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Research Article

Development of Functional Fermented Oat Milk by Using Probiotic Strains and Whey Protein

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Abstract

Background and Objective: In recent years, consumption of fermented vegetarian milk as a functional food was increased due to its beneficial health and there were many trials to improve the quality properties of fermented vegetarian milk by different modifications. So, the aim of this research was to enhance the functional attributes of fermented oat milk by using probiotic cultures and study the effect of fortifying oat fermented milk with whey protein on the quality of final product. **Methodology:** Three experimental fermented milk samples (labeled as FCM, FOM and FOM+DWPC) were made from cow milk, oat milk and oat milk fortified with 2% dried whey protein concentrate, respectively. ABT-3 starter culture containing *Streptococcus salivarius* sub sp. *thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was used for fermentation. Samples were taken when fresh and during cold storage for analyses. **Results:** Significant differences were observed in dry matter, fat, total nitrogen, ash, crude fibers, titratable acidity, diacetyl and acetaldehyde contents between fermented cow and oat milk. Fortification the FOM with whey protein caused a significantly increase in the dry mater, total nitrogen, titratable acidity, diacetyl and acetaldehyde contents of the final products. Dynamic viscosity of fermented oat milk had significant higher than that in fermented cow milk. The viability of ABT culture strains in all samples with or without whey protein was higher during the cold storage than the recommended minimum levels (10^6 CFU mL⁻¹ or g). Fortification fermented oat milk with whey protein had enhanced the viability of ABT culture strains in final product along the cold storage period. All the fermented cow and oat milk samples gave a good total impression. **Conclusion:** The results of this study indicate that oat milk could be used as good raw material for producing new functional fermented product with high acceptability and a high viability of bio-starter culture along the cold storage. Fortification oat milk with whey protein could be enhancing nutritional values and viability of probiotic strains in the functional fermented oat milk.

Key words: Fermented oat milk, probiotics, whey protein, functional properties, quality characteristics, fortification

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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

Functional foods are defined as the food or dietary components that may provide a health benefit beyond basic nutrition. Food can be functional through the application of any technological or biological means to increase the concentration, addition, removal or modification of a particular element as well as to improve its biological availability, provided that the ingredient has a functional impact¹. Arpita *et al.*² confirmed that, because of continuous health awareness and easily available information about the benefits of different diets and their direct link with health, the demand for functional food is increasing day by day. Functional foods include food or food ingredients that have a beneficial effect on host health and/or reduce the risk of chronic diseases beyond basic nutritional functions. Increased awareness of consumer health and attention to functional foods to achieve healthy lifestyles has led to the need for food products with multifaceted health benefits.

In recent years, cereals and their components have been accepted as a functional food and nutraceuticals due to its provision of dietary fiber, protein, energy, minerals, antioxidants and vitamins required for human health. Also, cereals can be used as fermentable substances for the growth of probiotic micro-organisms³. Oat (*Avena sativa* L.) is a well known annual crop in temperate climates. There is a lot of research ensures that oat is rich in biological substances such as soluble dietary fiber, β -glucan, vitamin E and polyunsaturated fatty acids and their consumption in the human diet is beneficial to human well-being. Many researchers have recognized the beneficial effects of consuming oat and oat based food products.

There has been increasing interest during the past decade to add intestinal *Lactobacillus* spp. and *Bifidobacterium* spp. to fermented food products⁴. Food products containing probiotic bacteria, e.g. "probiotic foods", recently defined as "foods containing live and specific bacteria, which when given in sufficient numbers, exert beneficial effects by altering the micro-flora in the host"⁵.

On the other hand, an increasing number of consumers choose vegetarian milk (plant-based milk substitutes) for medical reasons or lifestyle choices. Vegetarian milk is often seen as healthy, possibly because of negative perceptions of the nutritional properties of cow's milk. Also, vegetarian milk can be fermented to produce fermented dairy-free products and making raw materials in a more acceptable form. In order to prepare functional fermented products, the starter culture strains must be able to grow and dominate the micro-flora in the final product and produce the desired properties such as

texture and flavor. Generally, lactic acid bacteria such as lactobacilli, streptococci etc., have been used for various cereal fermentations for centuries and many kinds of cereal known to support their growth⁶.

Addition of whey proteins in food formulations is motivated mainly by their health benefits. Moreover, whey proteins may enhance technological characteristics in food products, since they can increase the protein content and improve the viscosity values in fortified product. Therefore, the fortification with whey proteins may modify the characteristics of the fermented vegetarian product such as its nutritional values, acceptance and the survival of the probiotic micro-organisms. Using some probiotic strains such as *L. acidophilus* and *Bifidobacterium* spp., for fermenting the vegetarian milk, also fortification the vegetarian milk with a source of protein may be enhance the functional properties of final product compared to traditional fermented vegetarian milk made with yoghurt culture without any fortification. Therefore, the aim of the present research was to improve the nutritional values and functional properties of fermented oat milk by using probiotic cultures and fortification with whey protein.

MATERIALS AND METHODS

Materials

Ingredients: Fresh cow's milk was purchased from the herd of the dairy cattle at Faculty of Agriculture, Cairo University, Cairo, Egypt. Skim milk powder (97% DM) produced in Poland was purchased from the local market of Cairo. Dried whey protein concentrate (DWPC) was purchased from Mullins Whey Company, USA origin. Oat flakes purchased from local market. Food grade α -amylase from *Bacillus subtilis* was purchased from Sigma Aldrich which had an activity of 2000IU in a powder form.

Some chemical composition (%) of fresh cow milk, oat milk and dried whey protein concentrate (DWPC) used in manufacture of different fermented samples are presented in Table 1.

Bacterial starter cultures: The bacterial starter culture used in this study, commercially named ABT-3 DIP 50 μ consists of a *Streptococcus salivarius* sub sp. *thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in the form of freeze-dried culture obtained from Chr. Hansen's Laboratories, Denmark and prepared as the mother culture by adding 1% of lyophilized cell culture into 12% sterilized reconstituted skim milk powder and incubated at 39°C for 4-6 before 24 h.

This study was carried out in Food Science Department, Faculty of Agricultural, Ain Shams University, Cairo, Egypt during August and September, 2018. The fermented products were prepared in Dairy production Unit, Faculty of Agricultural, Ain Shams University, Cairo, Egypt.

Experiment of procedures

Preparation of oat milk sample: Oat milk was prepared according to enzymatic method described by Deswal *et al.*⁷. About 1 kg of rolled oats was ground into a laboratory food processor to produce finely granulated oat flour and then mixed with 2.7 kg of water. Calcium chloride at a concentration of 0.04% (w/w) was added as a catalyst for the enzyme. Oat slurry was treated with α -amylase (77.78 mg kg⁻¹ of Rolled oats) for liquefaction for 49 min at 75°C. The liquefied oat solids were then filtered through muslin cloth to get the oat milk. At the end of the treatment, the enzyme was inactivated by heating at 100°C for 5 min. The total yield was (78%) estimated as the percentage of filtrate obtained.

Production of fermented milk samples: Three experimental fermented milk samples (labeled as FCM, FOM and FOM+DWPC) were made from cow milk fortified with 2% skim milk powder, oat milk and oat milk fortified with 2% dried whey protein concentrate, respectively. All milk samples were heated at 90°C for 10 min and subsequently cooled to 40°C and inoculated with 3% ABT-3 starter culture (*S. salivarius* subsp. *thermophilus*, *L. acidophilus* and *B. bifidum*). All samples were aseptically transferred into 100 mL plastic containers. Inoculated cow milk samples were incubated at 39°C till coagulation (pH 4.7) then cooled to 4°C. However, different inoculated oat milk samples were incubated at 39°C for 16 h. Three replicates were done for each treatment. The resulting fermented samples were stored at 4°C for 21 days. Samples were taken when fresh and after 3, 7, 14 and 21 days of the cold storage period for analysis.

Analytical methods for different fermented milk: Dry matter, ash, total nitrogen, fat, crude fibers and titratable acidity as lactic acid (TA) of different fermented milk treatments were determined according to AOAC⁸. Acetaldehyde and diacetyl contents were determined according to Lee and Jago^{9,10} using the Conway micro diffusion-Semi carbazide method.

Dynamic viscosity was measured using a rotational coaxial viscometer (RHEOTEST II-Medingen, Germany) at shear rates ranging from 1.000-437.4 sec⁻¹ according to Toledo¹¹, at 20±1°C. Dynamic viscosity of different samples was calculated at share rate 145.8 sec⁻¹.

Table 1: Some chemical composition (%) of fresh cow milk, oat milk and dried whey protein concentrate (DWPC) used in manufacture of different fermented samples

Parameters	Cow milk	Oat milk	DWPC
Dry matter	12.14	21.51	95.23
Protein	3.42	2.20	87.21
Fat	3.10	1.88	0.10
Ash	0.721	0.342	2.47
Crude fibers	ND	1.92	ND
Acidity	0.16	0.09	0.25

ND: Not detected

Table 2: Some chemical attributes of different fresh fermented cow and oat milk samples

Component (%)	FCM	FOM	FOM+WP
Dry matter	13.95±0.14 ^C	21.63±0.95 ^B	23.42±0.80 ^A
Fat	3.00±0.10 ^A	1.80±0.01 ^B	1.70±0.01 ^B
Total nitrogen	0.59±0.02 ^A	0.45±0.01 ^B	0.58±0.01 ^A
Crude fibers	ND	1.90±0.03 ^A	1.80±0.09 ^A
Ash	0.87±0.08 ^A	0.34±0.02 ^B	0.39±0.02 ^B

FCM: Fermented cow's milk, FOM: Fermented oat milk, FOM+WP: Fermented oat milk fortified with 2% dried whey protein concentrate, ^{A,B,C} Means with same letter among treatments are not significantly different at p≤0.05. Data presented as Mean±SE (n = 3), ND: Not detected

Bifidobacteria spp. was enumerated according to Dave and Shah¹² using modified MRS agar supplemented with 0.05% L-cysteine and 0.3% lithium chloride. The plates were incubated in anaerobic conditions at 37°C for 48-72 h. The *L. acidophilus* count was determined using Bile MRS Agar according to Vinderola and Reinheimer¹³. The plates were incubated in aerobic conditions at 37°C for 72 h. The *S. thermophilus* count was determined using M17 agar medium¹⁴. The plates were incubated in aerobic conditions at 37°C for 48 h.

The sensory properties of the various samples of fermented milk types were evaluated through a regular tasting panel for members of the Department of Food Science, Faculty of Agriculture, Ain Shams University. Fermented samples were evaluated for appearance, consistency, odor, flavor and general evaluation. According to these criteria, evaluation forms graded between 1 and 5 were given to the panelists and were asked to fill for the evaluation¹⁵.

Statistical analysis: Statistical analysis was performed according to SAS¹⁶ using General Linear Model (GLM) with main effect of treatments. Duncan's multiple range was used to separate among means of three replicates at p≤0.05.

RESULTS AND DISCUSSION

Chemical composition of fresh, fermented cow and oat milk samples: Table 2 presented some chemical properties of different fresh fermented cow milk, oat milk and oat milk

Table 3: Acidity, diacetyl and acetaldehyde contents different fermented cow and oat milk samples along the storage at 4°C for 21 days

Treatments	Storage period (days)				
	Fresh	3	7	14	21
Acidity (%)					
FCM	0.83±0.01 ^{Ae}	0.87±0.02 ^{Ad}	0.91±0.02 ^{Ac}	1.03±0.08 ^{Ab}	1.21±0.11 ^{Aa}
FOM	0.30±0.01 ^{Cad}	0.35±0.01 ^{Ccd}	0.40±0.02 ^{Cc}	0.46±0.05 ^{Cb}	0.53±0.06 ^{Ca}
FOM+WP	0.41±0.04 ^{Bad}	0.49±0.01 ^{Bc}	0.51±0.05 ^{Bbc}	0.56±0.04 ^{Bb}	0.64±0.04 ^{Ba}
Acetaldehyde (µmL/100 g)					
FCM	258.35±7.30 ^{Aa}	247.23±7.2 ^{Ab}	223.16±4.70 ^{Ac}	157.78±3.50 ^{Ad}	131.22±1.20 ^{Ae}
FOM	201.81±7.50 ^{Ca}	189.14±6.5 ^{Cb}	169.03±1.10 ^{Cc}	106.42±2.90 ^{Cd}	89.71±1.50 ^{Ce}
FOM+WP	210.80±5.30 ^{Ba}	196.86±5.0 ^{Bb}	179.78±0.86 ^{Bc}	118.70±1.80 ^{Bd}	94.90±0.80 ^{Be}
Diacetyl (µmL/100 g)					
FCM	18.26±0.98 ^{Ab}	20.18±0.70 ^{Aa}	17.64±0.13 ^{Ab}	12.88±0.11 ^{Ac}	8.43±0.20 ^{Ad}
FOM	11.21±1.10 ^{Cb}	12.78±0.65 ^{Ca}	11.09±0.25 ^{Cb}	8.65±0.08 ^{Bc}	5.89±0.20 ^{Bd}
FOM+WP	13.70±0.54 ^{Bb}	14.40±0.93 ^{Ba}	13.65±0.78 ^{Bb}	9.14±0.80 ^{Bc}	6.10±0.12 ^{Bd}

FCM: Fermented cow's milk, FOM: Fermented oat milk, FOM+WP: Fermented oat milk fortified with 2% dried whey protein concentrate, Means with same capital letter among treatments in the same storage period are not significantly differed, Means with same small letter for same treatment during storage periods are not significantly differed. Data presented as Mean ± SE (n = 3)

fortified with DWPC. Fat, total nitrogen, ash contents were significantly higher in FCM compared with fermented oat milk. Crude fiber were not detected in FCM while, it was 1.9 and 1.8% in FOM and FOM+WP. It is clear that, significant differences were observed in dry matter, fat, total nitrogen, ash and crude fibers contents between fermented cow and oat milk. These could be due to the differences of compositional properties between cow and oat milk which used for production of the fermented products (Table 1). These outcomes are similar to that reported by Singhal *et al.*¹⁷, who found that significant differences were observed in chemical composition between cow milk and other non-dairy beverages such as almond, cashew, coconut, hazelnut, hemp, oat, rice and soy and cow's milk was higher protein content compared to oat beverage and most of these products.

As it is cleared in Table 2, fortification of FOM with DWPC caused a significantly increase in the dry matter and total nitrogen contents of the final products. These results might be due to the higher protein content in DWPC (87.21%) compared with oat milk.

Acidity, diacetyl and acetaldehyde contents different fermented samples:

Data in Table 3 show that, titratable acidity was significantly lower in both fermented oat milk samples with or without 2% DWPC than other fermented sample made from cow milk. The differences were remarkable along of the cold storage period. The lower acidity of fermented oat-based milk samples compared to fermented cow milk product might be due to their lower buffering capacity¹⁸. The results agree with Bernat *et al.*¹⁹, who reported that titratable acidity values of fermented oat milk were lower than standard yoghurt. Also, Dinkci *et al.*²⁰ found that, the kefir sample with the highest amount of oat milk had the

lowest lactic acid concentration, while the control sample containing cow milk solely had the highest amount. Generally, a gradual increase in the acidity was recorded for all fermented samples all over the cold storage period. This may be due to the activity of fermented milk cultures²¹.

From data in Table 3, it can be observed that there were significant differences in diacetyl and acetaldehyde contents along all fermented treatment. Moreover, diacetyl and acetaldehyde contents were significantly ($p \leq 0.05$) higher in fermented cow milk samples than samples made from oat milk. This is may be due to the high content of citrate in cow milk compared with oat milk. Citrate is present in many of the substrates which are used for food fermentation such as milk. It can be fermented by limited number of lactic acid bacteria. Its degradation usually results in the formation of unusual fermentation products such as diacetyl, acetone, butanediol and acetaldehyde²².

The content of acetaldehyde gradually decreased in all fermented samples as the storage period progressed. While the diacetyl content increased until the 3rd day of storage followed by a gradual decrease until the end of the cold storage period (21 days). The reduction in acetaldehyde content during the storage period may be due to the ability of some bacterial strains of lactic acid to reduce acetaldehyde to ethanol or oxidize it to acetic acid²¹. On the other hand, the decrease in diacetyl mostly is due to slow reduction of diacetyl to acetone as reported by Diressen and Puhan²³ and Roushdy *et al.*²⁴. Generally, adding 2% DWPC to oat milk during fermentation caused a significant increase in the titratable acidity, diacetyl and acetaldehyde contents in the final product. This is may be due to the positive effect of addition DWPC on the growth and/or activity of ABT starter cultures²⁵⁻²⁷.

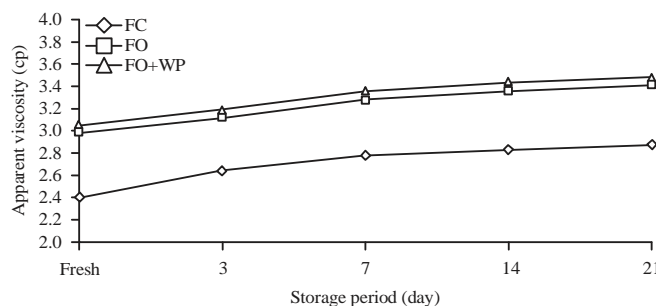


Fig. 1: Dynamic viscosity (cp) of different fermented cow and oat milk samples along the storage at 4°C for 21 days

Table 4: Survival of bacterial starter culture (log CFU mL⁻¹) in different fermented cow and oat milk samples along the storage at 4°C for 21 days

Treatments	Storage period (days)				
	Fresh	3	7	14	21
<i>S. thermophilus</i>					
FCM	7.81 ± 0.15 ^{Ba}	7.65 ± 0.10 ^{Bab}	7.21 ± 0.09 ^{Bb}	6.71 ± 0.08 ^{Bc}	6.40 ± 0.10 ^{Bd}
FOM	8.06 ± 0.20 ^{Aa}	7.92 ± 0.21 ^{Aa}	7.58 ± 0.22 ^{Ab}	7.18 ± 0.10 ^{Ac}	6.94 ± 0.06 ^{Ad}
FOM+DWPC	8.13 ± 0.20 ^{Aa}	7.98 ± 0.19 ^{Aa}	7.61 ± 0.24 ^{Ab}	7.21 ± 0.16 ^{Ac}	7.02 ± 0.18 ^{Ad}
<i>L. acidophilus</i>					
FCM	7.32 ± 0.08 ^{Aa}	7.26 ± 0.20 ^{Aab}	7.01 ± 0.18 ^{Ab}	6.76 ± 0.08 ^{Ac}	6.25 ± 0.03 ^{Ad}
FOM	7.01 ± 0.04 ^{Ca}	6.87 ± 0.21 ^{Cb}	6.58 ± 0.09 ^{Cc}	6.24 ± 0.10 ^{Cd}	6.08 ± 0.04 ^{De}
FOM+DWPC	7.13 ± 0.10 ^{Ba}	7.04 ± 0.15 ^{Ba}	6.87 ± 0.20 ^{Bb}	6.54 ± 0.16 ^{Bc}	6.11 ± 0.02 ^{Bd}
<i>Bifidobacteria spp.</i>					
FCM	7.00 ± 0.10 ^{Ba}	6.85 ± 0.05 ^{Bb}	6.50 ± 0.10 ^{Bc}	6.23 ± 0.01 ^{Bd}	6.01 ± 0.05 ^{Be}
FOM	7.15 ± 0.10 ^{ABa}	7.02 ± 0.09 ^{Aa}	6.75 ± 0.13 ^{Ab}	6.42 ± 0.02 ^{Ac}	6.21 ± 0.09 ^{Ad}
FOM+WP	7.25 ± 0.15 ^{Aa}	7.10 ± 0.10 ^{Aa}	6.81 ± 0.15 ^{Ab}	6.51 ± 0.15 ^{Ac}	6.28 ± 0.10 ^{Ad}

FCM: Fermented cow's milk, FOM: Fermented oat milk, FOM+WP: Fermented oat milk fortified with 2% dried whey protein concentrate, Means with same capital letter among treatments in the same storage period are not significantly differed, Means with same small letter for same treatment during storage periods are not significantly differed. Data presented as Mean ± SE (n = 3)

Dynamic viscosity: Dynamic viscosity plays an important role in the definition of textural and sensorial perception of a new fermented product. Figure 1 illustrates the change in dynamic viscosity values of different functional fermented product during the storage at 4°C for 21 days. It could be observed that, the dynamic viscosity of FOM was significantly ($p < 0.05$) higher than FCM. The higher dynamic viscosity in FOM compared to FCM could be due to the higher dry matter and β -glucan contents in oat milk than cow milk. The effect of β -glucan on increase the viscosity might be due to the high ability of β -glucan as soluble fiber to make the molecule flexible and contribute to its high water binding, solubility and viscosity²⁸. These results agree with the results obtained by Bernat *et al.*¹⁹, who found that the oat milk's microstructure could be organized as a polysaccharide network where fat and protein are embedded. This arrangement is associated to the gelling properties of β -glucans, once it is heated. The results indicated that, fortification fermented oat milk with 2% of DWPC caused insignificant increase in dynamic viscosity compared with unfortified fermented oat milk.

In this case, the dynamic viscosity in all different fermented products slight increased even during storage,

this is possibly due to increasing hydration²⁹. These results were confirmed with data obtained by Donkor *et al.*³⁰ and El Batawy and Khalil³¹, who found that the viscosity values of various probiotic fermented products increased during storage at 5°C.

Survival of lactic acid bacteria: The results in Table 4 indicated that, the viability of ABT culture strains (*Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium* spp.) in fermented oat milk fortified with or without whey protein were higher during the storage at 4°C for 21 days than the recommended minimum levels (10^6 CFU mL⁻¹ or g). In general, the food industry has targeted populations over 10^6 probiotics/g at the time of consumption of strain added to food³². Hekmat and McMahon³³, FAO and WHO³⁴ and Salem *et al.*³⁵ reported that the standard for any food sold with health claims from the addition of probiotics that it must contain at least 10^6 - 10^7 CFU g⁻¹ or mL of viable probiotic bacteria. Therefore, it will be reported that oat milk can be used for produce functional fermented product containing a high count of probiotic strain along storage at 4°C for 21 days. This is could

Table 5: Sensory quality of different fermented cow and oat milk samples along the storage at 4 °C for 21 days

Treatments	Storage period (days)				
	Fresh	3	7	14	21
Appearance (5)					
FCM	4.45±0.04 ^{Aa}	4.50±0.09 ^{Aa}	4.54±0.04 ^{Aa}	4.00±0.04 ^{Ab}	3.82±0.01 ^{Ac}
FOM	4.50±0.05 ^{Aa}	4.50±0.08 ^{Aa}	4.52±0.8 ^{Aa}	4.05±0.02 ^{Ab}	3.81±0.01 ^{Ac}
FMO+DWPC	4.45±0.01 ^{Aa}	4.50±0.02 ^{Aa}	4.50±0.06 ^{Aa}	4.10±0.02 ^{Ab}	3.85±0.01 ^{Ac}
Consistency (5)					
FCM	4.00±0.01 ^{Bd}	4.15±0.08 ^{Bc}	4.28±0.05 ^{Bb}	4.30±0.02 ^{Bab}	4.35±0.08 ^{Ba}
FOM	4.52±0.01 ^{Aa}	4.50±0.08 ^{Aa}	4.50±0.04 ^{Aa}	4.52±0.05 ^{Aa}	4.55±0.01 ^{Aa}
FMO+DWPC	4.54±0.02 ^{Aa}	4.55±0.10 ^{Aa}	4.50±0.09 ^{Aa}	4.54±0.06 ^{Aa}	4.55±0.05 ^{Aa}
Odour (5)					
FCM	4.46±0.08 ^{Aa}	4.51±0.10 ^{Aa}	4.56±0.12 ^{Aa}	4.31±0.09 ^{Ab}	4.15±0.01 ^{Ac}
FOM	4.15±0.06 ^{Ba}	4.20±0.08 ^{Ba}	4.21±0.08 ^{Ca}	4.00±0.05 ^{Bb}	3.74±0.01 ^{Cc}
FMO+DWPC	4.35±0.08 ^{ABa}	4.42±0.09 ^{Ca}	4.45±0.15 ^{Ba}	4.25±0.09 ^{ABb}	4.00±0.08 ^{Bc}
Flavor (5)					
FCM	4.45±0.08 ^{Aa}	4.55±0.11 ^{Aa}	4.52±0.18 ^{Aa}	4.40±0.10 ^{Ab}	4.15±0.06 ^{Ac}
FOM	4.20±0.02 ^{Ba}	4.32±0.15 ^{Ba}	4.35±0.10 ^{Ca}	4.10±0.08 ^{Cb}	4.00±0.04 ^{Ac}
FMO+DWPC	4.33±0.02 ^{ABa}	4.50±0.16 ^{Aa}	4.45±0.09 ^{Ba}	4.35±0.04 ^{Bb}	4.12±0.04 ^{Ac}
General evaluation (5)					
FCM	4.73±0.11 ^{Aa}	4.75±0.15 ^{Aa}	4.60±0.04 ^{Aa}	4.20±0.05 ^{Ab}	3.77±0.02 ^{Ac}
FOM	4.12±0.06 ^{Ca}	4.20±0.08 ^{Ca}	4.00±0.03 ^{Bb}	3.85±0.05 ^{Bc}	3.67±0.05 ^{Ad}
FMO+DWPC	4.45±0.09 ^{Ba}	4.56±0.08 ^{Ba}	4.55±0.09 ^{Aa}	4.28±0.08 ^{Ab}	3.80±0.04 ^{Ac}

FCM: Fermented cow's milk, FOM: Fermented oat milk, FOM+WP: Fermented oat milk fortified with 2% dried whey protein concentrate. Data presented as Mean±SE (n = 3), Means with same capital letter among treatments in the same storage period are not significantly differed, Means with same small letter for same treatment during storage periods are not significantly differed

be due to the ability of ABT starter culture to ferment maltose sugar, which is the main fermentable carbohydrate in the oat milk substrate. This conclusion is in agreement with Bekers *et al.*³⁶, Martensson *et al.*³⁷ and Dinkci *et al.*²⁰, who stated that oat is a suitable substrate for different types of lactic acid bacteria and functionality of fermented products, mainly yogurts could be improved by production of oat-based milk or mixture of cow and oat milk.

However, it is clear that counts of *Streptococcus salivarius* subsp. *thermophilus* and *Bifidobacterium* spp. in both oat fermented milk samples (with or without whey protein) were significantly ($p \leq 0.05$) higher than the counts of fermented cow milk along the cold storage period. This is might be due to the high content of oat-bases with different mono-and disaccharide, which can be used to support the growth of human intestinal bacteria and also maintain high cell viability during cold storage³⁸. The obtained results were basically in agreement with Abou-Dobara *et al.*³⁹, who found that the counts of *S. thermophilus* and *Bifidobacterium* spp. were higher in fermented Rayeb product prepared from oat milk individually or mixture of cow milk and oat milk (50:50) than those of cow milk Rayeb.

From these results, it could be observed that the addition of 2% dried whey protein concentrate during the manufacture of fermented oat milk had enhanced the viability of ABT culture strains along the cold storage period. The

obtained results agree with Janer *et al.*²⁵, who found that an increase in *Bifidobacterium lactis* population in milk supplemented with 2% WPC. According to these authors, the effect of WPC on the multiplication of bifidobacteria was due to the enzymatic cleavage process, producing bifidogenic compounds. Also, Antunes *et al.*²⁶ reported that *L. acidophilus* populations increased by 1.8 log in yogurt supplemented with WPC. Frederico *et al.*²⁷ observed that, higher *L. acidophilus* strain populations in the formulation containing the highest WPC content when compared to the other formulations.

Sensory attributes of different unfermented and fermented milk types:

To produce healthy fermented milk products, it must be organoleptically acceptable. Therefore, sensory attributes of fermented cow milk, oat milk and oat milk fortified with whey protein were evaluated during the cold storage at 4 °C for 21 days. As shown in Table 5, there were slight differences in appearance scores along all treatments during storage period (21 days). These results agree with Pallavi *et al.*⁴⁰, who found that the color and appearance scores of fermented Dahi was not affected significantly by increasing the levels of oat milk in cow milk. Data in Table 5 revealed that fermented samples made from oat milk had significantly ($p \leq 0.05$) higher consistency score than fermented samples made from cow milk along cold storage

period. This may be attributed to the differences in chemical composition and total solids between cow and oat milk. Total solids in prepared oat milk were higher than total solid in cow milk. Also, the increase in consistency could be due to the content of fibers and soluble fibers (β -glucan) in oat milk and this may have led to the interactions of the fibers and other components in the fermented milk.

Data in Table 5 indicated that, fermented cow milk samples recorded higher odor, flavor and general evaluation scores than that of fermented samples made from oat milk with or without whey protein. This could be due to the oat milk left a slight cereal taste in mouth. It could be observed that, sensory quality of fermented oat milk was slightly enhanced by adding the 2% DWPC.

In general, sensory quality for all fermented cow and oat milk were slightly increased during the first 7 days of storage period and then gradually decreased till the end of the storage period. This decrease may be due to the acidity development or the production of other microbial exerted metabolism which affect on sensory properties⁴¹.

All the fermented cow and oat milk samples gave a good total impression were smooth, glossy surface, no cracks or holes on the top of the final product, no whey syneresis, no off flavor or odor, clean layer on the surface of the fermented product. These results confirmed with Martensson *et al.*³⁷, who stated the potential for a new, fermentable, oat-based product with high acceptance and high final β -glucan content. Dinkci *et al.*²⁰ indicated that the possibility for a new cow/oat milk based kefir with good acceptability.

CONCLUSION

Finally, it could be concluded that, oat milk could be used as good raw material for preparing a new functional fermented product with high acceptability and high viability of bio-starter culture along the storage at 4°C for 21 days. Fortification of oat milk with whey protein caused a significant increase in nutritional value and improvement the viability of probiotic bacteria in final functional oat product.

SIGNIFICANCE STATEMENT

This study discovers the importance of fortifying oat milk with probiotic bacteria and whey protein (to the best of researchers' knowledge, no researchers used such additives in previous studies) in the production of functional fermented oat milk. Mixing whey protein with oat milk will improve nutrient value via increasing the protein content and increase the viability of probiotic bacteria in the final product. This

study will help the researcher to produce the novel fermented product from oat milk containing on high count of probiotics and high content of protein similar with fermented cow milk and suitable for some special individuals such as lactose intolerant individuals.

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