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Bioactivity, Physicochemical and Sensory Properties of Probiotic Yoghurt Made from Whole Milk Powder Reconstituted in Aqueous Fennel Extract

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Abstract: Fermented dairy products have long been associated with positive health benefits. The present study was undertaken to evaluate the physicochemical and sensory properties, viable probiotic counts, antioxidant activity and total phenolic content of probiotic yoghurt made by reconstituting of whole milk powder in aqueous fennel seed extract. Different concentrations of fennel aqueous seeds extract (2%, 4% and 6% *w*/*v*) were used as a substitute for water to reconstitute whole milk powder in formulations of yoghurt as functional additives. Interestingly, the use of aqueous extract of fennel seeds in the reconstituted yoghurt milk did not affect yoghurt composition (moisture, protein, fat and ash contents) compared to plain yoghurt. The titratable acidity significantly decreased after using aqueous fennel seed extract in the yogurt manufacture. In this regard, the titratable acidity value was 0.85 in the control yogurt at the fresh period and 1.14 after 21 days of storage, while this value significantly decreased in the yogurt treatments with 2%, 4% and 6% aqueous fennel seed extract to 80, 0.77 and 0.72, respectively, at fresh period and reached 1.03, 0.96 and 0.94, respectively, after 21 days of storage ($p < 0.05$). Conversely, the pH values significantly increased ($p < 0.05$) following the addition of aqueous fennel seed extract in the yogurt manufacture. Moreover, the total phenolic content significantly increased ($p < 0.05$) from 38.60 (mg GAE/L), in fresh plain yogurt, to 44.80, 53.20 and 64.30 (mg GAE/L), in 2% fennel extract yoghurt (FEY_2), 4% fennel extract yoghurt (FEY_4) and 6% fennel extract yoghurt (FEY_6), respectively. Likewise, the antioxidant activity significantly increased ($p < 0.05$) from 0.11 (mM TE) in fresh plain yogurt to 0.18, 0.26 and 0.32 (mM TE) in (FEY_2), (FEY_4) and (FEY_6), respectively. The survival of *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum* decreased during storage time in all yoghurt treatments, although it stood at recommended levels for health effects (at least 10^6 cfu/mL in traditional yoghurt). For sensory evaluation, FEY_4 was more acceptable, followed by FEY_6, FEY_2 and PY, respectively. Collectively, the present study provides useful information about the bioactivity, physicochemical and sensory properties of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract.

Keywords: yoghurt; *Foeniculum vulgare*; total phenolic; antioxidant activity; sensory evaluation; health effects

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1. Introduction

The last few decades have witnessed there a growing interest in research concerned with the therapeutic effect of dairy products, particularly those containing probiotics, which reduce symptoms associated with chronic diseases [1,2]. Among others, yoghurt is considered one of the most important dairy products; yoghurt is produced by fermenting lactose to lactic acid by the action yoghurt starter culture [3–5]. This starter culture, *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus*, interacts with milk protein and, therefore, improves the texture and sensory properties of the product [3–5].

Recently, there is a growing interest in improving the nutritional and health value of fermented milk by incorporating probiotic bacteria with health benefits [6]. In this respect, some additives have been used in food industry to improve the characteristics and properties of processed foods. These substances include nutritional and preservatives additives besides flavoring, coloring, and miscellaneous agents [1,7–9]. However, several previous studies have indicated that excessive consumption of industrial food additives results in problems related to the respiratory system, nervous system and skin diseases, causing alarm about random and excessive use [8,10]. Taken into account, yogurt has limited antioxidant activity. Many attempts have been conducted to fortify this issue with antioxidants from natural sources, which represented a good impact and a new approach for yogurt development. Furthermore, several natural extracts such as herbs and fruits, which have health-promoting properties such as antioxidant and antimicrobial effects, have been used as natural additives during yogurt manufacture to improve the functional and nutritional value of the final product [11,12]. An increased demand for natural antimicrobial substances as alternatives to replace the additives, and replacing additives with herbal extracts in food products, has attracted remarkable attention [9].

Interestingly, the fennel plant (*Foeniculum vulgare*) is a flowering plant species which showed potent activity against a wide range of disorders, including reproduction [13], respiratory [14], gastrointestinal [15] and endocrinal problems [16], besides its role as a lactation-improving agent [17]. Furthermore, fennel has shown potent antioxidant, antibacterial, antifungal, anti-inflammatory, and antiparasitic activities [18,19]**.** Fennel seed can also be used as a natural flavoring for some foods such as bread, cheese and pickles. It is therefore not suspiring to state that fennel seed is widely used as a food additive and for medicinal purposes, while all parts of the plant are important in the medicinal industry. In accordance with the composition and characterization of fennel seeds, they constitute 13.4% minerals, 10% fats, 42.3% carbohydrates, 9.5% protein, and 18.5% is as follows: vitamin C riboflavin, thiamine, niacin and phenolic contents including 1,3-*O*-dicaffeoylquinic acid, 5-*O*-caffeoylquinic acid, 1,5-*O*-di-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, and 1,4-*O*-di-caffeoylquinic acid [20]. Importantly, the antioxidant and antimicrobial action of fennel is due to the presence of 23 phenolic compounds in fennel essential oil extract, the most important of which are trans anethole, fenchone, estragole and limonene [21]. Given the above information, the aim of this study was to investigate using the aqueous extract of fennel seeds as a substitute for water to reconstitute whole milk powder yoghurt, and its effect on bioactivity and sensory properties and probiotic culture viability of yoghurt storage at 5–7 °C for 21 days.

2. Materials and Methods

2.1. Materials and Reagents

Dried fennel (*Foeniculum vulgare*) seed was procured from Agricultural Research Center, Giza, Egypt. Whole milk powder (26.0% fat, 30.0% protein, pH: 6.59) was obtained from the Irish Dairy Board, Grattan House, and Lower Mount St. (Dublin, Ireland). The lyophilized starter culture ABT-5, containing *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*, was obtained from Chr. Hansen (Hørsholm, Denmark), Galic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and other chemicals and reagents were purchased from Sigma (St. Louis, MO, USA). M17 agar, Bifidobacterium agar and De Man-Rogosa-Sharpe agar (MRS) were obtained from HiMedia (Mumbai, India).

2.2. Preparation of Fennel Aqueous Extract

The seeds of dried fennel were milled to a fine powder, sieved on 120 mesh sieves by Cyclotec (CT 293 Cyclotec™, Hilleroed, Denmark) sample mill. The aqueous extracts were prepared as follows: 2% (*w*/*v*, 6 g dried fennel powder in 300 mL distilled water), 4% (*w*/*v*, 12 g fennel powder in 300 mL distilled water) and 6% (*w*/*v*, 18 g fennel powder in 300 mL distilled water). The mixtures were heated, allowed to boil for 5 min, left to stand for 5 min, filtered using Whatman filter paper 1 (11 μM) then assembled, and it was used to reconstitute the milk powder used in the yoghurt manufacture.

2.3. Yoghurt Manufacture

Low heat whole milk powder (WMP) with (26.0% fat, 30.0% protein, pH: 6.59) was used to prepare reconstituted milk with 12% (*w*/*w*) total solids using deionized water for plain yoghurt (PY) and using fennel aqueous extracts for others three treatments 2% fennel extract yoghurt (FEY_2), 4% fennel extract yoghurt (FEY_4) and 6% fennel extract yoghurt (FEY_6). Briefly, deionized water and fennel aqueous extracts were heated to 30–40 °C before adding WMP. The mixture was then heated to 50 °C while being continuously stirred to dissolve completely all the solid materials according to Tavakoli et al. (2019) [22]. The production of yoghurt was performed according to the protocol described elsewhere [23]. Briefly, reconstituted milk was heat treated at 85 °C for 10 min, allowed to cool (42–45 °C), a lyophilized starter culture ABF-5 (0.02%, 50 U) containing *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*, was added (initial counts of log 107 CFU/mL for each of the containing bacteria strains). The preparation mix was then transferred to 100-mL plastic containers and incubated at 43 °C until the pH reached 4.65. All containers were immediately cooled down at the end of the fermentation process and stored in a refrigerator at 4 °C. Yoghurt treatments were analyzed on four different storage periods as follows: fresh, and after 7, 14 and 21 days from storage.

2.4. Chemical Composition

Total solids, fat, total protein and ash contents were determined according to Association of Official Agricultural Chemists (AOAC) [24]. The same protocol was used determination of moisture content of yoghurt samples through drying the samples in the oven for 24 h at 100 °C. The percentage moisture content was calculated by the following formula.

$$\%\ moisture = W1 - (W2 \times 100)/W1$$

where, *W*1 = initial weight of sample; *W*2 = weight of the dried sample.

2.5. Physico-Chemical Parameters

The titratable acidity of yoghurt samples was determined according to AOAC [24]. In this respect, the titratable acidity values (%) (as lactic acid) of yogurt treatments were determined according to the following equation after mixing the yogurt sample with 10 mL of hot distilled water (90 °C) and titrating with 0.1 N NaOH containing 0.5% phenolphthalein as an indicator to an end point of faint pink color. The changes in pH in the yoghurt samples during storage were measured using a laboratory pH meter with glass electrode (HANNA Instruments, Amorim, Portugal). Color was measured using a Minolta colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan, observer angle 10°, with a measuring head hole of 8 mm, calibrated on a white standard L* 99.18; a* −0.07; b* −0.05).) based on three color coordinates, namely L*, a*, b*. The color values were expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness). Color L*, a* and b* values were recorded as the mean of triplicate readings.

$$\text{Titratable Acidity} = \frac{\text{Volume of titrant} \times \text{N} \times 90}{\text{Volume of sample} \times 1000} \times 100$$

where, N = normality of titrant; 90 = Equivalent weight for lactic acid.

2.6. Determination of Total Phenolic Content and Antioxidant Activity

Yogurt samples were centrifuged at 20,000× *g* for 60 min at 4 °C. The supernatants were filtered using a 0.45 µm syringes filter (MS® CA, Membrane Solution, Shanghai, China) and kept at −20 °C until further testing. The total phenolic content (TPC) and antioxidant activity (AO) of prepared yoghurt supernatants were assessed as described elsewhere [25]. In this respect, TPC was determined using Folin–Ciocalteu colorimetric method using one and half milliliters of Folin–Ciocalteu reagent (diluted 10 times) and 1.2 mL of Na_2CO_3 (7.5% *w*/*v*) were added to 300 µL of supernatant. Mixtures were shaken and kept at room temperature for 30 min before measuring absorbance at 765 nm using a spectrophotometer (Pg T80+, Leicestershire, UK) and tests were carried out in triplicate. On the other hand, AO was determined using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. The supernatant (100 µL) was added to 3 mL of 0.1 mM DPPH which was dissolved in ethanol and incubated of 60 min at room temperature. The absorbance was then recorded against control at 517 nm. Percentage of antioxidant potential of DPPH• was calculated as follows:

$$\text{Inhibition (\%)} = [(A0 - A1)/A0] \times 100$$

where, A0 is the absorbance of the control, and A1 is the absorbance in the extract. Samples were analyzed in triplicate. The results were expressed as g of gallic acid equivalents (GAE) per liter for TPC, and mmol Trolox equivalents (TE) per liter of supernatant for AO capacity, measured by DPPH assay.

2.7. Microbiological Analysis of Yoghurts

In this step, *B. bifidum*, *L. acidophilus* and *S. thermophiles* were counted using the pour plate technique and serial dilutions in phosphate-buffer saline (1% PBS). Plate counts of *B. bifidum* were performed in Bifidobacterium agar under anaerobic incubation at 37 °C for 72 h, while plate counts of *L. acidophilus* was counted on MRS agar (pH 6.2) containing 1 mg/L sorbitol under anaerobic incubation at 37 °C for 72 h. Plate counts of *S. thermophilus* were performed in M17 agar (pH 7.2) under aerobic incubation at 37 °C for 48 h.

2.8. Sensory Evaluation

The sensory properties of yoghurt samples were assessed following the methods described by Nelson and Trout [26] and modified by Al-Shawi et al. [9] by a team of 10 professional panelists from the Faculty of Agriculture, Zagazig University. The following scoring points were used for different properties: taste and flavor (45), textures (30), acidity (15) and general appearance (10).

2.9. Statistical Analysis

The data were statistically analyzed in terms of the effect of using aqueous extract of fennel seeds at different concentrations (2%, 4% and 6% *w*/*v*) on the properties of yoghurt produced in each storage period versus plain yoghurt. Data were expressed as mean ± SD and compared among treatment groups using one-way analysis of variance (ANOVA) followed by the least significant difference (L.S.D) test. Statistic version 9 (http://www.statistix.com/freetrial.html, accessed on 5 November 2021) was performed for analyses of the data [27]. The differences between the means of the treatments were considered significant ($p < 0.05$) when they were more than LSD at the 5% levels. All measurements were used in triplicate and statistically analyzed.

3. Results and Discussion

Fermented dairy products are an easy way to incorporate and deliver probiotics to the consumer. Incorporation of plant-based additives can be used to increase the bioactive compounds in dairy products [28]. As mentioned above, the aim of this study was to investigate the bioactivity, physicochemical and sensory properties of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract (2%, 4% and 6% *w*/*v*) within 21 days of storage at 5 ± 1 °C.

3.1. Chemical Composition of Probiotic Yoghurt Containing Fennel Aqueous Extract

Table 1 shows the moisture content for plain yoghurt (PY), 2% fennel yoghurt (FEY_2), 4% fennel yoghurt (FEY_4) and 6% fennel yoghurt (FEY_6) treatments, which were 87.40%, 87.36%, 87.32% and 87.28%, respectively, at fresh time. As the storage period progresses, no difference was observed in yoghurt moisture content in all yoghurt treatments to reach 86.74%, 86.60%, 86.52% and 86.38%, respectively, after 21 days of storage, and this decrease may be due to the evaporation during storage or may have resulted from the decrease in pH. These results are in line with those reported by Caleja et al. [4], who found that the moisture content yoghurt at fresh time was 87.6% and reached 87.3%, after 14 days of storage. As shown in Table 1, the protein content of (PY), (FEY_2), (FEY_4) and (FEY_6) treatments were non-significant ($p < 0.05$) and had values of 3.65%, 3.68%, 3.72% and 3.75%, respectively. As the storage period progressed, there was an increase in the protein content in all yoghurt treatments. The values, after 21 days of storage, were 3.88%, 3.92%, 3.96% and 4.0%, respectively, and without any significant differences ($p < 0.05$). The reason for this increase may be attributed to the continued decrease in the moisture content of the yoghurt during storage periods. The present results revealed that using fennel extract in yoghurt milk did not influence significantly ($p < 0.05$) on the protein content of the manufactured yoghurt. Similar results were obtained by Caleja et al. [4] and Al-Shawi et al. [9], who reported that adding fennel or thyme extracts to yoghurt milk did not have an effect on the protein content compared to the control sample.

Table 1. Chemical composition of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract during storage at 5–7 °C for 21 days.

Components (%)	Storage Period (Day)	Treatments				LSD
		PY	FEY_2	FEY_4	FEY_6	
Moisture	0	87.40 ^{A}	87.36 ^{A}	87.32 ^{A}	87.28 ^{A}	0.9950
	7	87.22 ^{A}	87.08 ^{A}	87.02 ^{A}	86.94 ^{A}	0.3122
	14	87.00 ^{A}	86.92 ^{A}	86.84 ^{A}	86.70 ^{A}	0.9976
	21	86.74 ^{A}	86.60 ^{A}	86.52 ^{A}	86.38 ^{A}	0.6272
Protein	0	3.65 ^{A}	3.68 ^{A}	3.72 ^{A}	3.75 ^{A}	0.1392
	7	3.68 ^{A}	3.74 ^{A}	3.78 ^{A}	3.84 ^{A}	0.3159
	14	3.75 ^{A}	3.78 ^{A}	3.82 ^{A}	3.88 ^{A}	0.2510
	21	3.88 ^{A}	3.92 ^{A}	3.96 ^{A}	4.00 ^{A}	0.2449
Fat	0	3.14 ^{A}	3.18 ^{A}	3.20 ^{A}	3.24 ^{A}	0.2091
	7	3.26 ^{A}	3.30 ^{A}	3.34 ^{A}	3.40 ^{A}	0.2828
	14	3.44 ^{A}	3.52 ^{A}	3.58 ^{A}	3.62 ^{A}	0.3265
	21	3.68 ^{A}	3.74 ^{A}	3.80 ^{A}	3.86 ^{A}	0.5655
Ash	0	0.68 ^{A}	0.72 ^{A}	0.75 ^{A}	0.78 ^{A}	0.1306
	7	0.72 ^{A}	0.76 ^{A}	0.80 ^{A}	0.84 ^{A}	0.1306
	14	0.75 ^{A}	0.80 ^{A}	0.84 ^{A}	0.90 ^{A}	0.1768
	21	0.80 ^{A}	0.84 ^{A}	0.88 ^{A}	0.94 ^{A}	0.1635

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: least significant difference. PY: probiotic yoghurt made from whole milk powder reconstituted in water. FEY_2: probiotic yoghurt made from whole milk powder reconstituted in 2% aqueous fennel extract. FYE_4: probiotic yoghurt made from whole milk powder reconstituted in 4% aqueous fennel extract. FYE_6: probiotic yoghurt made from whole milk powder reconstituted in 6% aqueous fennel extract.

The fat content of (PY), (FEY_2), (FEY_4) and (FEY_6) treatments was 3.14%, 3.18%, 3.20% and 3.24%, respectively (Table 1). As the storage period progressed, there was a slight increase in yoghurt fat content for all treatments. The fat content of (PY), (FEY_2), (FEY_4) and (FEY_6) treatments after 21 days of storage was 3.68%, 3.74%, 3.80% and 3.86%, respectively. Non-significant differences ($p < 0.05$) were found among yoghurt treatments in fat content when using fennel extract in yoghurt milk compared to the plain yoghurt. Similar results were obtained by Caleja et al. [4] and Al-Shawi et al. [9], who reported that adding fennel

or thyme extracts to yoghurt milk did not have an effect on the fat content compared to the control sample. As shown in Table 1, the ash content of (PY), (FEY_2), (FEY_4) and (FEY_6) treatments was 0.68%, 0.72%, 0.75% and 0.78%, respectively, without any significant differences ($p < 0.05$), and there was no significant difference in yoghurt ash content of all treatments during the storage periods. The ash content after 21 days of storage was 0.80%, 0.84%, 0.88% and 0.94%, respectively. These results agree with those reported by Caleja et al. [4], who found that using fennel extract in yoghurt milk slightly increased ash content compared to the control sample.

3.2. Titratable Acidity, pH Values and Color Parameters of Probiotic Yoghurt Made from Whole Milk Powder Reconstituted in Aqueous Fennel Extract

The pH values of PY, FEY_2, FEY_4 and FEY_6 treatments are shown in Table 2. As shown in this Table 2, the pH values in PY were less compared to FEY_2, FSY_4 and FSY_6 after 21 day of storage periods. This difference might be attributed to the effect of aqueous fennel extracts on the growth of microorganisms and subsequently on pH values. The pH values of all yoghurt treatments decreased during 21 days of storage. The pH reduction during storage could be because of storage duration, starter culture variety, lactic acid conversion into lactose, and temperature of fermentation [29]. A previous study [30] reported that the pH value of plain yoghurt slightly increased than essential oil-treated yoghurts, and there were significant differences ($p < 0.05$) among treatments during storage periods. Additionally, Caleja et al. [4] and Al-Shawi et al. [9] found that use fennel or thyme extract in yoghurt milk increased pH values compared to the control sample.

Table 2. Titratable acidity, pH values and color parameters of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract during storage at 5–7 °C for 21 days.

Parameters	Storage Period (Day)		Treatments				LSD
			PY	FEY_2	FEY_4	FEY_6	
Acidity %		0	0.85 ^{A}	0.80 ^{AB}	0.77 ^{BC}	0.72 ^{C}	0.0566
		7	0.90 ^{A}	0.84 ^{AB}	0.82 ^{AB}	0.76 ^{B}	0.0979
		14	1.02 ^{A}	0.92 ^{B}	0.88 ^{C}	0.82 ^{D}	0.0163
		21	1.14 ^{A}	1.03 ^{B}	0.96 ^{C}	0.94 ^{C}	0.0627
pH values		0	4.58 ^{B}	4.62 ^{AB}	4.68 ^{A}	4.70 ^{A}	0.0832
		7	4.56 ^{C}	4.60 ^{BC}	4.64 ^{AB}	4.68 ^{A}	0.0566
		14	4.48 ^{D}	4.52 ^{C}	4.55 ^{B}	4.62 ^{A}	0.0141
		21	4.42 ^{C}	4.46 ^{BC}	4.50 ^{B}	4.58 ^{A}	0.0566
Color parameters	L*	0	93.86 ^{A}	93.20 ^{AB}	92.70 ^{AB}	92.20 ^{B}	1.2668
		7	94.60 ^{A}	93.46 ^{AB}	92.94 ^{B}	92.80 ^{B}	1.2199
		14	94.00 ^{A}	93.38 ^{AB}	92.85 ^{B}	92.54 ^{B}	0.8791
		21	93.92 ^{A}	93.26 ^{AB}	92.76 ^{BC}	92.36 ^{C}	0.7786
	a*	0	−3.40 ^{C}	−3.10 ^{BC}	−2.70 ^{AB}	−2.30 ^{A}	0.5655
		7	−3.60 ^{C}	−3.30 ^{BC}	−2.90 ^{AB}	−2.60 ^{A}	0.5655
		14	−4.20 ^{D}	−3.50 C	−3.20 ^{B}	−3.0 ^{A}	0.1632
		21	−4.50 ^{B}	−3.80 ^{B}	−3.60 ^{AB}	−3.30 ^{A}	0.8162
	b*	0	12.30 ^{D}	12.80 ^{C}	13.20 ^{B}	13.70 ^{A}	0.1632
		7	12.90 ^{C}	13.30 ^{BC}	13.60 ^{AB}	14.20 ^{A}	0.6270
		14	12.60 ^{C}	13.0 ^{BC}	13.50 ^{AB}	14.00 ^{A}	0.8364
		21	12.40 ^{C}	12.90 ^{BC}	13.30 ^{AB}	13.80 ^{A}	0.7657

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference.

The titratable acidity values at zero time for (PY), (FEY_2), (FEY_4) and (FEY_6) were 0.85, 0.80, 0.77 and 0.72, respectively, without any significant differences ($p < 0.05$), and reached 1.14, 1.03, 0.96 and 0.94, respectively, after 21 day of storage period (Table 2). It was significant ($p < 0.05$) for (PY) compared to (FSY2), (FSY4) and (FSY6). The TA increased

as storage period progressed; this may be due to the activity of the yoghurt starter culture, which converted lactose into lactic acid [31]. The TA of aqueous extracts yoghurt decreased compared to plain yoghurt, which might be due to the fennel extract activity which reduced the growth of yoghurt bacteria. The influence of herbs and spices on TA was investigated in a previous study by Suliman et al. [32], who reported that the titratable acidity of cinnamon-treated yoghurt was affected by cinnamon addition, which had an effect on pH increase. Additionally, Caleja et al. [4] and Al-Shawi et al. [9] found that the use of fennel or thyme extract in yoghurt milk decreased TA compared to the control sample. Regarding the color parameters, a significant change in L*, a*and b* in the different samples and along storage time was noted. Fennel extract yoghurt treatments showed lower L* values and higher a* and b* values compared with plain yoghurt (Table 2) The significant difference of color value between the plain yogurt and fennel extract yoghurt could be attributed to the increase in the L value by increasing fennel extract level, which affects the lightness as a result of the yellowish color of the fennel extract. Similar results were obtained by Caleja et al. [4] observed a slight change in L*, a*and b* in the fennel extract yoghurt and plain yoghurt and along storage time.

3.3. Total Phenolic Content and Antioxidant Activity of Probiotic Yoghurt Made from Whole Milk Powder Reconstituted in Aqueous Fennel Extract

The TPC and the AO activity of the supernatants obtained from the probiotic yoghurts with and without fennel extract are shown in Table 3. It can be observed that the using of fennel extract as a substitute for water to reconstitute whole milk powder yoghurt increases the TPC and AO activity of yoghurt samples, and these increases were associated with an increase in the concentration of fennel extract. Dubrovskii et al. [33] demonstrated that an increase in fennel extract content in yoghurt samples is positively correlated with TPC. Furthermore, fennel extract yoghurt treatments showed significantly higher scavenging compared to plain yoghurt [4]. Telugu et al. [20] showed that fennel extract contained a high content of TPC and exhibited strong total AO activity. Additionally, an increase in fennel extract concentration led to a significant elevation in AO activity [33]. The antioxidant activity of yoghurt samples containing fennel extract is due to the presence of polyphenols in fennel extract. Phenolic acids (1,3-*O*-dicaffeoylquinic acid, 5-*O*-caffeoylquinic acid, 1,5-*O*-di-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, and 1,4-*O*-di-caffeoylquinic acid) were found in fennel extract [20,34], where there is a positive relationship between the antioxidant activity of fennel extracts and their content of total phenols and flavonoids [35].

Table 3. Total phenolic content and antioxidant activity of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract during storage at 5–7 °C for 21 days.

Items	Storage Period (Day)	Treatments				LSD
		PY	FEY_2	FEY_4	FEY_6	
TPC (mg GAE/L)	0	38.60 ^{D}	44.80 ^{C}	53.20 ^{B}	64.30 ^{A}	5.6550
	7	34.20 ^{D}	41.30 ^{C}	48.60 ^{B}	59.80 ^{A}	4.8585
	14	29.50 ^{D}	36.70 ^{C}	43.40 ^{B}	53.60 ^{A}	4.3498
	21	26.80 ^{D}	31.60 ^{C}	39.70 ^{B}	48.50 ^{A}	4.4069
DPPH (mM TE)	0	0.11 ^{D}	0.18 ^{C}	0.26 ^{B}	0.32 ^{A}	0.0566
	7	0.08 ^{D}	0.15 ^{C}	0.22 ^{B}	0.30 ^{A}	0.0141
	14	0.04 ^{C}	0.09 ^{C}	0.17 ^{B}	0.24 ^{A}	0.0566
	21	0.02 ^{C}	0.06 ^{C}	0.13 ^{B}	0.20 ^{A}	0.0566

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: least significant difference. PY: probiotic yoghurt made from whole milk powder reconstituted in water. FEY_2: probiotic yoghurt made from whole milk powder reconstituted in 2% aqueous fennel extract. FYE_4: probiotic yoghurt made from whole milk powder reconstituted in 4% aqueous fennel extract. FYE_6: probiotic yoghurt made from whole milk powder reconstituted in 6% aqueous fennel extract.

3.4. Viable Counts of L. acidophilus, S. thermophilus and B. bifidum in Plain and Fennel Extract Yoghurt

Table 4 shows viable counts of *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum* in PY, FEY_2, FEY_4 and FEY_6 during storage periods. At zero time, there was nearly an equal non-significant ($p < 0.05$) content of *Streptococcus thermophilus* and *Bifidobacterium bifidum* and there was significant ($p < 0.05$) content of *Lactobacillus acidophilus* for all treatments and this content decreased slightly till day 14 and reduced gradually after day 21. There was a significant difference ($p < 0.05$) between PY and the other three treatments. The counts of *Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium bifidum* decreased during storage, and this decline is noticeable from day 14–21, probably due to increased yoghurt acidity [22]. The presence of fennel extract in yoghurt led to lower LAB content, and this may be attributed to fennel antimicrobial components such as linoleic acid, undecanal, 1,3-benzenediol, oleic acid and 2,4-undecadienal in the extract with a great inhibitory effect on Gram-positive bacteria [21]. The results of the present study agree with those of Dubrovskii et al. [33], who found that the presence of fennel extract in yoghurt led to lower LAB content. Additionally, Al-Shawi et al. [9] found that the presence of thyme extract in yoghurt led to lower LAB content. In contrast, Suliman et al. [32] found that the presence of cinnamon herb improved the viability of LAB. *Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium bifidum* viability slightly decreased with fennel extract but its number was still within the recommended range for probiotic cultures (>log 7 CFU/mL) [36].

Table 4. Viable counts *of L. acidophilus, S. thermophilus* and *B. bifidum* (log CFU/mL) in plain and fennel extract yoghurts during storage at 5–7 °C for 21 days.

Viable Counts (log CFU/mL)	Storage Period (Day)	Treatments				LSD
		PY	FEY_2	FEY_4	FEY_6	
Streptococcus thermophilus	0	8.96 ^{A}	8.92 ^{A}	8.90 ^{A}	8.87 ^{A}	0.2970
	7	8.92 ^{A}	8.88 ^{AB}	8.84 ^{BC}	8.80 ^{C}	0.0653
	14	8.87 ^{A}	8.83 ^{AB}	8.75 ^{B}	7.94 ^{C}	0.1016
	21	8.36 ^{A}	8.10 ^{A}	8.06 ^{B}	7.82 ^{C}	0.0905
Lactobacillus acidophilus	0	8.22 ^{A}	8.18 ^{AB}	8.12 ^{BC}	8.08 ^{C}	0.0627
	7	8.20 ^{A}	8.15 ^{B}	8.04 ^{C}	8.00 ^{D}	0.0271
	14	8.14 ^{A}	8.09 ^{A}	7.96 ^{B}	7.90 ^{B}	0.0979
	21	7.58 ^{A}	7.50 ^{AB}	7.48 ^{B}	7.42 ^{B}	0.0905
Bifidobacterium bifidum	0	8.74 ^{A}	8.70 ^{A}	8.66 ^{A}	8.64 ^{A}	0.1110
	7	8.68 ^{A}	8.55 ^{B}	8.46 ^{C}	8.40 ^{D}	0.0271
	14	8.62 ^{A}	8.12 ^{B}	8.04 ^{BC}	7.96 ^{C}	0.0848
	21	8.14 ^{A}	7.88 ^{B}	7.76 ^{C}	7.68 ^{D}	0.0632

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: least significant difference. PY: probiotic yoghurt made from whole milk powder reconstituted in water. FEY_2: probiotic yoghurt made from whole milk powder reconstituted in 2% aqueous fennel extract. FYE_4: probiotic yoghurt made from whole milk powder reconstituted in 4% aqueous fennel extract. FYE_6: probiotic yoghurt made from whole milk powder reconstituted in 6% aqueous fennel extract.

3.5. Sensory Properties of Probiotic Yoghurt Made from Whole Milk Powder Reconstituted in Aqueous Fennel Extract

Table 5 shows the sensory evaluation results of PY, FEY_2, FEY_4 and FEY_6. The results showed that FEY_4 was more acceptable for flavor compared to the other two treatments during the different storage periods ($p < 0.05$). Due to the development of acidity during the increase in the storage period, the taste and flavor of the plain yoghurt PY began to change to an acidic flavor as a result of the increase in the number of proteolytic bacteria and the production of short-chain peptides. The lactic acid bacteria are responsible for the development of acidity to the desired extent during the progression of storage periods. FEY flavor was better than PY flavor due to the effect of fennel components on the microorganism's activity, which may allow for the retention of the desired flavor and taste. The preference

was for FEY_4 followed by FEY_6 followed by FEY_2 and PY. As a result of the activity of its bacterial content and the accumulation of organic acids, the acceptability scores of PY decreased. This result agrees with Joung et al. [37], who indicated that the presence of this high amount of acetic acid (19.64 ± 1.99 mg/mL) in the plain yoghurt negatively affected its overall acceptance. For the texture characteristics, FEY_2, FEY_4 and FEY_6 exceeded PY. For the acidity characteristic, there were slight changes among PY and all FEY treatments, with the superiority of FEY treatments during different storage periods. The presence of some plant extracts improves the sensory properties of yoghurt by increasing its flavor and good texture. Clearly, yoghurt is a good carrier of bioactive compounds [4,9,37]. In contrast, the addition of cinnamon herb to yoghurt resulted in an undesirable overall taste compared to the plain yoghurt [38].

Table 5. Sensory properties of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract during storage at 5–7 °C for 21 days.

Sensory Attributes	Storage Period (Day)	Treatments				LSD
		PY	FEY_2	FEY_4	FEY_6	
Flavor (45)	0	43.40 ^{C}	43.80 ^{BC}	44.50 ^{A}	44.20 ^{AB}	0.6530
	7	42.60 ^{D}	43.50 ^{C}	44.10 ^{A}	43.80 ^{B}	0.1414
	14	42.00 ^{B}	43.00 ^{AB}	43.70 ^{A}	43.30 ^{A}	1.6325
	21	41.40 ^{B}	42.60 ^{A}	43.20 ^{A}	42.90 ^{A}	1.0063
Textures (30)	0	28.80 ^{B}	29.20 ^{AB}	29.60 ^{AB}	30.00 ^{A}	0.8324
	7	28.50 ^{A}	28.90 ^{A}	29.40 ^{A}	29.60 ^{A}	1.4783
	14	28.30 ^{A}	28.60 ^{A}	29.10 ^{A}	29.30 ^{A}	1.0674
	21	27.80 ^{C}	28.30 ^{BC}	28.90 ^{AB}	29.00 ^{A}	0.6270
Acidity (15)	0	15.00 ^{A}	14.60 ^{AB}	14.00 ^{B}	13.70 ^{B}	1.0674
	7	14.70 ^{A}	14.20 ^{AB}	13.80 ^{B}	13.50 ^{B}	0.7657
	14	14.30 ^{A}	14.00 ^{AB}	13.50 ^{AB}	13.20 ^{B}	0.8121
	21	13.90 ^{A}	13.60 ^{B}	13.10 ^{C}	13.00 ^{C}	0.2828
General Appearance (10)	0	10.00 ^{A}	10. 00 ^{A}	10.00 ^{A}	9.00 ^{B}	0.2828
	7	10.00 ^{A}	10.00 ^{A}	10.00 ^{A}	9.00 ^{B}	0.2828
	14	9.00 ^{A}	9.00 ^{A}	9.00 ^{A}	8.00 ^{B}	0.2828
	21	9.00 ^{A}	9.00 ^{A}	9.00 ^{A}	8.00 ^{B}	0.2828

Means followed by different superscript letters in the same column are significantly different ($p \leq 0.05$). LSD: least significant difference. PY: probiotic yoghurt made from whole milk powder reconstituted in water. FEY_2: probiotic yoghurt made from whole milk powder reconstituted in 2% aqueous fennel extract. FYE_4: probiotic yoghurt made from whole milk powder reconstituted in 4% aqueous fennel extract. FYE_6: probiotic yoghurt made from whole milk powder reconstituted in 6% aqueous fennel extract.

4. Conclusions

Probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract was successfully manufactured with viable probiotic starter counts, a suitable chemical composition, acceptable flavor and texture characteristics, and it was equal in general appearance, except for yoghurt treatment made from whole milk powder reconstituted in 6% aqueous fennel extract (FEY_6) compared to (PY). Clearly, the aqueous extract of fennel can be used up to 4% as a substitute of water to reconstitute whole milk powder in the manufacture of yoghurt to produce functional yoghurt with desirable properties compared to plain yogurt. Natural plant-based additives need further studies about their health and technological influence in dairy product manufacture to highlight their safety and high bioactive compounds content compared with synthetic additives.

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References

1. Walsh, H.; Ross, J.; Hendricks, G.; Guo, M. Physico-chemical properties, probiotic survivability, microstructure, and acceptability of a yogurt-like symbiotic oats-based product using pre-polymerized whey protein as a gelation agent. *J. Food Sci.* **2010**, *75*, M32–M337. [CrossRef] [PubMed]
2. Atwaa, E.; Sayed-Ahmed, A.; Eman, T.; Hassan, M. Physicochemical, Microbiological and Sensory Properties of Low Fat Probiotic Yoghurt Fortified with Mango Pulp Fiber Waste as Source of Dietary Fiber. *J. Food Dairy Sci.* **2020**, *11*, 271–276. [CrossRef]
3. Serafeimidou, A.; Zlatanos, S.; Kritikos, G.; Tourianis, A. Change of fatty acid profile, including conjugated linoleic acid (CLA) content, during refrigerated storage of yogurt made of cow and sheep milk. *J. Food Compos. Anal.* **2013**, *31*, 24–30. [CrossRef]
4. Caleja, C.; Barros, L.; Antonio, A.L.; Carocho, M.; Oliveira, M.B.P.; Ferreira, I.C. Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. *Food Chem.* **2016**, *210*, 262–268. [CrossRef]
5. Pakseresht, S.; Tehrani, M.M.; Razavi, S.M.A. Optimization of low-fat set-type yoghurt: Effect of altered whey protein to casein ratio, fat content and microbial transglutaminase on rheological and sensorial properties. *J. Food Sci. Technol.* **2017**, *54*, 2351–2360. [CrossRef]
6. Oliveira, M.; Sodini, I.; Remeuf, R.; Tissier, J.; Corrieu, G. Manufacture of fermented lactic beverages containing probiotic cultures. *J. Food Sci.* **2002**, *67*, 2336–2341. [CrossRef]
7. Dickson-Spillmann, M.; Siegrist, M.; Keller, C. Attitudes toward chemicals are associated with preference for natural food. *Food Qual. Prefer.* **2011**, *22*, 149–156. [CrossRef]
8. Carocho, M.; Morales, P.; Ferreira, I.C. Natural food additives: Quo vadis? *Trends Food Sci. Technol.* **2015**, *45*, 284–295. [CrossRef]
9. Al-Shawi, S.G.; Ali, H.I.; Al-Younis, Z.K. The effect of adding thyme extacts on microbiological, chemical and sensory characteristics of yogurt. *J. Pure Appl. Microbiol.* **2020**, *14*, 1367–1376. [CrossRef]
10. Randhawa, S.; Bahna, S.L. Hypersensitivity reactions to food additives. *Curr. Opin. Allergy Clin. Immunol.* **2009**, *9*, 278–283. [CrossRef]
11. Martins, A.; Barros, L.; Carvalho, A.M.; Santos-Buelga, C.; Fernandes, I.P.; Barreiro, F.; Ferreira, I.C. Phenolic extracts of Rubus ulmifolius Schott flowers: Characterization, microencapsulation and incorporation into yogurts as nutraceutical sources. *Food Funct.* **2014**, *5*, 1091–1100. [CrossRef] [PubMed]
12. Bertolino, M.; Belviso, S.; Dal Bello, B.; Ghirardello, D.; Giordano, M.; Rolle, L.; Gerbi, V.; Zeppa, G. Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. *LWT-Food Sci. Technol.* **2015**, *63*, 1145–1154. [CrossRef]
13. Rahimikian, F.; Rahimi, R.; Golzareh, P.; Bekhradi, R.; Mehran, A. Effect of Foeniculum vulgare Mill.(fennel) on menopausal symptoms in postmenopausal women: A randomized, triple-blind, placebo-controlled trial. *Menopause* **2017**, *24*, 1017–1021. [CrossRef] [PubMed]
14. Saber, J.I.; Eshra, D.H. Using Fennel Seeds and their Oil as a Preservative and Functional Food to Produce Some Food and Drink Products to Alleviate Cough Symptoms. *Alex. Sci. Exch. J.* **2019**, *40*, 406–414.
15. Syed, F.Q.; Mirza, M.B.; Elkady, A.I.; Hakeem, K.R.; Alkarim, S. An Insight of Multitudinous and Inveterate Pharmacological Applications of Foeniculum Vulgare (Fennel). In *Plant and Human Health*; Springer: Berlin/Heidelberg, Germany, 2019; Volume 3, pp. 231–254.
16. Amiot, M.; Riva, C.; Vinet, A. Effects of dietary polyphenols on metabolic syndrome features in humans: A systematic review. *Obes. Rev.* **2016**, *17*, 573–586. [CrossRef]
17. Derin, D.; Ozel, E. An investigation of galactagogues from the perspective of mothers who have a newborn child. *Int. J. Health Sci. Res.* **2016**, *6*, 268–278.
18. Samadi-Noshahr, Z.; Hadjzadeh, M.A.; Moradi-Marjaneh, R.; Khajavi-Rad, A. The hepatoprotective effects of fennel seeds extract and transepatoprot in streptozotocinective e liver injury in rats. *Food Sci Nutr.* **2020**, *9*, 1121–1131. [CrossRef]
19. Asmat, U.; Abad, K.; Ismail, K. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm J.* **2016**, *24*, 547–553. [CrossRef]

20. Telugu, R.K.; Tehlan, S.; Mor, V. Assessment of seed quality and vigour in fifty genotypes of fennel (*Foeniculum vulgare* Mill.). *IJCS* **2019**, *7*, 362–366.
21. Arman, M.S.I.; Akter, S.; Mahaldar, K.; Tuly, F.A.; Hossain, M.S. Investigation of antiemetic, antimicrobial and anti-radical properties of methanolic extract of Foeniculum vulgare: A medicinal herb. *Discov. Phytomed.* **2019**, *6*, 70–76.
22. Tavakoli, M.; Najafi, M.B.H.; Mohebbi, M. Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. *Heliyon* **2019**, *5*, e01204. [CrossRef] [PubMed]
23. Tamime, A.Y.; Robinson, R.K. *Yoghurt: Science and Technology*; CRC Press: Boca Raton, FL, USA, 1999.
24. Horwitz, W. *Official Methods of Analysis of AOAC International. Volume I, Agricultural Chemicals, Contaminants, Drugs*; Horwitz, W., Ed.; AOAC International: Gaithersburg, Maryland, 2010.
25. Petrovic, M.; Suznjevic, D.; Pastor, F.; Veljovic, M.; Pezo, L.; Antic, M.; Gorjanovic, S. Antioxidant capacity determination of complex samples and individual phenolics-multilateral approach. *Comb. Chem. High Throughput Screen.* **2016**, *19*, 58–65. [CrossRef] [PubMed]
26. Nelson, J.; Trout, G. *Juding Dairy Products*, 4th ed.; The Olsen Pubulishing Company: Milwaukee, WI, USA, 1964.
27. Boyd, C.; Petersen, S.; Gilbert, W.; Rodgers, R.; Fuhlendorf, S.; Larsen, R.; Wolfe, D.; Jensen, K.; Gonzales, P.; Nenneman, M. Analytical Software. 2009. In *Evaluation of Methods Used to Improve Grasslands as Ring-Necked Pheasant (Phasianus colchicus) Brood Habitat*; Statistix 9: Tallahassee, FL, USA, 2018; Volume 72, p. 82.
28. Swelam, S.; Zommara, M.A.; El-Aziz, A.; Elgammal, N.A.; Baty, R.S.; Elmahallawy, E.K. Insights into Chufa Milk Frozen Yoghurt as Cheap Functional Frozen Yoghurt with High Nutritional Value. *Fermentation* **2021**, *7*, 255. [CrossRef]
29. Singh, G.; Kapoor, I.P.S.; Singh, P. Effect of volatile oil and oleoresin of anise on the shelf life of yogurt. *J. Food Processing Preserv.* **2011**, *35*, 778–783. [CrossRef]
30. Azizkhani, M.; Tooryan, F. Antimicrobial activities of probiotic yogurts flavored with peppermint, basil, and zataria against *Escherichia coli* and *Listeria monocytogenes*. *J. Food Qual. Hazards Control* **2016**, *3*, 79–86.
31. Kumari, A.; Ranadheera, C.; Prasanna, P.; Senevirathne, N.; Vidanarachchi, J. Development of a rice incorporated synbiotic yogurt with low retrogradation properties. *Int. Food Res. J.* **2015**, *22*, 2032.
32. Suliman, A.; Ahmed, K.; Mohamed, B.; Babiker, E. Potential of cinnamon (*Cinnamomum cassia*) as an anti-oxidative and anti-microbial agent in sudanese yoghurt (Zabadi). *J. Dairy Vet. Sci.* **2019**, *12*, 1–8.
33. Dubrovskii, I.; Arseneva, T.; Evstigneeva, T.; Gorshkova, S.; Bazarnova, Y.; Iakovchenko, N. Development of formulation and technology of yogurt with prolonged shelf life enriched with biologically active substances from fennel seed extract. *Agron. Res.* **2019**, *17*, 1313–1323.
34. Afifi, S.M.; El-Mahis, A.; Heiss, A.G.; Farag, M.A. Gas Chromatography–Mass Spectrometry-Based Classification of 12 Fennel (*Foeniculum vulgare* Miller) Varieties Based on Their Aroma Profiles and Estragole Levels as Analyzed Using Chemometric Tools. *ACS Omega* **2021**, *6*, 5775–5785. [CrossRef]
35. Anka, Z.; Gimba, S.; Nanda, A.; Salisu, L. Phytochemistry and Pharmacological Activities of Foeniculum Vulgare. *IOSR J. Pharm.* **2020**, *10*, 1–10.
36. Nagpal, R.; Kumar, A.; Kumar, M.; Behare, P.V.; Jain, S.; Yadav, H. Probiotics, their health benefits and applications for developing healthier foods: A review. *FEMS Microbiol. Lett.* **2012**, *334*, 1–15. [CrossRef] [PubMed]
37. Joung, J.Y.; Lee, J.Y.; Ha, Y.S.; Shin, Y.K.; Kim, Y.; Kim, S.H.; Oh, N.S. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. *Korean J. Food Sci. Anim. Resour.* **2016**, *36*, 90. [CrossRef] [PubMed]
38. Yadav, K.; Shukla, S. Microbiological, physicochemical analysis and sensory evaluation of herbal yogurt. *Pharma Innov.* **2014**, *3*, 1.