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Research Article
Effect of Incorporation of Marjoram Honey on the Sensory, Rheological and Microbiological Properties of Goat Yogurt

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Abstract

Background and Objective: Health benefits of honey have long been realized by humans to treat a variety of ailments. Besides its sugar composition, honey consists of a number of bioactive compounds. So, this study aimed to analyze the effect of adding bee honey (one of the most widespread of medical plant honeys, Marjoram bee honey) on the rheological, microbiological and organoleptic properties of goat yogurt which containing Probiotic *Lactobacillus casei* EMCC761 during 14 days of refrigerated storage. **Materials and Methods:** Fifteen formulations of goat yogurt were prepared, each varying in the added marjoram bee honey in amount [(0, 5, 10, 15 and 20 % (v/v)], all inoculated with *L. casei* EMCC761 (0.2 g L^{-1} of goat milk). **Results:** The incorporation of marjoram honey positively affected several characteristics in goat yogurt containing *L. casei* EMCC761, like the texture, viscosity and microbial quality. All yogurt formulations presented counts of *L. casei* EMCC761 above 6.0 log CFU g^{-1} by 14 days of storage but the presence of bee honey increased the counts (1 log CFU g^{-1}) of *L. casei* EMCC761 and yogurt starter bacteria during14 days of storage. **Conclusion:** The results of this study presented a successful incorporation of both the Probiotic *L. casei* EMCC761 and the marjoram bee honey as ingredients of a new goat dairy product with satisfactory nutritional, sensory and microbial quality.

Key words: Marjoram honey, *Lactobacillus casei*, goat yoghurt, microbial quality

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

INTRODUCTION

Honey is the sweet substance which produced by honeybee from the secretion parts of many plants[1]. Honey is produced worldwide by over 500 bee species described in 32 genera[2] and naturally presents some amounts of anti-oxidants (including flavonoids, phenolics and carotenoids), organic acids, Maillard reaction products and amino acids in its composition[3] and specific sugar profile and acidity that bestow unique sensory characteristics[4].

Marjoram natural bee honey has been used as an effective medicine around the world, it's also has unique mild taste and aroma, while acting alone as relaxing remedy and it was successfully used for nervous tension removing has relaxing effect, relieves headaches, dizziness, helps to treat stomach and intestinal ulcers, Improves digestion and cleanses the body from the slag. Health benefits of honey have been investigated from a variety of conditions including microbial infections, glucose tolerance, inflammation, wound healing and analgesia[5,6].

Goat milk is as versatile as cow milk in just about every area. But the problem that was faced us is a goaty flavour of yogurt which not preferred by consumer[7]. Sometimes described as "waxy", "tangy" or "animalic", goat yogurt almost always has a prominent flavour that is formally described as "goaty"[8]. This flavour is noticeable, due to the fatty acid composition of goat milk a few important fatty acids that make up goaty flavour include: 4-ethyl octanoic acid, 4-methyl octanoic acid, caproic acid and caprylic acid .

The addition of flavors and aromas using essences, fruit and honey may be a better option than artificial flavorings for use in goat dairy products because of increasing nutritional values, in addition to become less evident the presence of the goat aroma and aftertaste which associated with a decreased acceptance by consumers.

In recent years, some studies with yogurt made from goat milk (as much as bovine yogurt) have focused on adding artificial sweeteners, fruit juices and pulps[9,10]. But according to author's knowledge, no study is available about the incorporation of marjoram honey and Probiotic goaty yogurt.

So, this work aimed to estimate the effects of incorporation of marjoram bee honey on the rheological, microbiological and organoleptic (sensory) properties of goat yogurt containing *Lactobacillus casei* EMCC761 during refrigerated storage.

MATERIALS AND METHODS

Preparing materials: The present study was conducted during May and June months of 2018 from the apiary at Giza governorate which planting the marjoram crop from February to April months. Fifteen honey bee colonies which equal in strength and exposed to the routine work during the experimental period were used for this study to produce the marjoram bee honey for this work.

Physiochemical properties of marjoram bee honey samples: Determination of water content of honey was carried out by measurements of its refractive index value (ABBE WAY-IS) refractometer[11] at 20°C. The quantity of sugars (glucose and fructose) were performed by HPLC according to the method of Bogdanov *et al.*[12]. The electrical conductivity was determined by the method of Vorwhol[13], using EC meter model EN50081-1 at room temperature (2 g of honey sample was dissolved in 10 mL of distilled water and the results were expressed as ppm). The optical density and color of the honey samples were measured by using the relation between optical density and USDA standard as indicated by White[14].

The honey sample was classified according to their botanical source based on the method of Von Der Ohe *et al.*[15]. Pollen classification as: mostly pollen (more than 45%), important minor pollen (10-20%) and minority pollen (less than 3%) by Andrade *et al.*[16] physiochemical analysis was conducted in "Elements laboratory, Campus of research laboratories, FARP", Faculty of Agriculture, Cairo University Research Park.

The goat milk was obtained from the experiment station and the Agricultural Research, Faculty of Agriculture, Cairo University. Then the goat milk was heated and maintained under refrigeration (4±1°C) until the experiment (maximum period 6 h). The samples of some marjoram Egyptian honeys were collected directly from different apiaries of different Egyptian governorate and the weight of each sample was approximately 500 g, stored at room temperature overnight before preparing the yogurts.

The starter culture (YC-X11) contain *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and the probiotic culture of *Lactobacillus casei* EMCC761 was obtained from Cairo Mircen Microbiological Resources Center, Agriculture Faculty, Ain-Shams University, Cairo.

Characterization of raw materials: The raw goat milk used in preparing the yogurts was analyzed for total solids, fat, protein, fixed mineral residue, acidity and pH, while the samples of honey was analyzed for pH, total acidity, glucose, fructose content. All physiochemical measurements in goat milk was determined using standard procedures[17].

For the marjoram honey sample, the concentration of glucose and fructose were determined by High Performance

Liquid Chromatography (HPLC). Analytical stainless-steel column 4, 6 mm in diameter, 250 mm length, containing amine-modified silica gel with 5-7 µm particle size, according to the method of Bogdanov *et al.*[12].

Preparation of yogurts: Goat milk was pasteurized (90°C/10 min), then milk was cooled to 42°C which is the optimum temperature for culture inoculated at a concentration of 3% for the starter culture consisting of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and a concentration of 1% for the probiotic culture of *Lactobacillus casei* EMCC761 defined according to previous testing to guarantee a minimal final count of approximately 7 log CFU mL^{-1}.

Fifteen different goat yogurt formulations were prepared using different concentration of honey (0, 5, 10, 15 and 20%) in a volume of 225 mL for each sample. The fermentation process was performed at 42°C for 4 h and the end point of yogurt fermentation was based on verification of clot firmness and pH value, which should reach a maximum of 4.6. Subsequently, the product was cooled to 4±1°C and the clot was broken by manual stirring with a glass rod. Then, the honey samples were added to the different formulations (w/v). All yogurt formulations were prepared in triplicate. The yogurts were subjected to physicochemical and microbiological analyses at different time points during 14 days of refrigerated storage. The physiochemical analysis of yogurt was performed depending on standard procedures[17].

Microbiological analysis of yogurts: The raw goat milk was assessed for microbiological quality parameters like total count (T.C), coliform and mold and yeasts. All of these analyses were performed according to standard procedures. The analysis as curtained total count $<10^3$ CFU mL^{-1} and coliform counts <10 CFU mL^{-1} and mold and yeast counts <1 CFU mL^{-1}, confirming the goat milk as being suitable for human consumption and for use as raw material for preparing yogurts.

For the lactic acid bacteria counts at each pre-established time, DE MAN, ROGOSA, SHARPE (MRS) broth medium were used. The MRS agar plus 0.5 g L^{-1} cysteine were used for counting *L. casei* EMCC761 N and the starter bacteria group (*S. thermophilus* and *L. bulgaricus*), respectively by MRS agar. The counts were expressed as the log of the colony forming units per mL of yogurt (log CFU mL^{-1}).

Organoleptic properties: The sensory analysis was established for different yogurt formulations on the 14th day of refrigerated storage. The yogurts were subjected to tests of acceptance and relative preference. The panelists were recruited from master's degree students, laboratory technicians non-trained panelists from Department of Dairy Science, Faculty of Agriculture, Cairo University, Giza, Egypt.

All sensory evaluation assays were performed with the same panelists who worked in individual with controlled temperature and lighting conditions. Each panelist was served 50 mL of each yogurt formulation on a small white glass at 6°C coded with a random number immediately after being taken out of the refrigerated storage. The acceptability of appearance, colour, flavor, taste, texture and overall acceptance were evaluated on a nine-point and the scale ranging from 9 as like very much to 1 as dislike very much[18].

Rheological parameters measurements: Rheological measurements of milk inoculated with bacteria (viscosity, shear stress and torque) were carried out in triplicates over temperature of 25°C using a concentric cylinder Brookfield Programmable viscometer (Model DV-II; Brookfield Engineering Laboratories, USA) with UL adaptor and ULA spindle over a shear rate of 6.1 sec^{-1}. WinGather version 1.1 (Brookfield Engineering Laboratories, Inc.) software was used to collect, store and plot the data on a personal computer connected to the viscometer.

Statistical analysis: The two-way statistical analysis of variance (ANOVA), mean separation and correlation required subprogram of MSTAT microcomputer statistical program[19] was applied to evaluate physiochemical and microbiological parameters of goat yogurt fortified by some Egyptian marjoram honey samples. Simple and multiple linear regression analysis were applied and the student t-test was used to test mean at $p<0.05$.

RESULTS AND DISCUSSION

Physiochemical parameters of marjoram honey: The physiochemical properties of marjoram (*Majorana hortensis*) bee honey samples were determined. As shown in Table 1

Table 1: Physiochemical parameters of the marjoram honey samples

Physiochemical parameters	Mean±SE
Moisture (%)	18.10±0.17
Electrical conductivity (%)	0.01±0.00
pH	3.84±0.11
Glucose (%)	28.00±0.00
Fructose (%)	37.50±0.00
Optical density	0.25±0.00

Table 2: Total solids (T.s) and protein contents in the yogurts made from goat milk with marjoram

Parameters	Days of storage	Yogurt formulation (Yog. - 0%)	(Yog. - 5%)	(Yog. - 10%)	(Yog. - 15%)	(Yog. - 20%)
Total solids (g/100 g)	0	13.97±0.01	15.95±0.02	17.97±0.01	19.83±0.01	22.45±0.01
	7	14.00±0.05	16.34±0.06	18.58±0.01	20.27±0.02	22.71±0.05
	14	14.10±0.07	16.98±0.01	19.34±0.04	21.16±0.01	22.87±0.02
Protein (g/100 g)	0	3.86±0.06	3.77±0.01	3.79±0.03	3.80±0.01	3.69±0.04
	7	3.86±0.05	3.74±0.03	3.75±0.05	3.69±0.01	3.67±0.01
	14	3.86±0.03	3.74±0.01	3.79±0.02	3.70±0.01	3.64±0.03

there were clear significant differences in all tested parameters. The water content (moisture %) was 18.10% in marjoram honey where the Electrical Conductivity (EC) was 0.011%, also the pH value was 3.84 in marjoram honey. The obtained data showed that, the glucose content was 28.00% (marjoram honey) and the fructose content was recorded 37.50 in marjoram. The Optical Density (O.D.) of honey samples were measured in Table 1, the O.D. was 0.25 OD in tested samples of marjoram bee honey. The moisture values which obtained in this study were similar to those found in South Asia honey[20]: from 15.3-21.7 g/100 g and in North African honey (from 14.6-21.8 g/100 g)[21,22,1].

The E.C. values were in the same range as those reported by other authors in Burkina Faso honey[23-25]. The sugar content (glucose and fructose sugars) of bee honey samples are similar to those found with Escriche *et al.*[26] which detected the levels of glucose (from 27.8-31.9 g/100 g) and fructose (38.3 and 42.7 g/100 g). While pH value was agree with that obtained by Rateb[27].

Physicochemical analyses of yogurts: The mean values of the physicochemical parameters (Total solid, protein content %) of goat yogurts formulations were presented in Table 2. According to obtained data the yogurt formulations containing 20% added marjoram bee honey presented higher total solids content, due to the higher amount of honey contained. The same effect was observed during storage period 14 days. The protein content in yogurt decrease with increase the marjoram honey, due to the high amount of honey which contain low protein concentration (0.3 g/100 g honey).

During the storage period, pH of yogurt gradually decreased in the treatments may be due to post acidification. pH of yogurt can be decreased due to accumulation of lactic acid which had been produced by yogurt starter and other living micro-organisms during storage also noted that low pH, in yogurt formulations containing added marjoram honey compared to control (0% honey).

The acidity of bee honey comes from the naturally occurring organic acids in its composition[20]. The initial pH of the assessed yogurt formulations continuously decreased until the 14th day of storage and the yogurt samples containing added marjoram bee honey showed lesser pH that was proportional to the honey amount add during preparation as shown in Fig. 1a. At the same time, the acidity also increased during storage period in all yogurt tested formulations (Fig. 1b).

It was confirmed that, these changes in pH and acidity values observed in yogurt formulations containing added marjoram bee honey could be related to the presence of extra fermentable compounds in bee honey namely sugars also addition of bee honey seemed to stimulate the lactic acid metabolism and consequently the acidification of yogurt formulations. This increased in the acidity values in yogurts containing marjoram bee honey may be associated with the presence of prebiotic oligosaccharides in bee honey which may promote the growth and the metabolic activity of lactic acid bacteria.

Organoleptic analyses of yogurts: Sensory analysis is often used to assess the flavor, appearance, texture and other attributes of food products as a function of processing parameters[28]. The results given in Table 3 described the effect of mixing marjoram honey with goat's yogurt on the sensory evaluation. Yogurt with no addition of honey had the highest scoring points of color and appearance whether in fresh or after 7 and 14 days of the cold storage as shown in Table 3.

In contrast to the color and appearance evaluation, body and texture attributes of goat yogurt fortified by honey had negatively affected on body and texture. Panelists recorded the lowest scores of body and texture for sample 4 (15 %) and sample 5 (20%).

The greatest effect of supplementation of goat's yogurt with marjoram honey on the sensory characteristics was observed in flavor. In Egypt, the majority of consumers don't prefer the goaty flavor. The panelists showed that adding

Table 3: Effect of adding marjoram honey to goat's yogurt on sensory evaluation at room temperature

Goat yogurt	Storage period under cooling (14 days)	Colour and appearance (15)	Body and texture (35)	Flavor (50)	Total (100)
Yog 0%	0	13.33^{AB}	32.00^{A}	38.33^{I}	83.66^{A}
	7	13.33^{AB}	31.00^{AB}	38.66^{I}	83.00^{AB}
	14	13.67^{A}	30.00^{BC}	39.00^{HI}	82.67^{AB}
Yog 5%	0	12.33^{B}	30.00^{BC}	41.00^{GH}	83.33^{AB}
	7	12.67^{AB}	30.00^{BC}	43.00^{FG}	85.67^{A}
	14	13.00^{AB}	29.00^{CDE}	43.67^{EF}	73.33^{B}
Yog 10%	0	12.67^{AB}	29.33^{BCD}	44.67^{DEF}	86.67^{A}
	7	13.00^{AB}	28.67^{CDE}	46.33^{BCD}	88.00^{A}
	14	13.33^{AB}	28.00^{DEF}	45.67^{CDE}	87.00^{A}
Yog 15%	0	13.33^{AB}	28.67^{CDE}	46.67^{BCD}	88.67^{A}
	7	13.00^{AB}	28.33^{CDE}	47.67^{ABC}	89.00^{A}
	14	13.00^{AB}	27.33^{EF}	47.67^{ABC}	88.67^{A}
Yog 20%	0	12.67^{AB}	28.33^{CDE}	49.00^{A}	90.00^{A}
	7	13.00^{AB}	26.33^{FG}	48.00^{AB}	87.33^{A}
	14	13.00^{AB}	25.00^{G}	48.3^{AB}	86.33^{A}
Co. Var.%		5.61%	3.84%	3.14%	7.13%
LSD value		1.218	1.843	2.332	10.17

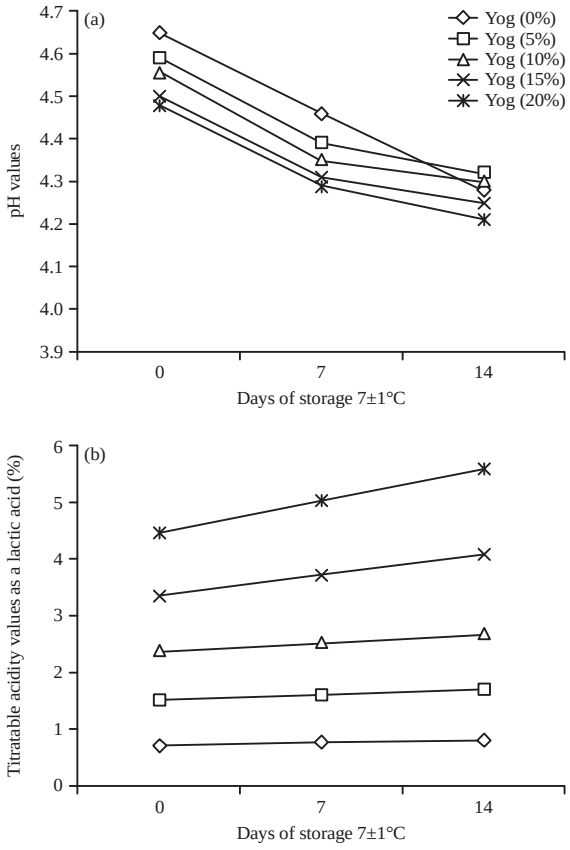

Fig. 1(a-b): Mean values of (a) pH and (b) Acidity of goat yogurt containing *L. casei* EMCC671 and marjoram honey at different concentrations, during 14 days of refrigerated storage

honey to goat's yogurt mask the goaty flavor with good sweetened one which increased the overall acceptability. The sensory evaluation showed that lower sensory acceptability was recorded for goat's yogurt stored for two weeks than the fresh products.

Moreover the added marjoram honey may had rendered the goat yogurt containing *L. casei* EMCC 671 more acceptable and attractive due to the presence of poly saccharides also the highest acidity observed in goat yogurts containing added marjoram honey did not affect negatively on the sensory acceptance due to honey sweetness that in combination with the higher acidity may provide an enjoyable flavour to yogurts this was the same result obtained by Baraka *et al.*[29].

Microbiological count analyses: The results of the viable counts of the starter bacteria group (composed of *S. thermophilus* and *L. bulgaricus*) YC-X11 and *L. casei* EMCC671 in goat yogurt containing or not containing marjoram bee honey during refrigerated storage were presented in Fig. 2 the counts of yogurt starter bacteria group YC-X11 at the first day of storage were approximately 8.8 log CFU mL^{-1} and decreased to approximately 7.1 log CFU mL^{-1} and 6.2 log CFU mL^{-1} on the 7th and 14th days of storage. The starter bacteria group presented a linear decrease in counts during the period in all yogurt formulations; however, these counts were higher in yogurts containing marjoram honey in 7th-14th day of storage.

The decreases in starter bacteria counts had been an expected behaviour during yogurt storage which was due to high acidity produced by microbial fermentation. Also a similar behavior of sharp reduction in counts of *L. casei* EMCC671 occurred from 7th-14th day of storage in all yogurt formulations as shown in Fig. 2b.

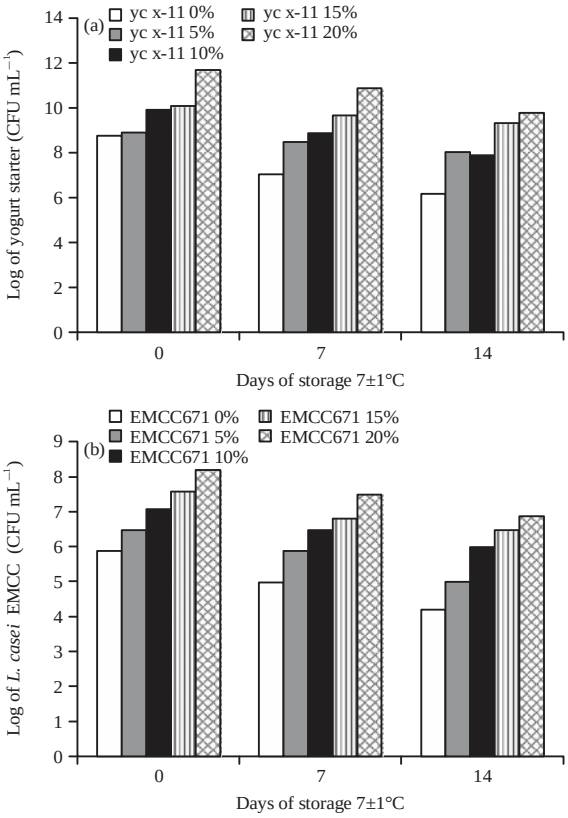

Fig. 2(a-b): Viable cell counts of bacteria starter group (a) Yc-X11 and (b) *L. casei* EMCC671 in goat yogurt containing or not marjoram honey at different concentrations during storage under cooling

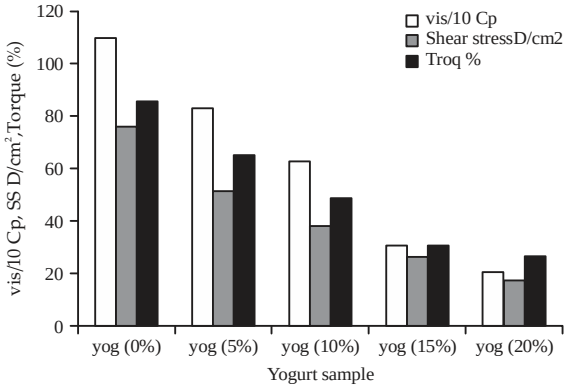

Fig. 3: Average viscosity/10 (cP), shear stress (D/cm^2) and torque (%) of yogurt sample (yog 0% control, yog 5%, yog 10%, yog 15%, yog 20%), respectively

Rheological characteristic: Bee honey is considered a high-viscosity fluid, also a pseudo-plastic offering greater resistance and higher viscosity for yogurts. Furthermore, the addition of marjoram bee honey increase total solids content and increase the consistency of the product but adding the honey led to decrease in the yogurt viscosity that was directly proportional to the amount of honey add. A corroding to Shaker *et al.*[30] who reported that yogurt is an emulsion of fat and protein suspended in water. Honey is a very concentrated solution of sugars. If you put honey and yogurt in contact, you create osmotic pressure because the number of solutes in the honey is much higher than the number of particles suspended in the yogurt. Osmosis causes the water to flow towards the greater concentration of solutes - the honey. The reason it gets kind of liquid in between the bee honey and the yogurt is because the honey is slow to dissolve.

Table 4: Mean, LSD value and coefficient of variation (Co. Var.) of rheological parameters of goat yogurt with marjoram honey

Concentrations of marjoram bee honey	Rheological parameters		
	Viscosity (Cp)	Shear stress (D cm^{-2})	Torque (%)
yog 0%	1098^{A}	67.37^{A}	85.47^{A}
yog 5%	835.0^{B}	51.83^{B}	65.37^{B}
yog 10%	628.0^{C}	38.46^{C}	49.10^{C}
yog 15%	309.0^{D}	26.67^{D}	31.73^{D}
yog 20%	206.0^{E}	17.67^{E}	26.73^{E}
Co. Var.%	0.24%	0.92%	0.56%
LSD value	2.597	0.6529	0.5075

At complete clotting pH 4.6±2, sample fortified by 20% marjoram honey showed the minimum values for viscosity (20.667 cP), shear stress (17.76 D cm^{-2}), torque (26.73 %) while the control sample without marjoram bee honey (yog 0%) had higher viscosity (1097.66 cP), shear stress (67.36 D cm^{-2}) and torque (85.60%) than both yog 5,10 and 15%, respectively as shown in Fig. 3.

Table 4 showed that, addition of marjoram bee honey in 20% v/v in goat yogurt recorded the lowest viscosity shear stress and torque value. The rheological parameters values in control sample was higher than 5 times as much as the value s recorded after marjoram honey addition in high concentration. The significant influence statistically found was for the concentration of bee honey add.

CONCLUSION

The addition of marjoram honey in goat yogurt containing the Probiotic *L. casei* EMCC671 has a positive effect on some of physical and rheological characteristics of the product during the 14 days of refrigerated storage. The *L. casei* EMCC 671 counts in all goat yogurt formulations remained in adequate amount during storage (>6 log CFU mL^{-1}) which had several health benefits to the consumer.

However, the formulation containing marjoram honey presented the highest counts of *L. casei* EMcc671 and yogurt starter YC-X11, indicating a growth promoting effect for starter bacteria. Moreover, the addition of marjoram honey affect directly on the acid profile of the goat yogurt during cooled storage, without negatively affecting its acceptance and its organolyptic properties. Otherwise, the yogurts containing marjoram honey presented the best sensory acceptance according to their score card. Finally, the results of this study presented a successful incorporation of both the Probiotic *L. casei* EMCC671 and the marjoram as ingredients of a new goat dairy product with satisfactory nutritional and sensory quality due to masking of goaty flavor, as well as added market value.

SIGNIFICANCE STATEMENT

This study confirmed that the addition of marjoram bee honey to the goat yogurt has led to the prolongation of the period of conservation also led to an increase in the survival of the yogurt and the increase of the period of vitality for *Lactobacillus casei*.

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