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## Research Article

# Assessment of Antimicrobial Activity of Myrrh Gum Extract to Merge in Soft Cheese

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## Abstract

**Background and Objective:** Urgent need for effective and natural preservative materials is still recommended. The target of the present study was to evaluate the myrrh gum extract (MGE) as a natural antimicrobial agent, then used it in preserving the soft cheese samples.

**Materials and Methods:** Myrrh (*Commiphora myrrha*) gum crystals were water-extracted (1:10 w/v) and exposed to ultra sonic-ray. Seven strains of bacteria and three strains of mold as well as two strains of yeast were used in this study to evaluate the antimicrobial effect of MGE. In another section, traditional soft cheese samples were prepared using different concentrations of MGE. Three treatments were prepared compared to control one. The first treatment (T<sub>1</sub>) was prepared using 5 mL MGE/1 L cheese milk. The second and third treatments (T<sub>2</sub> & T<sub>3</sub>) were conducted using 10 and 15 mL/1 L cheese milk, respectively. All cheese samples were stored at refrigerator (7°C) for three months and were analyzed for their chemical composition. Sensorial evaluation and textural assay were also conducted.

**Results:** Data revealed that MGE considered an effective and natural antimicrobial agent. Majority of tested microorganisms were susceptible to the action of MGE. *Sallmonella typhimurium* strain was the most resistant bacteria while *Escherichia coli* was the most sensitive and the most sensitive yeast was *Saccharomyces cerevisiae*. No clear differences between control and treated cheese samples were noticed in the chemical composition or texture properties. However, sensorial evaluation indicated that using of MGE gave a clean and slight acid taste and acceptable properties. It did not cause any gumminess in the body or any bitterness in the taste.

**Conclusion:** Myrrh extract could be used in preparing soft cheese samples and preserve them to 90 days without any critical changes in chemical or textural and sensorial properties. It could protect cheese samples from hazardous contaminant pathogenic strains. So it considered as effective, natural, safe and cheap antimicrobial agent. It is recommended to use it in food sector and other dairy products.

**Key words:** Myrrh gum extract, natural preservative, pathogenic strains, antimicrobial agent, dairy product

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**Competing Interest:** The authors have declared that no competing interest exists.

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

## INTRODUCTION

A recent and new trend to prevent the proliferation of microorganisms is using natural materials especially plant source<sup>1,2</sup>. Myrrh tree (*Commiphora myrrha*) is cultivated in Northeastern Africa. Myrrh gum is considered as natural and safe materials and it is one of the oldest medicines where it was commonly known by the ancient Egyptians as bitter gum<sup>3,4</sup>. It is likely used in pharmaceutical field and in traditional medicines<sup>5-8</sup>. It gained many health benefit against many diseases<sup>9,10,3,4</sup>. The granules of myrrh produce an oleo-gum resin that is 2-8% essential oil, 23-40% resin, 40-60% gum and 10-25% bitter principles<sup>7,4</sup>. The major constituents present in gum are furanoeudesma-1, 3-diene and curzarene that have analgesic effects<sup>9</sup>. Some active constituents of myrrh seemed to have widely antimicrobial effect<sup>4,10,11</sup>. Otherwise, important constituents of myrrh are terpenoids, steroids, flavonoids, lignans, carbohydrates and long chain aliphatic alcohol derivatives<sup>7</sup>.

Very little work or rarely research was achieved for using of myrrh in dairy products. The antimicrobial activity of myrrh-essential oil against different of pathogenic bacteria strains and its useful-used in processed cheese were only recently investigated by Mohamed *et al.*<sup>12</sup>. They recorded that using of 2% of its essential oil produced acceptable processed cheese spreads which can be stored till one year. They recommended that it could be used as a natural preservative agent in other dairy products.

In another hand, soft cheeses have limited shelf life which depending on different factors. Their storage can ranged from few days to one month. It has higher moisture content which promote the growth of various microorganism. So, many attempts were done to increase the cheese keeping quality or to extend its shelf life by various procedures<sup>13,14</sup>. Using of artificial preservatives is internationally prohibited and search for natural preservatives agents still need. In this regard, the goal of this research is assessment the antimicrobial activity of myrrh extract and using it as natural antimicrobial material in preparing soft cheese samples.

## MATERIALS AND METHODS

This study was carried out in Dairy chemistry Laboratory, Food Industries and Nutrition Division, National Research Centre, Giza, Egypt, in the period from June-November, 2016.

## Materials

**Myrrh gum crystals source:** Myrrh (*Commiphora myrrha*) belonging to family *Burseraceous* was purchased from Thailand as semi-white fine crystals.

**Bacterial strains source:** Seven pathogenic bacterial strains were obtained from Microbiological Resource Centre (Cairo MIRCEN). *Listeria monocytogenes* (598), *Staphylococcus aureus* (ATCC13565), *Bacillus cereus* (B-3711) and *B. subtilis* were used as Gram-positive bacteria. *Escherichia coli* O157:H7 (ATCC51659); *Salmonella typhimurium* (ATCC 25566) and *Yersinia enterocolitica* were used as Gram-negative.

**Mold and yeast source:** Strains of *Aspergillus flavus* (3357); *Saccharomyces cerevisiae* (Y2223) and *Candida albicans* were obtained from the Northern Regional Res. Lab. Illinois, USA (NRRL). However, *Aspergillus niger*, *Penicillium roqueforti* (J5) were obtained from Department of Microbiology, Swedish University of Agriculture Science Sweden.

**Starter culture source:** Strain of both *Lactococcus lactis* sub *lactis* and *L. delbrueckii* sub *bulgaricus* were obtained from Dairy Microbiology Laboratory, National Research Centre, Egypt.

**Milk source:** Fresh cow milk (12% TS and 4% Fat) as well as calf rennet, calcium chloride and commercial salt were purchased from Faculty of Agriculture, Cairo University, Egypt.

## Experiments

**Preparation of myrrh gum extract:** Myrrh gum crystals were fine milled, sieved and dissolved in distilled water (1:10 w/v), then were strongly stirred and left for 6 h at room temperature (25°C). The aqueous extract was exposed to ultra-sonication at 160 W power, 20 kHz frequency with 50% pulse for 20 min (Sonic Ruptor 400, OMNI International the Homogenizer Company, Kennesaw, GA, USA). During sonication, samples were placed in cold water bath at 4°C to prevent overheating of emulsion.

**Preparation of soft cheese samples:** Traditional methods for preparing Egyptian soft cheese were applied as recently mentioned by Farag *et al.*<sup>15</sup>. After pasteurization, the cheese- milk was cultured and kept for 30 min at 32°C. Three concentrations of prepared MGE (5, 10 and 15 mL/1 L milk) were added before renneting to serve three treatments (T1, T2 and T3, respectively) in addition to control sample. After completed coagulation and forming homogenous curd, all manufacture steps were traditionally completed and cheese

samples were stored at 7°C for 90 days. Cheese samples were chemically analyzed. Textural and sensorial evaluation were also assayed either fresh or during periodical storage periods (0, 30, 60 and 90 days).

### Methods of analysis

**Growth conditions of pathogenic bacteria:** Stock cultures of *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Yersinia enterocolitica*; *Escherichia coli* and *Salmonella typhimurium* were sub-cultured twice onto Tryptone Soya Agar (TSA) followed by incubation at 37°C/24 h. Cultures were prepared from subcultures and grown overnight in Tryptone Soya Broth medium (TSB, Oxoid, Basingstoke, UK) under optimal conditions for each microorganism.

Mold and yeast strains were cultivated in malt extract broth (Oxoid) then incubated at 25°C /72 h.

**Antimicrobial assay using the disc diffusion method:** The disc diffusion method according to Alabi *et al.*<sup>16</sup>, was applied as mentioned recently by Mohamed *et al.*<sup>17</sup>. The inhibition zones were measured in mm as recorded by Mohamed *et al.*<sup>1</sup>.

**Chemical analysis of soft cheese samples:** All cheese samples were analyzed for their total solids (TS), total protein (TP), fat, soluble nitrogen (SN) contents as well as acidity percentage according to AOAC<sup>18</sup>. The pH values were determined using a digital laboratory pH meter (HI 93 1400, Hanna instruments) with glass electrode. Total Volatile Fatty Acids (TVFAs) contents expressed as 0.1 N NaOH/100 g cheese were also determined according to Kosikowski<sup>19</sup>.

**Textural properties assaying of soft cheese samples:** Texture profile analysis (TPA) was performed on cheese samples using the double compression apparatus (Multi test 1d Memesin, Food Technology Corporation, Slinfold, W. Sussex, UK). Experiments were carried out as recently mentioned by Abbas *et al.*<sup>2</sup> using a compression test that generated a plot of force (N) versus time (s). A 25 mm diameter perplex conical-shaped probe was used to perform the TPA analysis at five different points on the sample surface. In the 1st stage, the sample was compressed by 80% of their original depth at a speed of 2 cm min<sup>-1</sup> during the pretest, compression and relaxation of the sample. From the force-time curve, the following parameters were determined according to the definition given by the International Dairy Federation<sup>20</sup>.

Firmness (N) = Maximum force of the 1st compression  
 Cohesiveness = Area under the 2nd compression/area under the 1st compression (A2/A1)  
 Adhesiveness (N.s) = Negative area in the curve  
 Springiness (mm) = Length of 2nd compression/length of 1st compression (L2/L1)  
 Gumminess (N) = Hardness × cohesiveness  
 Chewiness (mJ) = Gumminess × springiness

### Sensory properties evaluation of soft cheese samples:

Sensorial properties of soft cheese samples were panel-evaluated by 20 of the trained staff members at Dairy Department of Science, National Research Centre, Egypt. Appearance and color scores were 20 points while flavor scores were 50 points and body and texture scores were 30 points. This scheme was suggested by the authors and recommended by the staff members to be more suitable for the product and to focus on the desirable property.

## RESULTS

### Assaying of Antimicrobial activity of myrrh gum extract

**(MGE):** Obtained data indicated that the prepared MGE showed highly inhibition activity against the tested microorganisms as shown in Table 1.

The results revealed that Gram-positive bacteria were more susceptible than Gram-negative one. Majority of tested microorganisms were susceptible to the action of MGE. *Bacillus cereus* strain was the most resistant bacteria while *Salmonella typhimurium* and *Yersinia enterocolitica* strains were the most resistant bacteria while, *Bacillus subtilis* and *E. coli* O157:H7 followed by *Listeria monocytogenes* and *Basilus cereus* were the most sensitive strains. The most

Table 1: Antimicrobial activity of Myrrh Gum Extract (MGE) against different microorganisms as diameter of inhibition zone (mm)

Microbial strains	Average inhibition diameter zone (mm)
<i>Bacillus cereus</i> (B-3711)	13
<i>B. subtilis</i>	15
<i>Staphylococcus aureus</i> (ATCC13565)	4
<i>Listeria monocytogenes</i> (598)	14
<i>Salmonella typhimurium</i> (ATCC 25566)	Nil
<i>Escherichia coli</i> O157:H7 (ATCC51659)	15
<i>Yersinia enterocolitica</i>	Nil
<i>Aspergillus niger</i>	Nil
<i>Aspergillus flavus</i> (3357)	3
<i>Candida albicans</i>	2
<i>Penicillium roqueforti</i> (J5)	14
<i>Saccharomyces cerevisiae</i> (Y2223)	3

sensitive mold and yeast were *Penicillium roqueforti* and *Saccharomyces cerevisiae*, respectively. The most sensitive yeast was *Saccharomyces cerevisiae*. Myrrh extract had a highly inhibitory effect.

### DISCUSSION

The results revealed that MGE had antimicrobial activity against the pathogenic bacteria and inhibited their growth as well as mold and yeast. The same trend was also observed by various researchers and by different means. Mohamed *et al.*<sup>1</sup>, observed the same action of Dill and Caraway essential oils against pathogenic bacteria. Mohamed *et al.*<sup>12</sup>, also reported that myrrh essential oil had highly antimicrobial action against pathogenic microorganisms which lead to preserve processed cheese samples and increased their shelf life to one year. While, Brieskorn and Nobel<sup>11</sup> and Dolara *et al.*<sup>10</sup>, also reported that the essential oils of *Commiphora* species such as myrrh are rich in furano sesquiterpenoids and sesquiterpenes which have been possess anesthetic, antibacterial and antifungal properties.

**Chemical composition of soft cheese samples:** The TS; TP; SN; Fat; TVFA and pH values as well as acidity percentage of all cheese samples are shown in Table 2. There were no paramount variations in the general chemical composition or other studied parameters between treated samples and control as shown in Table 2. Myrrh gum extract seemed to have any effect on the composition of cheese samples.

The obtained data revealed that there were no clear variations on the chemical composition of treated cheese

samples and control. They indicated that the chemical composition of cheese samples lied in the normal ranges of Egyptian soft cheese<sup>2,15,21</sup>. The level of acidity percentage and pH values had the normal trends which indicated that addition of MGE did not affect the starter culture activity. Mohamed *et al.*<sup>1</sup> mentioned also that essential oil of caraway and dill did not affected the activity of starter culture in Labenah samples.

**Texture profile of soft cheese samples:** Data listed in Table 3 reflected the textural properties of soft cheese samples. It could be noticed that there were no clear differences in the studied parameters either in Firmness, Gumminess or Chewiness.

The MGE did not affect the textural properties of resultant soft cheese.

These data were in agreement with that obtained by Abbas *et al.*<sup>2</sup>, Mohamed *et al.*<sup>12</sup> and Foda *et al.*<sup>21</sup>. The concentration of Myrrh extract which was used in this study and the behavior of this material did not affect the textural properties. Logically, it was expected that myrrh may be increased the gumminess of samples because it have some gumming materials<sup>3</sup> or indigenous gums and resins<sup>8</sup> but the procedure that used in preparation and extraction was success in absent of this defect.

- Firmness (N) = Maximum force of the 1st compression
- Cohesiveness = Area under the 2nd compression/area under the 1st compression (A2/A1)
- Gumminess (N) = Firmness × cohesiveness

Table 2: Chemical composition of soft cheese samples supplemented with different ratios of myrrh gum extract (MGE)

Ratio (MGE)	Storage period (days)	TS (%)	Fat (%)	Protein (%)	Acidity (%)	pH	TVFA*	SN** (%)	SN/TN***
0 (Control)	0	39.63	15.00	12.50	0.37	5.60	7.910	0.123	6.279
	30	40.11	15.50	12.65	0.41	5.53	11.82	0.143	7.212
	60	41.23	16.50	13.11	0.52	5.41	15.68	0.185	9.003
	90	43.50	18.00	13.60	0.61	5.25	23.67	0.215	10.09
0.50%	0	40.11	15.33	12.45	0.36	5.63	8.010	0.121	6.201
	30	40.65	15.50	12.70	0.39	5.55	11.60	0.138	6.932
	60	41.50	16.00	12.95	0.53	5.43	15.13	0.159	7.833
	90	42.95	17.50	13.39	0.58	5.31	19.36	0.185	8.810
1.00%	0	40.65	15.50	12.41	0.35	5.65	8.050	0.119	6.100
	30	41.20	16.00	12.50	0.38	5.56	11.22	0.128	6.534
	60	42.65	16.50	12.89	0.49	5.41	14.83	0.146	7.226
	90	43.71	17.00	13.20	0.53	5.33	17.22	0.169	8.170
1.50%	0	41.23	15.66	12.38	0.34	5.68	8.290	0.116	6.000
	30	42.11	16.00	12.41	0.37	5.52	10.91	0.125	6.426
	60	43.29	17.50	12.60	0.46	5.44	13.35	0.132	6.684
	90	44.15	18.33	12.95	0.50	5.39	12.30	0.146	7.190

\*TVFA: Total volatile fatty acids, expressed as 0.1 N NaOH/100 g of cheese, \*\*SN: Soluble nitrogen, \*\*\*SN/TN: Soluble nitrogen/total nitrogen

Table 3: Textural properties of soft cheese samples supplemented with different ratios myrrh gum extract (MGE)

Treatments	Firmness	Cohesiveness	Gumminess	Chewiness	Springiness	Adhesiveness
Control	3.626	0.669	0.195	1.430	0.676	0.142
0.5% MGE	3.625	0.667	0.194	1.425	0.673	0.138
1.0% MGE	3.601	0.659	0.193	1.422	0.670	0.133
1.5% MGE	3.590	0.652	0.192	1.413	0.665	0.129

Table 4: Sensorial evaluation (Degree) of soft cheese samples supplemented with different ratios of myrrh gum extract (MGE)

Treatments	Storage period (days)											
	Fresh			30			60			90		
	A (10 d)	B (40 d)	F (50 d)	A (10 d)	B (40 d)	F (50 d)	A (10 d)	B (40 d)	F (50 d)	A (10 d)	B (40 d)	F (50 d)
Control	9	35	45	9	35	41	3	30	25	0	25	0
0.5% MGE	9	35	45	9	35	45	9	35	45	9	35	40
1.0% MGE	9	35	45	9	35	45	9	35	45	9	35	40
1.5% MGE	9	35	45	9	35	45	9	35	45	9	35	45

A: Appearance, B: Body and texture, F: Flavor d: Degree

Chewiness (mm) = Gumminess × springiness

Springiness (mm) = Length of 2nd compression/length of 1 compression (L2/L1)

Adhesiveness (N.s) = Negative area in the curve

**Sensorial behavior of soft cheese samples:** Effect of using MGE in organoleptic properties of cheese samples is clear in Table 4. Soft cheese samples seemed to be natural and had no odd character either in the body and texture or flavor. No bitter taste was observed or gummy body and compact texture was noticed. Addition of MGE gave clean and acceptable acid taste.

So, Myrrh Gum Extract can be used in preparing soft cheese samples which had relative high moisture content (about 60%) and increase its shelf life without any risk in their chemical, textural or sensorial properties. This study recommended using of Myrrh gum extract as an antimicrobial agent in food sector and dairy products as natural, safe and effective material.

### CONCLUSION

It could be concluded that the urgent need for natural and safe antimicrobial agents is still a requirement, however, myrrh is a natural and safe material which could be used as food preservative. The potential effect of myrrh gum extract (MGE) was observed as its antimicrobial effect against various examined-strains of pathogenic microorganisms. MGE did not affect the starter culture or development of acidity through 90 days of storage. Obtained results reveal that using 10 mL MGE L<sup>-1</sup> cheese milk in preparing soft cheese is more beneficial and gave the favorite acceptance. So, this study recommended its use in preparing cheese with high

content of moisture, where the chemical; rheological and sensorial properties of samples did not impressed by its using.

### SIGNIFICANCE STATEMENT

This study deals with using myrrh gum extract as a natural, safe and cheap preservative agent to produce healthy and safe soft cheese.

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