/L of the both extract and thereafter these organisms disappeared when the amount of essential oil decrease. *S. aureus* and *Salmonella*, yeast and mould were absent in all the samples analysed.

The complete abolition of the germs can be attributed to the presence of linalool, linalyl acetate, 1, 8-cineole, beta-ocimene (usually both *cis*- and *trans*-), terpinen-4-ol and camphor major components of *Lavandula* spp. (Cavanagh and Wilkinson, 2002; Dob et al., 2005; Imelouane et al., 2009) and α-bisabolol, bisabolol oxides, chamazulene of *Chamaemelum* spp. (Raal et al., 2011; Sharafzadeh and Alizadeh, 2011) which have been found *in vitro* to have antimicrobial properties on these germs responsible for deterioration of nutritional quality and taste of food and producers as toxins responsible for food poisoning infection.

Table 4 show the results obtained for the sensory analysis carried out at the end (day 21) of the assay. The yogurt samples containing *Lavandula* spp. extract had significantly higher flavor score, than the one containing *Chamaemelum* spp. oil and the control.

The increase in the essential oil amount did not affect the flavor of yoghurt. The highest percentage (100%) was obtained in the samples with 0.36 g/L (C_4) of *Lavandula* spp. oil.

The enriched yoghurt with 0.14 g/L of the two essential oils showed a significant better score for overall taste compared to the control (yoghurt without extract), while the remainder samples (C_2, C_3, C_4), showed a decrease in the percentage of acceptability by panelists. This might be due to the addition of both essential oil that enhanced the yogurt taste at a low concentration, but affect it at a high concentration. Yogurt texture characterization is important for product and process development, quality control and to ensure consumer acceptability (Tarakçi, 2010).

All yoghurt samples with *Chamaemelum* spp. oil received significantly higher texture scores than yoghurts with *Lavandula* spp. extract and control. Among the samples with added essential oil, the highest score was obtained for the samples with 0.29 and 0.36 g/L *Chamaemelum* spp. extract, while the lowest was obtained for the samples with 0.29 and 0.36 g/L *Lavandula* spp. oil.

In conclusion, the addition of essential oil to yoghurt formula was successfully achieved. Oils incorporation significantly decreased pH, protein, lactose, fat, and increase the titratable acidity values, the mass fraction of total solids and ash in the yoghurts.

Considering the physico-chemical properties of the yoghurt, it was found that essential oils have a marked effect on bacteria, yeast and mould. Panelists gave the highest flavour, taste and texture scores to the yoghurts with 0.14 g/L of essential oil among the yoghurts.

Although the two plant extracts tested in this study have been widely used in many food preparations, further studies addressing the safety and toxicity of using such natural extracts in yoghurt will be necessary.

ABBREVIATIONS

C, Control; **C_1,** 0.14 g/L; **C_2,** 0.21 g/L; **C_3,** 0.29 g/L; **C_4,** 0.36 g/L; ***C.* spp,** *Chamaemelum* spp.; ***L.* spp,** *Lavandula* spp.; **T.C,** total coliforms; **F.C,** faecal coliforms; ***S. aureus,*** *Staphylococcus aureus*; ***Sal,*** *Salmonella;* **Y&M,** yeast and mold.

Table 4. Effect of Addition of different concentrations (C_1- C_4) of *Chamaemelum* spp. and *Lavandula* spp. extracts on sensory characteristics of yoghurt samples after 21 days of storage.

			Excellent (%)	Good (%)	Acceptable (%)	Bad (%)	Insupportable (%)
Flavor	*Chamaemelum* spp.	C	-	40	40	10	10
		C_1	40	20	10	30	-
		C_2	20	20	40	20	-
		C_3	20	40	40	-	-
		C_4	-	40	60	-	-
	Lavandula spp.	C_1	60	40	-	-	-
		C_2	40	30	20	10	-
		C_3	30	40	30	-	-
		C_4	-	100	-	-	-
Taste	*Chamaemelum* spp.	C	40	30	30	-	-
		C_1	60	10	10	20	-
		C_2	20	20	40	20	-
		C_3	-	10	10	30	50
		C_4	-	-	-	-	100
	Lavandula spp.	C_1	50	30	20	-	-
		C_2	40	30	20	10	-
		C_3	-	10	30	40	20
		C_4	-	-	-	30	70
Texture	*Chamaemelum* spp.	C	-	20	80	-	-
		C_1	90	10	-	-	-
		C_2	50	50	-	-	-
		C_3	100	-	-	-	-
		C_4	100	-	-	-	-
	Lavandula spp.	C_1	50	50	-	-	-
		C_2	20	60	20	-	-
		C_3	-	10	30	60	-
		C_4	-	-	10	10	80

C: Control, C_1: 0.14 g/L, C_2: 0.21 g/L, C_3: 0.29 g/L, C_4: 0.36 g/L.

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