Short communication: Influence of an aqueous myrrh suspension on yogurt culture bacteria over yogurt shelf life

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ABSTRACT

Myrrh is an essential oil and natural flavoring approved by the US Food and Drug Administration, and it has antibacterial and antifungal activity against pathogens. Our objective was to determine the effect of an aqueous myrrh suspension on *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* counts in peptone solution and yogurt, as well as pH and titratable acidity of yogurt during 5 wk of storage at 1 to 4°C. The myrrh suspension (10% wt/vol) was prepared and incorporated into a pure culture dilution in peptone and into yogurt mix at a 1% (vol/vol) level. A control with no myrrh was also prepared, and 3 replications were conducted. *Streptococcus thermophilus* were enumerated using *Streptococcus thermophilus* agar with aerobic incubation at 37°C for 24 h, and *Lactobacillus delbrueckii* ssp. *bulgaricus* were enumerated using de Man, Rogosa, and Sharpe agar adjusted to pH 5.2, with anaerobic incubation at 43°C for 72 h. During the 8-h period after inoculation, *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* counts in peptone solution at 37°C and 43°C, respectively, were not significantly different in the presence or absence of the aqueous myrrh suspension. Counts of *S. thermophilus* in yogurt containing myrrh (mean $\pm$ SD; 4.96 ± 0.58 log cfu/mL) were not significantly different from those in the control yogurt (4.87 ± 0.39 log cfu/mL). The log counts for *L. delbrueckii* ssp. *bulgaricus* in yogurt containing myrrh (5.04 ± 1.44 log cfu/mL) and those of the control (5.52 ± 1.81 log cfu/mL) did not differ, and the counts remained within 1 log of each other throughout 5 wk of storage. The pH of the yogurts containing the aqueous myrrh suspension was not significantly different from that of the control yogurts, and their pH values were within 0.1 pH unit of each other in any given week. Titratable acidity values remained steady around 1.1 to 1.2% lactic acid for both yogurt types throughout the

storage period, with no significant differences between them. Yogurt culture bacteria can survive in the presence of a myrrh suspension in yogurt with no significant change in pH or titratable acidity. Therefore, it may be beneficial to add an aqueous myrrh suspension to yogurt.

Key words: myrrh, antimicrobial, yogurt culture

Short Communication

Myrrh is extracted from *Commiphora* trees of the Burseraceae family. Species in the *Commiphora* genus are small trees or shrubs with short, thorny branches and number more than 200 (Hanuš et al., 2005); *Commiphora myrrha* is a variable species found in parts of Saudi Arabia and northeast Africa as far south as northeast Kenya. Myrrh, a brown powder, contains gum, resin, volatile oil, and other compounds (Mohamed et al., 2016).

Myrrh is a safe, natural flavoring substance approved by the US Food and Drug Administration (FDA, 2018; 21CFR172.510) and functions as a preservative. Extracts of many types of plants have been used as flavoring and seasoning agents in foods and beverages and as folk medicines and food preservatives since ancient times. Myrrh adds characteristic flavor and prolongs the shelf life of foods by its antioxidant, bacteriostatic, and bactericidal activities (Kim et al., 2011).

Myrrh has considerable antimicrobial activity and is used to cure a variety of diseases. It has antibacterial activity against standard pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* (Lotfy et al., 2006). Furthermore, it shows strong inhibitory activity against *Salmonella enterica* serovar Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes* on lettuce (Kim et al., 2011). Myrrh has antibacterial and antifungal properties because it contains sesquiterpenes (furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one) and furanosesquiterpenoids [3-methoxy-4-furanogermacrene-10(15)-dien-6-one, 2-methoxy-4-furanogermacrene-1(10)-en-6-one, furanogermacrene-1(10)-4-dien-6-one, and curzerenone

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(6,7-dihydro-5-isopropenyl-3,6-dimethyl-6-vinyl benzofuran-4-one)] (Mohamed et al., 2016). Myrrh, along with honey and bee propolis, can help cure chronic nonhealing wounds such as diabetic foot disease (Lotfy et al., 2006). In the Middle Eastern region, the traditional way of taking myrrh as a medicine to treat a sore throat or a cold is by adding 1.67 mL of myrrh to 113 g of boiled water and consuming a teaspoon of 1% myrrh supernatant suspension daily (Fetrow and Avila, 2004). Boiling water enhances the medicinal (antimicrobial) efficacy of the myrrh supernatant compounds and does not inhibit the antimicrobial effect of the myrrh supernatant compounds (Fetrow and Avila, 2004).

Several studies have evaluated the health benefits provided by consumption of yogurt starter culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) and the safety of yogurt consumption. *Streptococcus thermophilus* has many health benefits including reducing the occurrence of acute diarrhea and the level of nasal colonization of the pathogenic bacteria *Streptococcus pneumoniae*, *Staphylococcus aureus*, and β -hemolytic streptococci. Furthermore, *S. thermophilus* along with *L. delbrueckii* ssp. *bulgaricus* can reduce cholesterol assimilation (Sharma et al., 2014). The consumption of lactic acid bacteria (LAB) has several benefits, including enhancing the immune system; improving the intestinal tract by controlling irritable bowel syndrome, inflammatory bowel disease, and suppressing endogenous and exogenous pathogens; reducing symptoms of lactose intolerance; and reducing the risk of certain cancers (Parvez et al., 2006). Although yogurt consumption provides various health benefits and is almost always safe because of its low pH and inhibitory substances produced by added cultures, food poisoning outbreaks from *E. coli* O157:H7, *Salmonella* Typhimurium, staphylococci, and other pathogens in yogurt have been reported, as summarized by Aryana and Olson (2017). Myrrh can act as a pathogen inhibitor, flavoring agent, and a preservative. Pathogenic mutations that can survive low pH conditions are a possibility. Myrrh would add an additional barrier or hurdle for a potential pathogen, creating another layer of safety in this widely consumed product for gut health. Therefore, it would be beneficial to find ways to further decrease food safety risks by using myrrh.

The effect of various essential oils (EO) on the viability of LAB in dairy starter cultures and dairy products and on the viability of spoilage and pathogenic microorganisms has been studied. Marcial et al. (2016) reported that oregano EO did not affect LAB starter culture viability in cheese but protected it from growth of spoilage organisms. Likewise, Moro et al. (2015) reported that rosemary EO did not affect growth of

LAB in fortified cheese but inhibited *Clostridium* spp. However, Kostova et al. (2014) found that *Lactobacillus* species and *S. thermophilus* with *Lactococcus lactis* were inhibited by a 0.05% concentration of dill EO and killed by a 0.5% concentration. Çakmakçı et al. (2014) reported that black cumin EO decreased LAB counts in butter during storage. De Carvalho et al. (2015) recommended exercising caution when adding *Thymus vulgaris* L. EO to cheese models because of the more severe inhibiting effects on cheese starter cultures than on pathogenic bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*). Therefore, different EO at various concentrations have different effects on viability of LAB.

Because of the many potential beneficial effects provided by myrrh (flavor, preservative, and activity against pathogenic microorganisms), we hypothesized that an aqueous myrrh suspension could be incorporated into yogurt with potentially beneficial results if *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* are not adversely affected by myrrh. To the best of our knowledge, whether myrrh has an antimicrobial effect on yogurt culture bacteria has not been reported in the literature. Therefore, the objective was to determine whether myrrh has antimicrobial effects on *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in pure form and in yogurt. We also investigated whether pH and titratable acidity in yogurt would be affected by the presence of myrrh.

Myrrh gum (*Commiphora myrrha*; country of origin: Eritrea; packed by Mond Trading Company, Toronto, ON, Canada) was dissolved in boiling distilled water to make a 10% (wt/vol) suspension. The myrrh suspension was set for 1 h at room temperature for cooling. The suspension was centrifuged ($3,677 \times g$, 5 min, 20°C; Accuspin 400 centrifuge, Fisher Scientific, Osterode, Germany), and only the supernatant was collected.

A 0.3-mL aliquot of myrrh supernatant (10% wt/vol) was added to 3 mL of either freshly thawed pure cultures of *S. thermophilus* ST-M5 (approximately 1×10^{11} cfu/g; Chr. Hansen, Milwaukee, WI; Muramalla and Aryana, 2011) or *L. delbrueckii* ssp. *bulgaricus* LB-12 (2.5×10^{10} cfu/g according to the certificate of analysis from Chr. Hansen) separately diluted with 26.7 mL of 0.1% sterilized peptone solution (Bacto Peptone, Difco/Becton, Dickinson and Co., Sparks, MD) to form a 1% myrrh supernatant suspension, with a total myrrh concentration of 0.1%. The peptone solution was stored at 37°C for the *S. thermophilus* ST-M5 assay and at 43°C for the *L. delbrueckii* ssp. *bulgaricus* LB-12 assay. The inoculated myrrh supernatant was pour plated with M17 agar (Oxoid, Basingstoke, UK) containing 1% lactose (Oxoid) for *S. thermophilus* ST-M5 and

with de Man, Rogosa and Sharpe (MRS) agar (Difco) for *L. delbrueckii* ssp. *bulgaricus* LB-12 in duplicate at 0, 2, 4, 6, and 8 h after inoculation. All M17-lactose plates were incubated aerobically at 37°C for 24 h, and all MRS plates for *L. delbrueckii* ssp. *bulgaricus* were incubated anaerobically at 43°C for 72 h. After incubation, a Quebec Darkfield Colony Counter (Leica Inc., Buffalo, NY) was used to enumerate the colonies.

Whole milk (7.57 L) in each of 2 pails was heated to 85°C, held at that temperature for 30 min, cooled to 40°C, and then inoculated with 2 mL of freshly thawed pure cultures of *S. thermophilus* ST-M5 (Chr. Hansen) and *L. delbrueckii* ssp. *bulgaricus* LB-12 (Chr. Hansen). Myrrh supernatant (75.7 mL) was added to 1 of the 2 pails to form a 1% supernatant suspension, and the contents in the other pail served as a control. After mixing, yogurt mixes were poured into 355-mL Reynolds RDC212 Del-Pak Combo-Pak containers (Alcoa Inc., Pittsburgh, PA) and incubated at 40°C to pH 4.6 before cooling to between 1 and 4°C. Three replications were performed.

Yogurt was serially diluted with 0.1% (wt/vol) sterilized peptone solution. Each dilution was pour plated with *Streptococcus thermophilus* agar made in our laboratory (Dave and Shah, 1996) for enumerating *S. thermophilus* and MRS agar adjusted to pH 5.2 (Dave and Shah, 1996) for enumerating *L. delbrueckii* ssp. *bulgaricus*. Plating was conducted in duplicate at 0, 1, 2, 3, 4, and 5 wk after yogurt manufacturing. All *Streptococcus thermophilus* agar plates were incubated aerobically at 37°C for 24 h, and all MRS plates were incubated anaerobically at 43°C for 72 h. A Quebec Darkfield Colony Counter (Leica Inc.) was used to assist in enumerating the colonies.

The pH of the yogurts was measured with an Ultra-Basic pH/mV Meter (Denver Instrument Co., Arvada, CO) at 5°C at 0, 1, 2, 3, 4, and 5 wk of storage. The pH electrode was calibrated with pH 7.00 and 4.00 buffer solutions (VWR International, West Chester, PA) before use. Titratable acidity was measured weekly for 5 wk by weighing 9 g of yogurt in a white evaporating dish, adding 3 drops of phenolphthalein indicator, and titrating with 0.1 N NaOH to a faint pink color; titratable acidity was expressed as percent lactic acid (Wehr et al., 2004).

The log plate count, pH, and titratable acidity data were analyzed as a randomized block design with repeated measures using PROC MIXED in SAS software (version 9.4; SAS Institute Inc., Cary, NC). Time was considered a random effect. Slopes of regression lines of log counts as a function of time represent the net growth or decline of the bacteria over time, and a comparison of the slopes of the regression lines between

the myrrh-containing yogurt and the control would provide information about the effect of the presence of myrrh on the growth of these bacteria in yogurt. Significant differences in slopes of regression lines were tested using a random coefficient model by examining the significance of the myrrh (presence vs. absence) and time (hours after inoculation) interaction term in the ANOVA. PROC REG was also used to determine whether the regression lines were a good fit to the data points (R^2 values). Significance was set at $\alpha = 0.05$.

We did not analyze the aqueous extracts of myrrh in this study. Although myrrh contains antimicrobial substances, the amount of active compounds present in the aqueous extract was not known and it is unclear whether sufficient quantity exists to exert an antimicrobial effect. Also, the effect of various extraction procedures and solvents on the amount of active compounds that enter the suspension is not known and could be the subject of a future study. Compounds within extracts of myrrh resin have been identified by GC-MS analysis, as reported by Hanuš et al. (2008) and Fraternali et al. (2011).

Log counts of *S. thermophilus* ST-M5 in peptone solution as influenced by myrrh at 0, 2, 4, 6, and 8 h are shown in Figure 1a. Counts in the presence and absence of myrrh remained steady around 9.9 log cfu/mL, ranging from (mean $\pm$ SD) 9.80 ± 0.03 to 10.08 ± 0.05 log cfu/mL. We detected no significant differences in *S. thermophilus* log counts between the myrrh samples and controls or in the slopes of the regression lines for *S. thermophilus* log counts versus time for myrrh samples and control. The 1% myrrh supernatant was not strong enough to kill *S. thermophilus* ST-M5 bacteria suspended in a peptone solution.

Log counts of *L. delbrueckii* ssp. *bulgaricus* LB-12 in peptone solution as influenced by myrrh at 0, 2, 4,

Figure 1. Log counts of (a) *Streptococcus thermophilus* ST-M5 and (b) *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 counts in peptone water in the presence ($\blacktriangle$) and absence ($\blacksquare$) of myrrh over the 8-h experiment. Error bars represent SD.

6, and 8 h are shown in Figure 1b. Counts decreased slightly from (mean ± SD) 9.39 ± 0.09 to 9.13 ± 0.06 log cfu/mL for the control yogurt and from 9.56 ± 0.03 to 9.17 ± 0.02 log cfu/mL for the myrrh-containing yogurt during the 8-h experiment. As observed for *S. thermophilus*, we detected no significant difference in *L. delbrueckii* ssp. *bulgaricus* log counts between the myrrh samples and controls or in the slopes of the regression lines for *L. delbrueckii* ssp. *bulgaricus* log counts versus time after inoculation of the myrrh-containing samples versus controls. Again, the 1% myrrh supernatant concentration was not strong enough to kill the *L. delbrueckii* ssp. *bulgaricus* LB-12 bacteria.

For yogurt shelf-life study, *S. thermophilus* ST-M5 log counts as influenced by myrrh throughout 5 wk of storage are shown in Figure 2a. Initial counts (mean ± SD) for *S. thermophilus* ST-M5 (4.68 ± 0.33 log cfu/g for the control and 4.40 ± 0.26 log cfu/g for the myrrh-containing yogurt) in the present study were lower than the approximately 8 to 9 log cfu/g reported by Dave and Shah (1997) and Vinderola et al. (2000). Microbial counts for myrrh and control increased 1.01 and 0.63 log cfu/g, respectively, over time from 0 to 5 wk. The intercept, slope of the regression line, *t*-value, *P*-value, and *R*² value for *S. thermophilus* log counts as a function of storage time are shown in Table 1. We detected no significant difference in *S. thermophilus* log counts between the myrrh-containing yogurts (mean ± SD; 4.96 ± 0.58 log cfu/mL) and the control (4.87 ± 0.39 log cfu/mL) or in the slopes of the regression lines for *S. thermophilus* log counts versus time during storage of the yogurts. The 1% myrrh supernatant was not strong enough to kill *S. thermophilus* ST-M5 bacteria in yogurt. The symbiotic relationship between *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in yogurt may have protected the *S. thermophilus* in yogurt.

The *L. delbrueckii* ssp. *bulgaricus* LB-12 log counts for the yogurt shelf life study as influenced by myrrh over 5 wk of storage are shown in Figure 2b. Log microbial counts for the myrrh and control yogurts decreased by 3.77 and 3.92 log cfu/g, respectively, between 0 and 5

Figure 2. Log counts of (a) *Streptococcus thermophilus* ST-M5 and (b) *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 counts in yogurt in the presence (▲) and absence (■) of myrrh over 5 wk of storage. Error bars represent SD.

wk. The intercept, slope of the regression line, *t*-value, *P*-value, and *R*² value for *L. delbrueckii* ssp. *bulgaricus* counts as a function of storage time are shown in Table 1. As observed for the *S. thermophilus* log counts, we detected no significant difference in *L. delbrueckii* ssp. *bulgaricus* log counts between the myrrh-containing yogurts (5.04 ± 1.44 log cfu/mL) and the control (5.52 ± 1.81 log cfu/mL) or in the slopes of the regression lines for *L. delbrueckii* ssp. *bulgaricus* log counts versus time during storage of the myrrh and control samples. The log counts remained within 1 log of each other during 5 wk of storage. The 1% myrrh supernatant was not strong enough to kill the *L. delbrueckii* ssp. *bulgaricus* LB-12 bacteria in yogurt.

The pH of yogurt during its 5 wk shelf life study is shown in Figure 3. We observed no significant difference in pH between the myrrh-containing yogurts and the controls at different storage times or in the slopes of the regression lines for pH versus time during storage of the yogurt samples. The pH values of all samples were

Table 1. The intercept (log count) and slope of the regression line (log count/wk), *t*-values, *P*-values, and *R*² values for *Streptococcus thermophilus* ST-M5 log counts and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 log counts by week

Organism	Treatment	Parameter	Parameter estimate	<i>t</i> -value	<i>P</i> -value	<i>R</i> ²
<i>S. thermophilus</i> ST-M5	Control	Intercept	4.530	34.24	<0.001	0.3799
		Slope	0.137	3.13	0.007	
	Myrrh	Intercept	4.402	24.18	<0.001	0.462
		Slope	0.223	3.71	0.002	
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> LB-12	Control	Intercept	7.625	17.13	<0.001	0.673
		Slope	−0.844	−5.74	<0.001	
	Myrrh	Intercept	6.929	28.73	<0.001	0.849
		Slope	−0.754	−9.47	<0.001

Figure 3. The pH of yogurts containing myrrh (▲) and control (■) over 5 wk of storage. Error bars represent SD.

within 0.1 pH unit of each other in any given week. This may be due to the addition of the free hydroxyl ions originating from the myrrh/water supernatant. The free hydroxyl ions may have neutralized some of the lactic acid produced by *S. thermophilus* ST-M5 and *L. delbrueckii* ssp. *bulgaricus* LB-12 bacteria, resulting in a slight increase of the yogurt's pH compared with the control. Dairy products are able to bind or release ions, causing a small change in pH upon addition of an acid or alkali substance. Furthermore, dairy products generally have a high protein content, ensuring a relatively high buffering capacity due to the buffering effect of amino groups (Al-Dabbas et al., 2011). According to Mohamed et al. (2016), the pH values increased with increasing EO concentration. This increase may be due to the action of EO inhibition of acidity formation and the alkaline effect of the EO itself. During storage, pH values decreased slightly for all treatments. The decrease in pH during the first week of storage in the present study could be related to the production of lactic acid arising from lactose hydrolysis that likely occurred during storage.

Titrateable acidity values for myrrh and control yogurts throughout 5 wk of storage are shown in Figure 4. The titrateable acidity values remained steady around 1.1 to 1.2% (expressed as % lactic acid) for myrrh and control yogurts throughout the 5-wk storage period. We detected no significant differences in titrateable acidity values between myrrh and control yogurts, among the

Figure 4. Titrateable acidity expressed as percent lactic acid of yogurts containing myrrh (▲) and control (■) over 5 wk of storage. Error bars represent SD.

various storage times, or in the slopes of the regression lines for pH versus time during storage of the myrrh-containing samples versus the controls.

In this study, we investigated the effect of an aqueous myrrh suspension on *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* counts in both peptone and yogurt, and on resulting pH and titrateable acidities. Yogurt culture bacteria can survive in the presence of an aqueous myrrh suspension in pure form and in the yogurt matrix with no significant effect on pH or titrateable acidity of yogurt. Therefore, it may be possible to use an aqueous myrrh suspension in yogurt without adverse effects. Improvements in safety, shelf life, and organoleptic properties in yogurt containing an aqueous myrrh suspension could be subjects for further investigation.

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