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Research Article
Garlic Flavored Self-preserved and Vegetable Oil Based Strained Yoghurt

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Abstract

Background and Objective: Garlic essential oil (GO) is considered to be one of the effective natural antimicrobial agents for application in food preservation. Therefore the main purpose of this study was to extend the shelf-life of strained yoghurt (SY) by adding GO and compared it with plain skimmed SY which was not done before. **Methodology:** Garlic essential oil (GO) was prepared at three different concentrations in vegetable oil bases. The antimicrobial activity of these GO bases was evaluated against different pathogenic and food spoilage organisms. Then, each concentration of these GO bases was incorporated into a previously manufactured plain skimmed strained yoghurt (SY) made from buffalo milk to produce GO flavored and vegetable oil based SY containing 10, 50 and 100 ppm of GO. Chemical analysis and textural properties of the product was investigated. The shelf life stability in terms of microbial and sensorial evaluations was also studied during 45 days of storage at 4°C. **Results:** Addition of GO in vegetable oil bases showed that the antimicrobial activity against some pathogenic bacteria, mold and yeast. When these GO bases were incorporated into plain skimmed SY, it extended its shelf life to 38 days at 4°C compared with 21 days for the plain skimmed SY. **Conclusion:** The potentials of manufacturing SY with enhanced flavor and shelf life stability by using GO as a plant-based flavoring and preserving agent.

Key words: Strained yoghurt, garlic oil, antimicrobial activity, shelf life, flavor

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

INTRODUCTION

Essential Oils (EOs) isolated from aromatic plants are among the most important natural preserving and food flavoring agents. The antimicrobial activity of these EOs and their major compounds against a wide range of pathogenic microorganisms was investigated[1,2]. Garlic essential oil (GO) is considered to be one the effective natural antimicrobial agents for application in food preservation and in active antimicrobial packaging[3-6]. The antimicrobial activity of GO originates from the high content of ally sulfides that represent a major constituents of GO[7]. Beside its antimicrobial activity, GO also has a characteristic sulfuracious-spicy taste so it is used to flavor and preserve meat[8] and processed chicken products[9]. On the other hand garlic flavor is not one of the common tastes that usually used in fermented dairy products compared with fruit flavors which are more appreciated in such products.

Among the fermented dairy products, Strained Yoghurt (SY) is a well known food appetizer in different parts of the world. It has a relatively thick consistency between that of conventional yoghurt and cheese. The SY is produced by removing part of the serum (whey) from fresh yoghurt to reach a total solids content[10] around 23.0-25.0%, therefore it is also called concentrated yoghurt. There is an expanding demand for SY due to its appreciated flavor, richer texture and much higher protein content than traditional yoghurt. In addition, SY has less lactose than fresh yoghurt due to removal of some of this sugar during the straining process. For this reason, SY could be appealing for lactose intolerant consumers.

On the other hand, the shelf life[11] of SY is ~15 days at 4°C. Manufacturers are generally interested in increasing the shelf life of this product, therefore synthetic preservatives like benzoates and sorbates may be used in some occasions for the preservation of SY[12] or extending of its shelf life. This trend is considered to be a drawback for those consumers seeking for fermented dairy products with all natural ingredients.

The promising antimicrobial activity of essential oils as natural preservatives motivated the researchers of the current study to investigate their inherent preserving potentials and aroma attributes for extending the shelf life of SY and to make it more flavorful and appetizing. Garlic essential oil (GO) was chosen in the current study among the different other essential oils due to some reasons. First, its well known antimicrobial activity against pathogenic and food spoilage microorganisms is evident, as previously illustrated earlier in this section. Second, its flavoring attributes, which was tested in a preliminary evaluation was found to be sensorial acceptable and compatible with SY. Third, as far as the authors concerned there were no previous investigations approached the trend of incorporation of GO in SY for a dual preservation and flavoring purpose. Therefore, the current study was directed towards technological processing and shelf life evaluation of GO flavored SY after storage period of 45 days at 4°C, which is three times the normal shelf life of traditional SY.

Meanwhile milk fat will be replaced with vegetable oil in order to satisfy low cholesterol dairy seeking consumers.

MATERIALS AND METHODS

Materials: Fresh full fat buffalo's milk was obtained from Faculty of Agriculture, Cairo University. Garlic cloves were purchased from the local market in Cairo. Distilled monoglycerides of vegetable fatty acids (Palsgaard® DMG 0093) was purchased from Palsgaard, Denmark. Sodium chloride was obtained from El-Naser Company for salt, Egypt.

Pathogenic strains included: *Bacillus cereus* B-3711, *Bacillus subtilis*, *Aspergillus flavus* 3357 and *Saccharomyces cerevisiae* Y-2223 (provided by the Northern Regional Research Laboratory Illinois, USA). *Listeria monocytogenes* 598 (provided by the Department of Food Science, University of Massashusetts, Ambert MA, USA). *Escherichia coli* 0157: H7 and *Staphylococcus aureus* (isolated and serologically identified by dairy microbiological Lab., National Research Center, Egypt). *Yersinia enterocolitica* (obtained from Hungarian National Collection of Medical Bacteria, OKI, Gyaliut, Budapest, Hungary). *Aspergillus niger*, *Pseudomonas aeruginosa*, *Penicillium roqueforti* J5 (obtained from Department of Microbiology, Swedish University of Agricultural Sciences), *Candida albicans* (provided by the Institute of Applied Microbiology, University of Tokyo, Japan).

The pathogenic strains were routinely transferred into tryptone soya broth, incubated at 37°C for 24 h. Yeast and mold strains were activated in malt extract broth (Oxoid), incubated at 25°C for 72 h.

Starter culture strains: Yoghurt starter cultures *Lactobacillus delbrueckii* subsp., *bulgaricus* Lb-12 DRI-VAC (provided by Northern Regional Research Laboratory Illinois, USA). *Streptococcus thermophilus* CH-1 (obtained from Chr. Hansens's Lab., Denmark).

Isolation of garlic essential oil (GO): Garlic cloves were mixed with suitable quantity of distilled water and blended for 3 min using an electric blender to produce garlic puree. The

obtained puree was transferred into 5 L round bottom flasks and more distilled water was added to reach a final consistency suitable for hydro-distillation (~1:7 by weight, garlic cloves: Water, respectively). Clevenger-type apparatus was used to extract the essential oil by hydro-distillation for 2.5 h. The separated GO was collected from the bottom of the side arm of the Clevenger apparatus (as GO is denser than water), dried over anhydrous sodium sulfate and stored in dark brown vials at -4°C till used.

Formulation of GO in vegetable oil bases: Three different concentrations of GO flavor bases were prepared by diluting certain amounts of GO in sunflower oil (brand name: Crystal) to formulate 100, 500 and 1000 ppm GO in the three sunflower oil flavor bases.

Manufacturing of the plain skimmed strained yoghurt: The SY was prepared as described by the method previously described[13] with some modification. Milk fat was separated from fresh milk by centrifugation. The milk was heated at 95°C for 15 min, cooled to 42°C then inoculated with 2.0% yoghurt starter cultures and incubated at 42°C until the acidity decreased to pH 4.8. The obtained skimmed plain yoghurt was cooled to 10°C overnight, stirred and strained using cheese cloth which was suspended in refrigerator at 4°C to allow whey drainage for 12 h. Finally, NaCl (0.5%) was added to the contents of the cheese cloth bags after the straining process was completed to produce plain skimmed SY. Samples were manufactured in triplicates.

Incorporation of GO flavor bases into the skimmed plain strained yoghurt: Calculated amounts of a food grad monoglyceride emulsifier (Palsgaard® DMG 009) were dissolved in each of the three prepared GO flavor bases in order to bind these bases with the plain skimmed SY. Then calculated amounts of each of these GO bases/emulsifier mixture were added to the previously manufactured plain skimmed SY with continuous stirring for 5 min to ensure complete incorporation and equal distribution of the GO bases/emulsifier mixture into the plain SY.

This process lead to production of GO flavored vegetable oil based SY containing 10, 50 and 100 ppm of GO and ~11.5-12.0% vegetable oil content. Finally, that end product was filled into different plastic containers and stored at 4°C for assessing the shelf life and the keeping quality. Samples were manufactured in triplicates.

Chemical analysis: The plain skimmed SY (control) and GO flavored SY samples were chemically analyzed for moisture, total solids, total protein, ash, fat percentage and pH values according to AOAC[14]. The total volatile fatty acids were determined according to Kosikowiski[15].

Sensory evaluation: Fresh and stored plain SY and GO flavored SY samples were evaluated for their sensory properties according to the score card method[16]. Panelists (10 persons) from the researchers at the dairy department, National Research Center, Cairo, who are familiar with the original taste of SY were volunteered for this evaluation. The Flavor (50 points), body and texture (40 points) and appearance (10 points) were evaluated regularly for 45 days storage at 4°C. Samples were taken out of the refrigerator and left at the room temperature to equilibrate with the atmosphere. Then it was served with a cup of water to the panelists for rinsing mouth between samples.

Textural analysis: Texture measurements were carried out with universal testing machine (Cometech, B type, Taiwan) provided with software. Back extrusion cell with 35 mm diameter compression disc was used. Two cycles were applied, at a constant cross head velocity of 1.0 mm sec^{-1} to 25% of sample depth then returned. From the resulting force-time curve, the values for texture attributes, i.e., firmness, gumminess, chewiness, cohesiveness, springiness and resilience were calculated from the TPA graphic[17].

Microbiological analysis: Preparation of inoculum for assessment of antimicrobial activity: About 0.1 mL (approximately 10^9 cells mL^{-1}) of the tested microorganisms was grown on trypton soya broth medium at 37°C for 24 h. For preparation of mold and yeast, the strains were grown in malt extract broth medium at 25°C for 72 h and then spread on the nutrient agar medium found in the entire surface of the petri dish using a sterile swab.

Assessment of the antimicrobial activity of the GO flavor bases: Before incorporation of GO flavor bases into the plain skimmed SY, their antimicrobial activity against some food spoilage and pathogenic organisms was evaluated. For this purpose, portion of each GO flavor bases that contained 100, 500 and 1000 ppm GO were diluted for the second time in sunflower oil to give final concentration of GO corresponding to 10, 50 and 100 ppm in the vegetable oil base. These new concentration resemble that of GO in the final target product (GO flavored SY). Then each of these concentrations was further diluted 1:1 (by weight) with absolute isopropyl alcohol to facilitate mobility of the GO flavor bases through the agar

gel during the microbiological assay. The disc diffusion method[18] was used to determine the antimicrobial activity. About 20 µL of each concentration of these GO flavor bases/isopropanol was impregnated on different sterile paper discs (Whatman, 1.6 mm) and placed on the surface of nutrient agar in petri dishes. According to this procedure, each paper disc carried a load of GO equals to 0.1, 0.5 and 1.0 µg, corresponding to GO flavor base/isopropanol concentrations of 5, 25 and 50 ppm, respectively (after 1:1 dilution with isopropanol). The plates were incubated at 37°C for 24 h. After the incubation period the inhibition zones around the paper discs (mm) were measured (after deduction of 0.5 cm which is the diameter of the paper disc). The antimicrobial activity of GO flavor bases was assessed in triplicates.

Microbial count in the final product (GO flavored strained yoghurt): This trend was used to evaluate the effect of GO on the starter culture and also to determine the shelf life of the final product by counting the number of mold, yeast, total viable bacteria and coliform during storage. The different procedures used for microbial counts in each case were investigated as follows:

Starter culture count: *Lactobacillus bulgaricus* count was determined using MRS agar[19]. The plates were incubated at 37°C for 48 h under anaerobic condition. *Streptococcus thermophilus* count was determined using M17 agar[20]. The plates were incubated at 35°C for 48 h under aerobic conditions.

Mold and yeast count: It was determined using potato dextrose agar acidified to pH 3.5 with sterile lactic acid solution (10%)[21]. The plates were aerobically incubated at 25°C for 4 days.

Total viable bacterial count: It was determined using plate count agar medium (Oxoid). The plates were incubated aerobically at 37°C for 48 h[22].

Coliform count: It was determined using violet red bile agar medium[23]. The plates were incubated at 37°C for 18 h. All counts were determined in triplicates.

Statistical analysis: The data were analyzed according to Statistical Analysis System Users Guide b[24] (SAS Institute, Inc, U.S.A.). Separation among means in triplicates was carried out using ANOVA test followed by Duncan multiple tests. All tables result data analysis in two ways except for the antimicrobial table data analysis in one way.

RESULTS AND DISCUSSION

Chemical analysis: Chemical analysis revealed that fresh plain SY (control) contained 11.5±0.2% fat, 10.5±0.01% protein, 29.5±0.2% total solids and 70.5±0.2% moisture and 1.9±0.02% ash. There was no significant difference observed between these values and that of the end product (GO flavored SY). These results may differ from some other investigations[25,26] probably due to difference in milk type or manufacturing process.

Texture analysis of the fresh plain SY (control) showed that the firmness (N), gumminess (N), chewiness (mN), cohesiveness (A/B), springiness (mm) and resilience were 0.340, 0.176, 0.134, 0.568, 0.655 and 0.131, respectively. There was no significant difference in all parameters between fresh plain SY and GO flavored SY indicating no effect of GO on the texture. This observation could be due to the similarity of chemical composition between both products especially fat and total solid contents, as was previously shown earlier.

Assessment of the antimicrobial activity of GO flavor bases: The antimicrobial activity of the three GO flavor bases (10, 50 and 100 ppm GO in sunflower oil) diluted 1:1 with isopropanol was assessed using the agar disc diffusion method. Table 1 showed that these GO bases had various degrees of inhibition against the growth of some pathogenic and food spoilage microorganisms. From the Table 1, it is clear that GO bases were most effective against *Staphylococcus aureus*. This organism was previously found to be sensitive to GO as indicated previously[27]. That study showed that GO at 4x MIC can reduce the original inoculums of *Staphylococcus aureus* to <2 $\log_{10}$ within 6.0 h. The antimicrobial activity of GO originates from the high content of allyl sulfides[7]. The content of these sulfides depends on garlic cultivar and geographical origin and can ranges[28,29] from 42.6-80.0% of the total constituents of GO. Beside allyl sulfides, some methyl sulfide derivatives like dimethyl trisulfide were found to be inhibitory against yeast growth, but not to bacterial growth[5]. The same investigation found the opposite for dipropyl trisulfide and dipropyl tetrasulfide.

In the current investigation the authors did not intend to determine the composition of allyl or methyl sulfides in GO because the main subject of this research focused on the overall preserving potential of GO when it gets incorporated into SY.

Table 1 also showed that *Bacillus subtilis* was less sensitive against the GO flavor bases compared with the other tested bacterial species. This result came in accordance with

Table 1: Antimicrobial activity of GO flavor bases/isopropanol at different concentrations of GO

		Concentration of GO in the flavor base/isopropanol		
		Diameter of inhibition zone (mm)		
Strains	Control	5 ppm (0.1 μg GO disc^{-1})	25 ppm (0.5 μg GO disc^{-1})	50 ppm (1.0 μg GO disc^{-1})
Staphylococcus aureus	Nil	10.66±0.066^{a}	11.00±0.057^{a}	12.66±0.033^{a}
Bacillus cereus	Nil	4.33±0.033^{c}	8.33±0.066^{b}	9.66±0.033^{b}
Listeria monocytogenes	Nil	3.66±0.033^{d}	7.66±0.033^{c}	8.33±0.033^{c}
Escherichia coli	Nil	5.33±0.033^{c}	6.00±0.057^{c}	8.00±0.033^{c}
Pseudomonas aeruginosa	Nil	3.00±0.057^{b}	3.33±0.033^{b}	5.66±0.033^{a}
Yersinia enterocolitica	Nil	2.66±0.033^{d}	3.33±0.033^{d}	4.66±0.033^{d}
Bacillus subtilis	Nil	3.66±0.066^{d}	4.00±0.033^{d}	4.60±0.057^{d}
Aspergillus niger	Nil	4.33±0.066^{c}	9.00±0.057^{b}	11.33±0.066^{a}
Aspergillus flavus	Nil	6.66±0.033^{b}	8.33±0.033^{b}	9.66±0.033^{b}
Penicillium roqueforti	Nil	7.66±0.066^{b}	8.00±0.033^{b}	8.66±0.033^{c}
Candida albicans	Nil	6.66±0.057^{b}	8.66±0.033^{b}	10.00±0.033^{b}
Saccharomyces cerevisiae	Nil	2.33±0.033^{d}	2.66±0.057^{e}	3.33±0.033^{e}

Means with the same letters in the same column are significantly not different ($p \leq 0.05$), Nil: No inhibition zone observed

Table 2: pH values of plain and GO flavored strained yoghurt during storage

	[a]Storage period (day) at 4°C pH			
SY treatments	1	15	30	45
Plain (control)	4.62^{Ac}	4.50^{Ba}	Moldy^{b}	Moldy^{b}
Flavored with 10 ppm GO	4.66^{Ab}	4.45^{Bb}	4.41^{Ca}	4.33^{Db}
Flavored with 50 ppm GO	4.69^{Aa}	4.43^{Bc}	4.42^{Ba}	4.35^{Cb}
Flavored with 100 ppm GO	4.70^{Aa}	4.49^{Ba}	4.44^{Ca}	4.39^{Da}

[a]The first assessment of acidity was measured after day 1 from addition of GO bases to SY, means with the same capital letters in the same row are significantly not different ($p \leq 0.05$), means with the same small letters in the same column are significantly not different ($p \leq 0.05$), the standard error of all samples = ±0.01, [b]Became moldy after 21 days of storage

a previous study which indicate that *B. subtilis* was completely insensitive toward GO when tested in vitro using disc diffusion method[30]. Different degrees of sensitivity toward GO flavor bases were detected among the other tested bacteria as shown in Table 1. From the Table 1 it is also clear that GO flavor bases are antimicrobial active against some common food spoilage fungi and yeast particularly *Candida albicans*. This result was interesting because the growth of these superficial organisms on the surface of fermented dairy products is common leading to deterioration of the appearance, taste and general quality of the product.

The antimicrobial activity of GO flavor bases that was shown in Table 1 may suggest potential application as natural preservatives when they get incorporated into some fermented dairy products like SY. However, this advantage may turn into liability if that antimicrobial action extends to inhibit the activity of the starter bacteria (yoghurt culture). These organisms would better be kept active even after manufacturing and commercialization of SY for continuous production of lactic acid and other free fatty acids that give the product its characteristic flavor. Therefore, it was necessary to investigate the effect of GO flavor bases on the activity of starter culture in the final product (GO flavored SY).

Effect of GO on the starter culture in the final product: The effect of GO on the activity of the starter culture in the final product (GO flavored SY) was investigated by continuous monitoring of the decrease in pH values, increase of total volatile fatty acids (TVFA) and the viable count during storage for 45 days at 4°C. It should be also noted that some investigators used the titratable acidity to quantify lactic acid production and hence the activity of the starter culture[31]. Data shown in Table 2 indicated that the pH values of GO flavored SY decreased as the storage period of the product increased indicating an increase in acidity during storage. Although, that decrease in pH is not sharp at the end of the storage period compared with pH at day 1 but it was significant and gives an indication that the presence of GO did not totally inhibit the activity of the starter culture. This conclusion was also confirmed by assessment of the TVFA produced during storage. These acids are generated by the starter culture via bacterial fermentation which produces short and medium chain fatty acids. The amount of these acids influences the sensory properties of the fermented product and also indicates the viability of the starter culture. In the current study the TVFA of plain SY and GO flavored SY at day one was 10.0±0.4 (0.1 N NaOH/100 g). At the end of the storage period this value reached 18.0±0.5 (0.1 N NaOH/100 g) for SY flavored with the highest concentration of GO (100 ppm). This result indicated that the applied concentrations of GO in this study was sub-lethal to the activity of the starter culture.

Beside the above mentioned approaches (pH and TVFA), the effect of GO on the activity of the starter culture in the

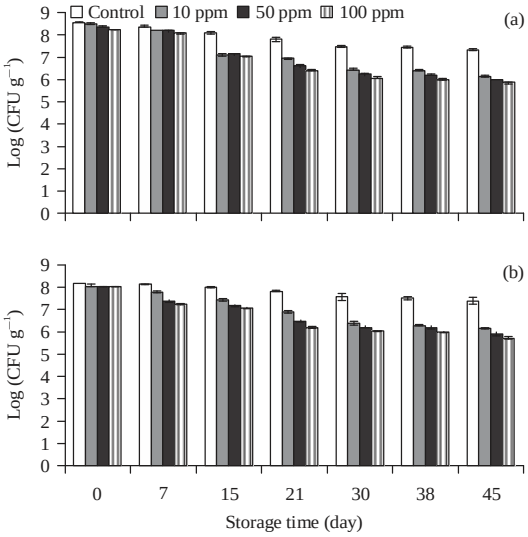

Fig. 1(a-b): Viability count of the starter cultures in plain (control) and GO flavored strained yoghurt at different concentrations of GO during storage of (a) *Lactobacillus bulgaricus* and (b) *Streptococcus thermophilus*

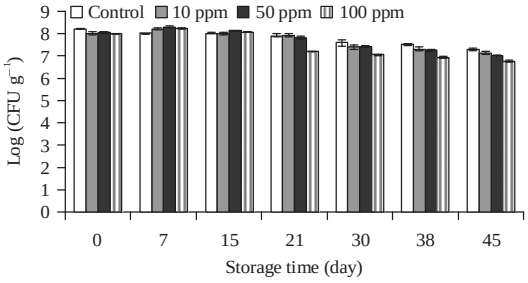

Fig. 2: Total viable bacteria counts in plain (control) and GO flavored strained yoghurt at different concentrations of GO during storage

final product was also investigated by determination of the viable count of the starter organisms during storage for 45 days at 4°C (Fig. 1). Data in the Fig. 1 indicated that the number of starter bacteria decreased at the end of storage period compared with the fresh product. However, the decrease did not affect on the production of lactic acid during storage, as evident from the decrease in pH and increase in TVFA, as indicated earlier. From the Fig. 1 it is also evident that at the end of the storage period *L. bulgaricus* count decreased to ~14.0% for control and 29.0% for GO flavored SY containing 100 ppm GO. On the other hand, *S. thermophilus* counts decreased from ~10.0% for control to ~28.0% for GO flavored SY containing 100 ppm GO.

The decrease in counts of starter culture of SY due to incorporation of essential oils was previously reported[32]. For

Fig. 3: Plain (central container) and GO flavored strained yoghurt samples after 21 days storage period at 4°C. Anticlockwise, from left: 10, 50 and 100 ppm GO. (Arrows are pointing to fungal/yeast growth)

Table 3: Total viable molds and yeasts counts (Log CFU g^{-1}) in plain and GO flavored strained yoghurt during storage

	SY treatments			
Storage periods (days)	Plain (control)	10 (ppm GO)	50 (ppm GO)	100 (ppm GO)
0	Nil	Nil	Nil	Nil
7	Nil	Nil	Nil	Nil
15	Nil	Nil	Nil	Nil
21	1.40±0.4	Nil	Nil	Nil
30	2.00±0.5	Nil	Nil	Nil
38	2.30±0.3	Nil	Nil	Nil
45	3.00±0.2	Nil	Nil	Nil

instance, essential oils like cinnamon lowered the viable cell counts of the starter culture of *L. rhamnosus* in fermented milk. However, the cell count in that study remained greater than the minimum count that required for a product to be considered as probiotic[32]. The same study also showed that clove and mint essential oils caused sub-lethal stress to the starter *L. rhamnosus*.

Effect of GO on shelf life of strained yoghurt: The hazard analysis is one of the methods used to estimate the shelf life of different food and dairy products[33,34]. The procedure requires fitting failure times to Weibull distribution and determining the time at which the product is expected to fail at different probability levels. On the other hand, the researchers of the current study used the counts of molds and yeasts (Table 3), total viable bacteria counts (Fig. 2), sensory properties (Table 4) and visual inspection (Fig. 3) as criteria for estimating the shelf life of the GO flavored SY.

Table 4: Sensory evaluation of plain and GO flavored strained yoghurt

	Storage period (days) at 4°C														
	1			7			15			30			38		
SY treatments	Body and texture (40)	Flavor (50)	Appearance (10)	Body and texture (40)	Flavor (50)	Appearance (10)	Body and texture (40)	Flavor (50)	Appearance (10)	Body and texture (40)	Flavor (50)	Appearance (10)	Body and texture (40)	Flavor (50)	Appearance (10)
Plain (control)	37^{Aa}	41^{Ba}	9^{Aa}	35^{Ab}	39^{Bb}	7^{Bb}	30^{Bc}	35^{Bc}	7^{Bb}	nd	nd	nd	nd	nd	nd
F10	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	36^{Aa}	44^{Aa}	9^{Aa}
F50	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	36^{Aa}	44^{Aa}	9^{Aa}
F100	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	37^{Aa}	45^{Aa}	9^{Aa}	36^{Aa}	44^{Aa}	9^{Aa}

F10: Flavored with 10 ppm GO, F50: Flavored with 50 ppm GO, F100: Flavored with 100 ppm GO, means in the same column showing the same capital letters are not significantly different ($p \leq 0.05$), means in the same row showing the same small letters are not significantly different ($p \leq 0.05$), nd: Not determined because the samples showed symptoms of contamination at day 21 (Fig. 2)

From Table 3 it can be seen that there were no detectable growth of mold or yeast in the final product during 45 days of storage at 4°C for all concentrations of GO. On the other hand, the plain SY samples (control) became moldy after 21 days (Table 3, Fig. 3). Data from Fig. 2 indicated that the total number of bacteria did not increase over 45 days of storage compared with that at the zero time. The highest total viable bacterial counts observed after 7 days of storage then increased gradually in all treatments up to 15 days of storage and slightly decreased at the end of storage probably due to the effect of GO. In addition all samples of GO flavored SY were free from coliform bacteria along the storage period which may be attributed to the pasteurization of milk beside the potential antimicrobial activity of GO on coliforms.

Based on the above mentioned, a storage period of 45 days at 4°C may be recommended as shelf life of GO flavored SY, however, that cannot be ascertained without performing continuous sensory evaluation on the final product along that storage period.

Sensory evaluation of the final product (GO flavored strained yoghurt): Data given in Table 4 showed that GO flavored SY was tastier and highly preferred compared with the plain SY (control) even after 38 days of storage at 4°C. There was no significant difference in flavor assessment and preference between GO flavored SY that contain 10, 50 or 100 ppm GO in the final product. This result indicated that less GO (10 ppm) in SY is required to get the same flavor preference as high concentrations (100 ppm) which could be economic. The GO flavored SY samples subjected to sensory evaluation at day 45 tasted yeasty and unacceptable although there was no observed mold or yeast growth detected on their surface.

Based on the above mentioned criteria that were revealed in the results section including counts of molds and yeasts, total viable bacteria and coliform counts, visual inspection and sensory properties, 38 days was recommended as a shelf life for GO flavored SY stored at 4°C.

Comparing our results with other shelf life studies on SY revealed that a storage period between 7-10 days at 4°C has been recommended as shelf life for SY made from cow milk using cloth straining bags[35]. In another investigation, the nominal shelf-life of SY using sensory changes and microbiological counts as failure criteria has ranged between 18.5-18.9 days for samples[36] stored at 5°C. Interestingly, an investigation on SY made from goat's milk reported that a storage period up to 45 days at 4°C did not change the flavor attribute of the product[31]. This result was challenging and gave the reader an impression that a preservative was added,

which was actually not. Therefore our potential justification of the long shelf life of that SY with no added preservative is probably due to the formation of antimicrobial peptides that form upon fermentation of goat's milk with the starter culture[37].

CONCLUSION

The current investigation demonstrated the potentials of manufacturing SY with enhanced flavor and shelf life stability by using GO as a plant-based flavoring and preserving agent. In addition the product was made from vegetable oil that replaced milk fat which could be satisfying for consumers seeking for low cholesterol fermented dairy products.

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