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Research Article

Impact of Myrrh Essential Oil as a Highly Effective Antimicrobial Agent in Processed Cheese Spreads

¹A.G. Mohamed, ¹Hayam M. Abbas, ¹Jihan M. Kassem, ²Walid A. Gafour and ³A.G. Attalah

¹Department of Dairy, National Research Centre, 33th El-Tahrir St., Cairo, Egypt

²Institute of Food Technology Research, Ministry of Agriculture, Giza, Egypt

³Department of Microbial Genetics, National Research Centre, 33th El th El-Tahrir St Cairo, Egypt

Abstract

A new approach to prevent the proliferation of microorganisms or protect food from oxidation is using of essential oils or plant extracts. Among the antimicrobial agents, *Commiphora myrrha* is considered as natural and safe materials. The antimicrobial activity of *Commiphora myrrha*-essential oil against different species of pathogenic gram-positive as well as gram-negative bacteria were investigated. Data revealed that all tested microorganisms were susceptible to the action of *Commiphora myrrha*. Their Minimum Inhibitory Concentration (MIC) ranged from 2-5 $\mu\text{L mL}^{-1}$ for all microorganisms. Processed Cheese Spreads (PCSs) samples were prepared by using five ratios of *Commiphora myrrha*-Essential Oil (EO) to evaluate their properties and their acceptability. Their properties were estimated through one year of storage at $5 \pm 2^\circ\text{C}$. Obtained results showed that using 2% (w/w) *Commiphora myrrha*-EO for preparing PCS gave satisfactorily sensory properties. The appearance was well shiny; gumminess and oil separation were absent. The penetration of satisfied treatment of myrrh (2%) was (33.5, 32.0, 31.2, 30.00 and 29.20 mm) compared to control samples (33.0, 30.5, 26.5, 25.1 and 24.5 mm) when fresh and after 1, 3, 6 and 12 months, respectively. On the other hand, meltability took the same trend; the treated-samples gained 85.4, 81.6, 80.0, 79.1 and 78.2 mm comparing with control samples 81.6, 80.5, 78.7, 77.1 and 76.6 mm, respectively. Therefore, it could be concluded that using of 2% w/w *Commiphora myrrha*-essential oil produced acceptable and satisfied processed cheese spreads and it could be used as a natural preservation in dairy products.

Key words: *Commiphora myrrha*-essential oil, processed cheese spreads, antimicrobial action

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Corresponding Author: A.G. Mohamed, Department of Dairy, National Research Centre, 33th El-Tahrir St., Cairo, Egypt

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Commiphora myrrha (Myrrh) family (Burseraceae) is native to Northeastern Africa, especially Somalia. Myrrh is one of the oldest known medicines and was widely used by the ancient Egyptians. It is commonly known in Arabic as bitter gum. Myrrh has been traditionally used from northern Africa east through India to treat a huge range of ailments, as an anti-inflammatory, analgesic, emmenagogue, antibacterial and for oral maladies (Groom, 1981; Lewis and Elvin-Lewis, 2003). For instance, myrrh is an important product used in pharmaceutical industries, cosmetics and perfumery as well as in traditional medicines (Massoud *et al.*, 2001; Salah, 2014). It is an excellent remedy for mouth and throat problems with a drying, slightly bitter taste and it useful for skin problems, atherosclerosis, hemorrhoid, heptoses, high cholesterol, stomatosis, immune depression and hyperglycemia (Helal *et al.*, 2005). The myrrh's Gum-resin-volatile oil is the main used part, where it contains (30-60%) gum including acidic polysaccharides, resin (25-40%), volatile oil (3-8%), herabolene, eugenol and many furansesquiterpenes (Chevallier, 1996; Duke, 2002; Helal *et al.*, 2005). In another report, the plant produces an oleogum resin that is 2-8% essential oil (El-Shahat *et al.*, 2012) 23-40% resin, 40-60% gum and 10-25% bitter principles (Su *et al.*, 2011). Some active constituents of myrrh include the sesquiterpenes furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, which have antibacterial, antifungal and local anesthetic activities (Dolara *et al.*, 2000) and furanoedesma-1,3-diene and curzarene, which have potent analgesic effects (Dolara *et al.*, 1996). The furanoses-quiter-penoids lind-estrene and its analogues make up 19% of the essential oil and are responsible for the famous scent associated with myrrh (Jensen). Other important constituents are terpenoids, steroids, flavonoids, lignans, carbohydrates and long chain aliphatic alcohol derivatives; these various metabolites give myrrh its manifold biological activities (Su *et al.*, 2011).

Over the past few decades, many aspects of the manufacture of processed cheese have been reviewed by many authors (Shimp, 1985; Abd El-Salam *et al.*, 2005; Dimitreli and Thomareis, 2007; Kapoor and Metzger, 2008). The production of processed cheese started in Europe and could date to the mid-1890s. Natural cheeses have limited shelf life and depending on many factors (i.e., level of moisture content, sanitary conditions during the manufacturing stages and storage conditions of the product). Their storage can range from a few weeks to a couple of years. It is possible to suggest that the idea of processed cheese originated from a desire to extend the shelf life of natural cheese or to develop a new

type of cheese that was milder in taste or more stable (Berger *et al.*, 1989). On the other hand, spreadable processed cheese has higher moisture content than other processed cheese. The standard American FDA set was moisture content 44-60% and natural cheese content no less than 51%. Thanks to the heating, emulsifying and sterilization processing, this considered as preservative factors (Guo, 2004; Attalla *et al.*, 2014). In this regard, the content of sorbic acid/sorbate as preservative in cheeses with high moisture has been studied (Rinaldoni *et al.*, 2014).

The goal of this study is using E.O. of *Commiphora myrrha* as antimicrobial agent to produce extended shelf life spreadable processed cheese and evaluate their acceptability by evaluating their chemical, rheological, microbiological and sensory parameters.

MATERIALS AND METHODS

Materials:

- Ras cheese (one month old) was obtained from Arabic Food Industrial Co. (Dometty), 6th October City, Egypt
- Matured Cheddar cheese (8 months old) and Kasomelas emulsifying salt (K-2394-Rhone-Poulenc Chimie, France) were obtained from International Dairy and Food Co. (Green Land), 10th Ramadan City, Egypt
- Low heat Skim Milk Powder (SMP) and butter were obtained from local market (purchased from Irish Dairy Board, Grattow Hawse, Ireland)
- Myrrha plant (*Commiphora myrrha*) belonging to family (Burseraceae) was purchased from Thailand as a white fine crystals
- **Bacterial strains:** Five pathogenic bacterial strains were obtained from Microbiological Resources Center (Cairo MIRCEN): *Listeria monocytogenes* (EMCC 1875), *Staphylococcus aureus* (ATCC13565) *Bacillus cereus* (EMCC1080), used as gram-positive bacteria. *Escherichia coli* O157:H7 (ATCC51659) and *Salmonella typhimurium* (ATCC 25566) used as gram-negative

The chemical composition of the ingredients, which used in the manufacturing of processed cheese spreads, is presented in Table 1.

Five concentrations of myrrh's-Essential-Oil (EO) were used in manufactured of spread-processed cheese to serve five treatments (T₁, T₂, T₃, T₄ and T₅) as well as control. Table 2 illustrated the components of different blends used in spread-processed cheese.

Table 1: Chemical composition of ingredients used in manufacture of processed cheese spreads

Composition	Ingredients			
	Cheddar cheese	Ras cheese	S.M.P	Butter
Total solids (TS) (%)	66.01	54.62	96.00	84.00
Fat/DM	52.95	45.00	1.04	97.61
Total protein (TP) (%)	25.44	22.22	37.13	ND
Total carbohydrate (%)	0.10	1.60	47.43	ND

Table 2: Components (kg/100 kg) of different blends with different ratios of EO used in manufacture of processed cheese spreads

Ingredients	Treatments					
	Control	T ₁	T ₂	T ₃	T ₄	T ₅
Cheddar cheese	12.80	12.80	12.80	12.80	12.80	12.80
Ras cheese	38.44	38.44	38.44	38.44	38.44	38.44
SMP	5.12	5.12	5.12	5.12	5.12	5.12
Butter	10.26	10.26	10.26	10.26	10.26	10.26
Emulsifying salt	2.50	2.50	2.50	2.50	2.50	2.50
EO-ratios (mL kg ⁻¹)	-	0.20	0.40	0.60	0.80	1.00
Water	30.88	30.68	30.48	30.28	30.08	29.88
Total	100.00	100.00	100.00	100.00	100.00	100.00

Methods

Essential oil extraction and (GC/MS) analysis: Hydro distillation in clevenger apparatus had been used for essential oil extraction. The GC/MS analysis was performed with a Hewlett Packard model 5890. Gas chromatography equipped with 5 series. Mass selective detector 9144 (HP) was also conducted as reported by Mohamed *et al.* (2013). Table 3 illustrated the main active compounds of *Commiphora myrrha*-essential oil.

Growth conditions of pathogenic bacteria: Stock cultures of *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157:H7 and *Salmonella typhimurium* were sub-cultured twice onto Tryptone Soya Agar (TSA) followed by incubation at 37°C. Cultures were prepared from subcultures and grown overnight in Tryptone Soya Broth medium (TSB, Oxoid, Basingstoke, UK) under optimal conditions for each microorganism as reported by Mohamed *et al.* (2013).

Antimicrobial assay using the disc diffusion method: Essential oil was evaluated for its antimicrobial activity on (TSA) at 37°C. Antimicrobial EO was tested undiluted as well as diluted in Tween 20 at concentrations of 25, 50 and 75 mL/100 mL, the amount of undiluted EO added to filter paper disc was 10 µL while 20 and 60 µL were added from diluted EO. The concentration of bacteria inoculated in TSA was 2 × 10⁸ CFU mL⁻¹ with inoculated volume (0.1 mL). Sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of TSA. The inhibition zone diameter was measured (the filter paper disc) using

Table 3: Concentration of main active compounds of hydro-distillates of *Commiphora myrrha*

Components (%)	<i>Commiphora myrrha</i>
δ-elemene41	21.0
β-elemene39	8.7
GermacreneDd 60	3.2
T-cadinol 69	1.6
Furanodieneb, c 36	19.7
2-methoxyfuranodiene 25	2.1
Lindrestrene16	12.0
Furanoedesma-1,3-diene 14	34.0

vernier calipers and expressed in millimeter (Mohamed *et al.*, 2013). All experiments were performed in duplicate.

Minimum Inhibitory Concentration (MIC) assay: Minimum Inhibitor Concentrations (MIC) was determined by using agar dilution methods (Prudent *et al.*, 1995). Series dilutions of oil, ranging from 0.001-0.007 mL mL⁻¹ were prepared in Nutrient Agar. Plates were dried at room temperature for 30 min prior to spot inoculation with 0.1 mL of culture containing approximately 10⁵ CFU mL⁻¹ of each organism. Inoculated plates were incubated at 37°C for 18 h and the MIC was determined. Inhibition of bacterial growth in plates containing the tested oil was judged by comparison with growth in blank control plates. The MIC were calculated as the lowest concentration of oil which inhibited visible growth of each organism on agar plate (Delaquis *et al.*, 2002). Experiments were carried out in duplicate.

Processed cheese spreads preparation: Processed Cheese Spreads (PCSs) were prepared as described by Abdel-Hamid *et al.* (2000). Five concentrations of EO (Table 2) were added at the blends before cooking (85°C/15 min) to serve 5 treatments (T₁, T₂, T₃, T₄ and T₅), respectively. Three replicates of each treatment were prepared. All samples were stored at 5 ± 2°C for 12 months.

Methods of analysis: Chemical composition of processed cheese: The resultants PCSs were freshly analyzed for their Total Solids (TS), Total Protein (TP), Soluble Nitrogen (SN) contents and fat according to AOAC. (2007), salt and ash as mentioned by IDF (1964). The pH value was determined during storage period using a digital laboratory pH meter (HI 93 1400, Hanna instruments) with glass electrode. Total Volatile Fatty Acids (TVFAs) contents (0.1 N NaOH/100 g) were also determined according to Kosikowski (1986).

Physicochemical properties: Melting quality was determined by the method of Savello *et al.* (1989). Oil separation index of

processed cheese was estimated according to Thomas *et al.* (1980). Penetration was also calculated, using penetrometer Cochler Co. INC. USA.

Color parameters: Color was measured using Hunter Colorimeter model D2s A-2 (Hunter, 1975). Tri-stimulus values of the color namely L, a and b were measured

Where:

L : Value represents darkness from black (0) to white (100)

a : Value represents color ranging from red (+) to green (-)

b : Value represents color ranging from yellow (+) to blue (-)

Sensory properties: Sensory properties of the processed cheese spreads were evaluated by panel test by the staff members at the Dairy Dept., National Research Centre, Cairo, Egypt; using the graphical descriptor scale limits scheme.

Statistical analysis: Statistical analysis was performed according to the User's Guide given by SAS (2004) using Least Significant Differences (LSD).

RESULTS AND DISCUSSION

Growth inhibition of some pathogens by myrrh-EO: The tested oil showed highly inhibition activity against the five bacterial strains using the disc diffusion method as mentioned in Table 4. The results revealed that gram-positive bacteria were more susceptible than gram-negative one. *Commiphora myrrha* essential oil had a highly enhanced inhibitory effect; the most susceptible was *Listeria monocytogenes* and the most resistant was *E. coli* O157:H7. Brieskorn and Nobel (1980, 1982, 1983) had mentioned that the essential oils of *Commiphora* species are rich in furanosesquiterpenoids, which have been found to possess anesthetic, antibacterial,

antifungal, anti-hyperglycemic properties. Five furanosesquiterpenoids; 3-methoxy-4-furanogermacra-10(15)-dien-6-one, 2-methoxy-4-furanogermacra-1(10)-en-6-one, furanogermacra-1(10)-4-dien-6-one and curzerenone (6,7-dihydro-5-isopropenyl-3, 6- dimethyl-6-vinyl benzofuran-4-one) were isolated from myrrh gum. Moreover, Hili *et al.* (1997) tested fifty-one essential oils extracted from *Commiphora myrrha* for their antimicrobial activity against three bacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and four yeasts, *Torulopsis utilis*, *Schizosaccharomyces pombe*, *Candida albicans* and *Saccharomyces cerevisiae* using the drop diffusion method. All showed antimicrobial activity against at least one of the micro-organisms. Also, the essential oil extract of *Commiphora myrrha* showed significant antimicrobial activity against *Escherichia coli*, *Proteus vulgaris*, *Shigella flexneri*, *Salmonella paratyphi B* and *Klebsiella pneumoniae* (Abdalla, 2005).

Related results were obtained by Friedman *et al.* (2002), Elaissi *et al.* (2011) and Mohamed *et al.* (2013).

Minimum Inhibitory Concentrations (MIC) assay: The application of Myrrh essential oil in manufacture of processed cheese spreads requires determination of the less concentration of the oil which gave the highly inhibition effect with highly acceptable sensory properties of the final product and low defects. Table 5 reflected that the concentration was ranged from 2-5 $\mu\text{L mL}^{-1}$ to achieve the complete inhibition effect.

Chemical composition of processed cheese spreads: Table 6 showed the gross chemical compositions of different blends of processed cheese spreads with different concentrations of myrrh's EO at fresh and during 1, 3, 6 and 12 months of storage periods compared to control. The

Table 4: Inhibition growth zone of some pathogenic bacterial strains by Commiphora EO

Agents	Zone diameter (mm)				
	<i>L. monocytogenes</i>	<i>E. coli</i> O157:H7	<i>S. aureus</i>	<i>B. cereus</i>	<i>S. typhimurium</i>
10 μL Eo	40.7	55.7	53.2	50.1	43.2

Three replicates were done

Table 5: Minimum inhibitory concentration of myrrh essential oil against some pathogenic bacteria

Agent cons ($\mu\text{L mL}^{-1}$)	<i>L. monocytogenes</i>	<i>E. coli</i> O157:H7	<i>S. aureus</i>	<i>B. cereus</i>	<i>S. typhimurium</i>
Control	++++	++++	++++	++++	++++
EO 0.001	+++	+	+	++	++
EO 0.003	-	-	-	-	-
EO 0.005	-	-	-	-	-
EO 0.007	-	-	-	-	-

Table 6: Chemical composition of processed cheese spreads fortified with Myrrh-EO when fresh and during storage periods at 5°C for 3 months

Ratios (mL kg ⁻¹)*	Storage periods (months)	TS (%)	Fat/DM	TP (%)	Salt/moistures	Ash (%)
Control	Fresh	44.69	50.92	13.83	3.10	5.00
	1	44.70	50.99	13.84	3.11	5.03
	3	44.82	51.01	13.86	3.16	5.11
	6	44.90	50.11	13.88	3.12	5.12
	12	45.08	50.22	13.89	3.07	5.13
T ₁ (2*)	Fresh	44.70	50.91	13.79	3.01	5.01
	1	44.75	51.00	13.81	3.07	5.05
	3	44.77	51.18	13.85	3.13	5.19
	6	44.80	51.22	13.87	3.10	5.20
	12	44.95	51.30	13.40	3.06	5.21
T ₂ (4*)	Fresh	44.72	50.94	13.70	3.04	5.04
	1	44.73	50.95	13.75	3.08	5.08
	3	44.76	51.12	13.86	3.11	5.18
	6	44.85	51.20	13.87	3.14	5.19
	12	44.99	51.29	13.90	3.10	5.20
T ₃ (6 *)	Fresh	44.75	50.90	13.67	3.05	5.06
	1	44.76	50.97	13.70	3.09	5.10
	3	44.79	51.13	13.79	3.12	5.18
	6	44.86	51.18	13.81	3.15	5.19
	12	44.91	51.30	13.83	3.11	5.21
T ₄ (8*)	Fresh	44.76	50.93	13.66	3.06	5.07
	1	44.74	50.44	13.70	3.10	5.11
	3	44.80	51.15	13.78	3.13	5.18
	6	44.85	51.20	13.80	3.15	5.19
	12	44.90	51.30	13.83	3.18	5.20
T ₅ (10*)	Fresh	44.79	50.95	13.63	3.09	5.08
	1	44.80	51.01	13.71	3.11	5.13
	3	44.86	51.16	13.80	3.13	5.19
	6	44.89	51.25	13.82	3.17	5.20
	12	44.42	51.32	13.84	3.19	5.21

*Filter paper disc diameter was 6 mm

similarity in chemical composition of all studied samples could be explained with the uniformity of the ingredients used. The results indicated that there were slightly significant differences between fresh samples and during storage compared to control in TS, Fat/DM, TP, salt/ moisture and ash contents. The TS in all samples were slightly increased and this would possibly be as a result from the loss of moisture during storage. The results were in accordance with Krumov *et al.* (2010), Mansour *et al.* (2011) and Suleiman *et al.* (2011). In addition, the data demonstrated that both Fat/DM and salt/moisture slightly increased during storage while there were slightly differences between control and treatment samples in TP. Total protein was slightly higher in control samples either fresh or during storage periods compared to treated samples. Increasing concentrations of myrrh's essential oil causing significantly increase ($p > 0.05$) in soluble nitrogen at fresh and during storage periods compared to control samples as shown in Fig. 1. The changes in SN during storage could be the result of enzymatic activity of resistant proteinases present in the product. It could be also due to the hydrolysis of polyphosphate in emulsifying salts which caused more solubilization of proteins. These results are in agreement with Awad *et al.* (2014).

On the other hand, data which presented in Fig. 2 showed the difference in pH values between control compared to different samples when fresh and during 12 months of storage period. It could be noticed that pH values increased with increasing of essential oil concentrations. This increase may be due to the action of EO in inhibition of acidity forming and the alkaline effect of EO itself. During storage, pH values slightly decreased in all treatments. The decrease in pH during storage could be related to the hydrolysis occurred in emulsifying salts and their interaction with proteins. These data were agreed with Mansour *et al.* (2011) and Awad *et al.* (2014).

In Fig. 3, the data illustrated the TVFAs values in control and treated samples. There were clear differences between control and treatments when fresh and during storage period. TVFAs took an increasing trend during storage in all samples. This increase was mainly attributed to the residual activity of heat resistant lipases in cheese formula. Consequently, hydrolysis of purified polysaccharides of gum myrrh gave high yields of a mixture of neutral sugars and acidic oligosaccharides. Moreover, Foda *et al.* (2010) indicated that increasing of spearmint essential oil concentration, more than 1.0 mL kg⁻¹ retentate, significantly increased these values.

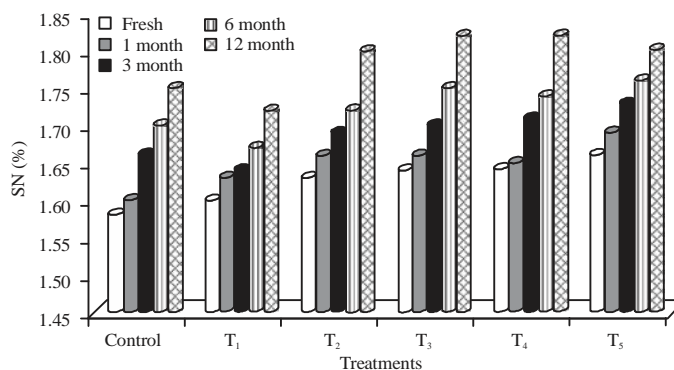


Fig. 1: Changes of SN (%) of processed cheese spreads fortified with different concentration of myrrh's EO when fresh and during storage period for 12 months at $5 \pm 2^\circ\text{C}$

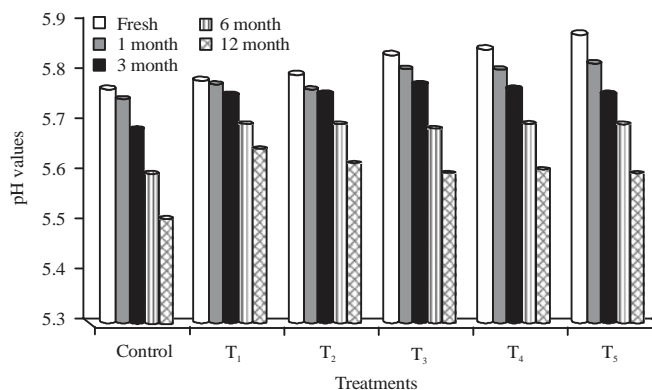


Fig. 2: Changes of pH values of processed cheese spreads fortified with different concentration of myrrh's EO when fresh and during storage period for 12 months at $5 \pm 2^\circ\text{C}$

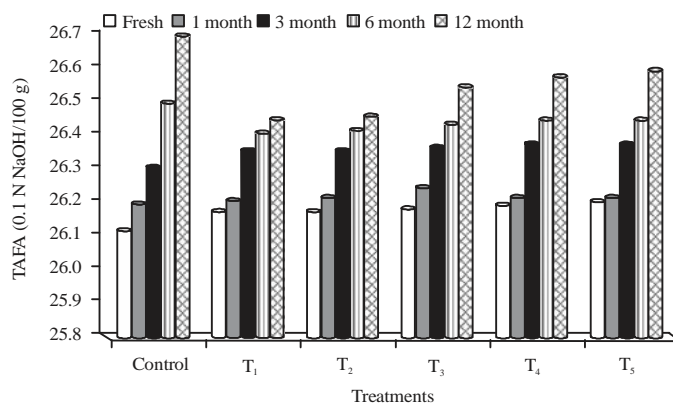


Fig. 3: Changes of TVFAs values of processed cheese spreads fortified with different concentration of myrrh's EO when fresh and during storage period for 12 months at $5 \pm 2^\circ\text{C}$

Presented data also indicated that prolonging the cold storage for 12 months significantly ($p > 0.05$) increased (TVFAs) values compared with fresh processed cheese spreads. These results in agreement with those obtained by Abd El-Salam *et al.*

(1993), who reported that the changes in TVFAs occur during the first 15-30 days of storage which related with maximum bacterial growth and high concentration of total volatile free fatty acids in cheese.

Physical properties of processed cheese spreads

Penetration: Figure 4a indicated that all treated samples had higher penetration values than control samples. The data revealed that increasing the concentration of myrrh's essential oil ratios caused significant increasing ($p>0.05$) of penetration values, so the highest sample was (T_5) which had the highest concentration compared to control. This increase could be due to the unique composition of myrrh. The main constituents of the myrrh oleo-gum resin are 2-8% volatile oil (El-Shahat *et al.*, 2012) 23-40% resin and 40-60% gum (Su *et al.*, 2011). The gum is water-soluble and contains polysaccharides, proteoglycans and proteins, as well as the compounds D-galactose, L-arabinose and 4-methyl

D-glucuronic acid. The volatile oil is consists of steroids, sterols and terpenes (Hanus *et al.*, 2005) including the furanosesquiterpenoids lindestrene (Table 3) and its analogues.

On the other hand, Fig. 4b also represented meltability values in processed cheese made with different concentrations of myrrh essential oil. It could be noticed that as increasing EO concentrations, the meltability was increased compared to control samples. It could be attributed to the specific composition of that EO that contains about 40-60% gum (Su *et al.*, 2011). The cheese meltability showed a tendency to decrease along the storage period and the decrease was more noticeable in the control samples.

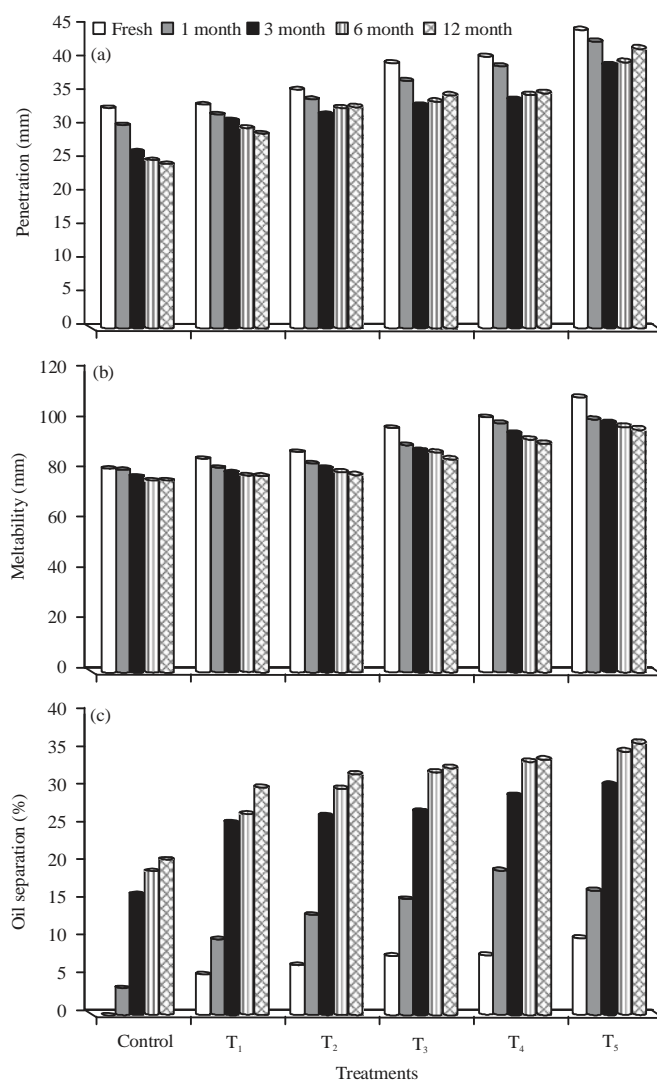


Fig. 4(a-c): Physical properties (a) Penetration, (b) Meltability and (c) Oil separation of processed cheese spreads fortified with different concentration of myrrh's EO when fresh and during storage period for 12 months at $5\pm 2^\circ\text{C}$

Table 7: Color parameters of different processed cheese spreads fortified with myrrh EO when fresh and during storage period for 12 months at 5±2°C

Treatments*	Storage period (months)	L	a	b
Control	Fresh	87.88	-1.1	22.20
	1	87.87	-1.2	22.50
	3	87.10	-1.5	23.10
	6	87.00	-1.4	23.30
	12	86.82	-1.3	23.50
T ₁	Fresh	86.20	-1.2	22.80
	1	86.00	-1.2	23.10
	3	85.77	-1.5	23.90
	6	85.61	-1.4	24.10
	12	85.55	-1.3	24.50
T ₂	Fresh	85.71	-1.6	24.60
	1	85.50	-1.7	25.00
	3	84.91	-1.9	25.80
	6	84.80	-1.9	26.00
	12	84.72	-1.8	26.01
T ₃	Fresh	81.90	-1.8	25.20
	1	81.01	-1.9	26.10
	3	80.78	-2.1	26.90
	6	80.70	-2.0	27.00
	12	80.61	-2.0	27.01
T ₄	Fresh	77.79	-1.9	26.00
	1	76.99	-2.1	26.70
	3	75.88	-2.4	27.10
	6	75.80	-2.3	27.20
	12	75.70	-2.1	27.30
T ₅	Fresh	75.90	-2.0	26.30
	1	74.99	-2.3	27.10
	3	74.01	-2.7	27.90
	6	73.60	-2.6	28.00
	12	73.50	-2.6	28.10

*See Table 2, L: Value of darkness from black (0) to white (100), a: Color ranging from red (+) to green (-), b: Color ranging from yellow (+) to blue (-)

Oil separation took the same trend of meltability which had been showed in Fig. 4c. Increasing concentration of Myrrh's essential oil caused increasing of oil separation when fresh and during storage compared to control samples. The highest oil separation values was in treatment 5 compared to control samples which had the lowest values. The oil separation value depends on the state of fat and protein in resultant processed cheese emulsion that can be affected by type and amount of raw materials in the base formula, pH value, cooking time and temperature. These results agree with Awad *et al.* (2014). The increase in oil separation value with prolonged storage time could be due to the drop in the pH value and changes that occurred in soluble nitrogen contents (Abdel-Hamid *et al.*, 2000).

Color parameters: Table 7 reflected the color parameters of control and processed cheese spreads samples with different myrrh's EO ratios when fresh and during cold storage for 12 months. Control samples had gained higher values of whiteness degree when fresh and during storage than the rest

of treatments. Whiteness had been decreased with increasing of myrrh's EO ratios in samples. As increasing ratios of myrrh's EO in the samples the parameters (a and b) increasing. Parameter (b) which reflected, yellowish was higher pronounced than parameter (a) which reflected reddish, this may be due to the slightly yellow color of myrrh crystal.

Sensory evaluation of processed cheese spreads:

Organoleptic properties were evaluated using graphical descriptor scale limits. Table 8 indicated that control samples had moderate shiny surface appearance, soft spreadable body and moderate strong flavor. Guminess and oil separation were absent in control sample and overall preference was like. Obtained data also revealed that no clear differences were observed between the four treatments (T₂, T₃, T₄ and T₅). The best result was obtained by 2% bitter gum (T₁). This treatment had very much shiny appearance, very soft and spreadable body. The flavor was strong as well as guminess and oil separation was absent and overall preference was like very much.

Table 8: Sensory properties of processed cheese spreads fortified with Myrrh EO when fresh and during storage period for 12 months at 5±2°C

Sensory attribute	Storage period (months)	Treatments					
		Control	T ₁	T ₂	T ₃	T ₄	T ₅
Surface appearance	Fresh	Shiny moderate	Shiny very much	Shiny moderate	Shiny very much	Shiny very much	Shiny very much
	1	Shiny moderate	Shiny very much	Shiny moderate	Shiny very much	Shiny very much	Shiny very much
	3	Shiny moderate	Shiny very much	Shiny moderate	Shiny very much	Shiny very much	Shiny very much
	6	Shiny moderate	Shiny very much	Shiny moderate	Shiny very much	Shiny very much	Shiny very much
	12	Shiny moderate	Shiny very much	Shiny moderate	Shiny very much	Shiny very much	Shiny very much
Firmness of body	Fresh	Soft	Very soft	Soft	Very soft	Soft	Soft
	1	Soft	Very soft	Soft	Very soft	Soft	Soft
	3	Soft	Very soft	Soft	Very soft	Soft	Soft
	6	Soft	Very soft	Soft	Very soft	Soft	Soft
	12	Soft	Very soft	Soft	Very soft	Soft	Soft
Spreading quality	Fresh	Spreadable	Spreadable	Easy to spread	Easy to spread	Spreadable	Easy to spread
	1	Spreadable	Spreadable	Easy to spread	Easy to spread	Spreadable	Easy to spread
	3	Spreadable	Spreadable	Easy to spread	Easy to spread	Spreadable	Easy to spread
	6	Spreadable	Spreadable	Easy to spread	Easy to spread	Spreadable	Easy to spread
	12	Spreadable	Spreadable	Easy to spread	Easy to spread	Spreadable	Easy to spread
Stickiness	Fresh	Not sticky	Not sticky	Not sticky	Not sticky	Not sticky	Sticky slightly
	1	Not sticky	Not sticky	Not sticky	Not sticky	Not sticky	Sticky slightly
	3	Not sticky	Not sticky	Not sticky	Not sticky	Not sticky	Sticky slightly
	6	Not sticky	Not sticky	Not sticky	Not sticky	Not sticky	Sticky slightly
	12	Not sticky	Not sticky	Not sticky	Not sticky	Not sticky	Sticky slightly
Smoothness of texture	Fresh	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Smooth	Smooth
	1	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Smooth	Smooth
	3	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Smooth	Smooth
	6	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Smooth	Smooth
	12	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Very smooth lacks sandiness	Smooth	Smooth
Break down properties	Fresh	Dissolve readily very well	Dissolve readily	Dissolve readily very well	Dissolve readily	Dissolve readily	Dissolve readily
	1	Dissolve readily very well	Dissolve readily	Dissolve readily very well	Dissolve readily	Dissolve readily	Dissolve readily
	3	Dissolve readily very well	Dissolve readily	Dissolve readily very well	Dissolve readily	Dissolve readily	Dissolve readily
	6	Dissolve readily very well	Dissolve readily	Dissolve readily very well	Dissolve readily	Dissolve readily	Dissolve readily
	12	Dissolve readily very well	Dissolve readily	Dissolve readily very well	Dissolve readily	Dissolve readily	Dissolve readily
Chewiness	Fresh	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing
	1	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing
	3	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing
	6	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing
	12	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing	Not chewing
Gumminess	Fresh	Absent	Absent	Absent	Absent	Absent	Absent
	1	Absent	Absent	Absent	Absent	Absent	Absent
	3	Absent	Absent	Absent	Absent	Absent	Absent
	6	Absent	Absent	Absent	Absent	Absent	Absent
	12	Absent	Absent	Absent	Absent	Absent	Absent
Oil separation	Fresh	Absent	Absent	Absent	Absent	Absent	Absent
	1	Absent	Absent	Absent	Absent	Absent	Absent
	3	Absent	Absent	Absent	Absent	Absent	Absent
	6	Absent	Absent	Absent	Absent	Absent	Absent
	12	Absent	Absent	Absent	Absent	Absent	Absent

Table 8: Continue

Sensory attribute	Storage period (months)	Treatments					
		Control	T ₁	T ₂	T ₃	T ₄	T ₅
Flavor	Fresh	Moderately strong	Strong	Strong	Strong	Strong	Strong
	1	Moderately strong	Strong	Strong	Strong	Strong	Strong
	3	Moderately strong	Strong	Strong	Strong	Strong	Strong
	6	Moderately strong	Strong	Strong	Strong	Strong	Strong
	12	Moderately strong	Strong	Strong	Strong	Strong	Strong
Overall preference	Fresh	Like	Like very much	Like	Like	Like	Like
	1	Like	Like very much	Like	Like	Like	Like
	3	Like	Like very much	Like	Like	Like	Like
	6	Like	Like very much	Like	Like	Like	Like
	12	Like	Like very much	Like	Like	Like	Like

CONCLUSION

It could be concluded that using 2 mL kg⁻¹ Myrrah essential oil produced satisfied properties; highly antimicrobial activity with best acceptable organoleptic behavior in processed cheese spread.

REFERENCES

- AOAC., 2007. Association of Official Analytical Chemists. 17th Edn., Official Methods of Analysis, Gaithersburg, MD., USA.
- Abd El-Salam, M.H., E. Alihanids and G.K. Zerfiridis, 1993. Domiati and Feta Type Cheeses. In: Cheese, Chemistry, Physics and Microbiology, Volume 2, Fox, P.F. (Ed.). Chapman and Hall, London, UK., pp: 132-144.
- Abd El-Salam, M.H., S. El-Shibiny and N.S. Ahmed, 2005. Studies on processed cheese in Egypt: A review. Egypt. J. Dairy Sci., 33: 129-141.
- Abdalla, B.M., 2005. Antibacterial activity of essential oils extracted from selected Sudanese medicinal and aromatic plants. M.Sc. Thesis, Omdurman Islamic University, Sudan.
- Abdel-Hamid, L.B., S.A. El-Shabrawy, R.A. Awad and R.K. Singh, 2000. Chemical properties of processed ras cheese spreads as affected by emulsifying salt mixtures. J. Food Process. Preserv., 24: 191-208.
- Attalla, N.R., E.F. Mohamed, W.H.M. El-Reffaei and N.I. Bassyoni, 2014. Production and evaluation of sweet spreadable goat cheese. Int. J. Nutr. Food Sci., 3: 79-90.
- Awad, R.A., W.M. Salama and A.M. Farahat, 2014. Effect of lupine as cheese base substitution on technological and nutritional properties of processed cheese analogue. Acta Sci. Pol. Technol. Aliment., 13: 55-64.
- Berger, W., H. Klostermeyer, K. Merkenich and G. Uhlmann, 1989. Processed Cheese Manufacture: A Joha Guide. BK Ladenburg, Ladenburg, Germany.
- Brieskorn, C.H. and P. Noble, 1980. Drei neue furanogermacrene aus myrrhe. Tetrahedron Lett., 21: 1511-1514.
- Brieskorn, C.H. and P. Noble, 1982. [Constituents of the essential oil of myrrh]. Planta Medica, 44: 87-90, (In German).
- Brieskorn, C.H. and P. Noble, 1983. Two furanoeudesmanes from the essential oil of myrrh. Phytochemistry, 22: 187-189.
- Chevallier, A., 1996. The Encyclopedia of Medicinal Plants. DK Publishing, UK., ISBN: 9780789410672, Pages: 336.
- Delaquis, P.J., K. Stanich, B. Girard and G. Mazza, 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol., 74: 101-109.
- Dimitreli, G. and A.S. Thomareis, 2007. Texture evaluation of block-type processed cheese as a function of chemical composition and in relation to its apparent viscosity. J. Food Eng., 79: 1364-1373.
- Dolara, P., C. Luceri, C. Ghelardini, C. Monserrat and S. Aiolfi *et al.*, 1996. Analgesic effects of myrrh. Nature, 379: 29-29.
- Dolara, P., B. Corte, C. Ghelardini, A.M. Pugliese, E. Cerbai, S. Menichetti and A. Lo-Nostro, 2000. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. Planta Med., 66: 356-358.
- Duke, J.A., 2002. Hand Book of Medicinal Herbs. 2nd Edn., CRC Press, USA., ISBN: 9781420040463, pp: 15-519.
- El-Shahat, M., S. El-Abd, M. Alkafafy and G. El-Khatib, 2012. Potential chemoprevention of diethylnitrosamine-induced hepatocarcinogenesis in rats: Myrrh (*Commiphora molmo*) vs. Turmeric (*Curcuma longa*). Acta Histochemica, 114: 421-428.
- Elaissi, A., K.H. Salah, S. Mabrouk, K.M. Larbi, R. Chemli and F. Harzallah-Skhiri, 2011. Antibacterial activity and chemical composition of 20 *Eucalyptus* species' essential oils. Food Chem., 129: 1427-1434.
- Foda, M.I., M.A. El-Sayed, A.A. Hassan, N.M. Rasmy and M.M. El-Moghazy, 2010. Effect of spearmint essential oil on chemical composition and sensory properties of white cheese. J. Am. Sci., 6: 272-279.
- Friedman, M., P.R. Henika and R.E. Mandrell, 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. J. Food Prot., 65: 1545-1560.

- Groom, N., 1981. Frankincense and Myrrh: A Study of the Arabian Incense Trade. International Book Centre, USA., ISBN-13: 978-0866855938, Pages: 285.
- Guo, B.H., 2004. Modern Dairy Products Processing Technology-Cheese. Chemical Industry Press, Beijing, China, pp: 32, (In Chinese).
- Hanus, L.O., I. Rezanka, V.M. Dembitsky and A. Moussaieff, 2005. Myrrh-commiphora chemistry. Biomed Pap., 149: 3-28.
- Helal, E.G.E., A. Mahmoud, E.E. El-Badawy and A.A. Kahwash, 2005. Effect of *Commiphora myrrha* extract on some physiological parameters and histological changes in diabetic albino rats. Egypt. J. Hosp. Med., 20: 148-162.
- Hili, P., C.S. Evans and R.G. Veness, 1997. Antimicrobial action of essential oils : The effect of dimethylsulphoxide on the activity of cinnamon oil. Lett. Applied Microbiol., 21: 269-275.
- Hunter, R.S., 1975. Scales for Measurements of Color Difference. In: The Measurement of Appearance, Hunter, R. and R.W. Harold (Eds.). Wiley-Interscience, New York, pp: 162-192.
- IDF., 1964. Determination of the ash content of processed cheese products. Standard FIL-IDF 25:1964, International Dairy Federation (IDF), Brussels, Belgium.
- Kapoor, R. and L.E. Metzger, 2008. Process cheese: Scientific and technological aspects-A review. Comprehen. Rev. Food Sci. Food Saf., 7: 194-214.
- Kosikowski, F.V., 1986. Cheese and Fermented Milk Foods. 3th Edn., F.V. Kosikowski and Associates, New York, Pages: 225.
- Krumov, K., G. Ivanov, A. Slavchev and N. Nenov, 2010. Improving the processed cheese quality by the addition of natural spice extracts. Adv. J. Food Sci. Technol., 2: 335-339.
- Lewis, W.H. and M.P.F. Elvin-Lewis, 2003. Medical Botany: Plants Affecting Human Health. 2nd Edn., Wiley, New Hersey, ISBN-13: 978-0813329550, Pages: 832.
- Mansour, A.I.A., M.A.M. Omar and W.I. El-Dosoki, 2011. Studies on uusing of ultrafiltered rretentated milk in processed cheese making. Internet J. Food Saf., 13: 303-309.
- Massoud, A., S. El Sisi, O. Salama and A. Massoud, 2001. Preliminary study of therapeutic efficacy of a new fasciolicidal drug derived from *Commiphora molmol*(myrrh). Am. J. Trop. Med. Hygiene, 65: 96-99.
- Mohamed, S.H.S., W.M. Zaky, J.M. Kassem, H.M. Abbas, M.M.E. Salem and H.A.H. Said-Al Ahl, 2013. Impact of antimicrobial properties of some essential oils on cheese yoghurt quality. World Applied Sci. J., 27: 497-507.
- Prudent, D., F. Perineau, J.M. Bessiere, G.M. Michel and J.C. Baccou, 1995. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.)-Evaluation of its bacteriostatic and fungistatic properties. J. Essent. Oil Res., 7: 165-173.
- Rinaldoni, A.N., D.R. Palatnik, N. Zaritzky and M.E. Campderros, 2014. Soft cheese-like product development enriched with soy protein concentrates. LWT-Food Sci. Technol., 55: 139-147.
- SAS., 2004. SAS User's Guide: Statistics, Version 6.04. 4th Edn., SAS Inst. Inc., Cary, NC., USA.
- Salah, Y.M.S., 2014. The role of indigenous gums and resins in pastoralists' livelihood security and climate change adaptation in Garba Tula area of Northern Kenya. Early Career Fellowship Programme, February 2014.
- Savello, P.A., C.A. Ernstrom and M. Kalab, 1989. Microstructure and meltability of model process cheese made with rennet and acid casein. J. Dairy Sci., 72: 1-11.
- Shimp, L.A., 1985. Process cheese principles. Food Technol., 39: 63-69.
- Su, S., T. Wang, T. Chen, J.A. Duan, L. Yu and Y. Tang, 2011. Cytotoxicity activity of extracts and compounds from *Commiphora myrrha* resin against human gynecologic cancer cells. J. Med. Plants Res., 5: 1382-1389.
- Suleiman, T.A.E., M.O.M. Abdalla, N.H.M. El Haj and H.M.O. Elsiddig, 2011. Chemical and microbiological evaluation of processed cheese available in Khartoum market, Sudan. Am. J. Food Nutr., 1: 28-33.
- Thomas, M.A., G. Newell, G.A. Abad and A.D. Turner, 1980. Effect of emulsifying salts on objective and subjective properties of processed cheese. J. Food Sci., 45: 458-459.