

## Article

# Developing New High-Protein-Content Traditional-Type Greek Yoghurts Based on Jersey Cow Milk

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**Abstract:** In the past few years, Jersey cow milk has been considered an effective commodity for the production of dairy products, such as cheese and yoghurts, due to its high nutrient density. Initially, the microbial safety and physicochemical properties of pasteurized milk from Jersey cows raised in Evrofarma S.A. dairy facilities were monitored and compared to Holstein Friesian milk over a six-month period. Subsequently, a new traditional-type Greek yoghurt was prepared using Jersey cow milk and the physicochemical, microbiological and sensory properties were monitored throughout its shelf time and compared to commercially available traditional-type Greek yoghurts made with Milk Protein Concentrate powder (MPC)-fortified Holstein Friesian milk. Our study confirmed the significantly higher fat (29.41%), protein (20.6%) and calcium content (19.9%) compared to Holstein Friesian milk. The new yoghurt demonstrated several technological advantages and shared similar physicochemical properties with the commercially available products. Importantly, high protein concentration (4.03 g/100 g) was documented without the addition of MPC. Finally, the new yoghurt was accepted during the preliminary sensory evaluation, while similar scores with the commercially available products were noted during the texture, flavour, aroma and overall quality assessment.

**Keywords:** jersey cow milk; holstein friesian cow milk; traditional greek yoghurt; milk protein; calcium



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## 1. Introduction

Greek yoghurt is a strained-type yoghurt that has become popular in recent years mainly for its creamy texture, rich flavour, high protein content and low lactose levels [1,2]. Typically, it is produced from cow's milk, and its standard production process involves bacterial fermentation (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and whey straining from regular yoghurt to create a thicker and creamier product with high total solids and a characteristic strong tangy flavour [3,4]. Traditional Greek yoghurt, on the other hand, is a set-style yoghurt variant allowed to thicken naturally through bacterial fermentation, without straining out the whey and can be produced with either cow, goat or ewe's milk [5,6]. It is produced without homogenization, leading to the development of a characteristic layer of crust, formed by fat and milk's serum proteins, on the surface of the product [5,7]. Generally, this type of yoghurt contains at least 3.25% milk fat [5] and tends to be substantially more acidic than strained-type Greek yoghurt, with its pH ranging from 4.0 to 4.4 [8].

Dairy product manufacturing in Europe relies predominantly on the use of milk from the Holstein Friesian breed [9]. In several cases, in order to achieve the desired levels of protein content and gel firmness in dairy products, such as strained or set yoghurt, fortification with skim milk powder, whey protein concentrates or Milk Protein Concentrates (MPC) is carried out [1]. MPC is a protein-rich powder with an identical ratio of casein to whey

protein of milk, and it is manufactured by either milk ultrafiltration or diafiltration, optional evaporation and spray drying [10]. The addition of MPC to yoghurt has been reported to yield higher total solids and protein content in the final product, resulting in firm yoghurt gels with a smooth texture [11], increasing, however, the overall production cost.

In recent years, the superior properties of Jersey milk compared to Holstein Friesian milk have been uncovered [12], thus making it an attractive commodity available to the dairy industry for the production of diverse and nutrient-dense dairy products [13]. In particular, Jersey milk has been well documented for its higher density of solids and other nutrients, such as fat, protein, casein and calcium content [14], which can be used for higher yields in dairy product manufacturing, increased coagulation strength [13] and faster formation rates [15,16]. As a result, reduced quantities of milk are required, leading ultimately to a positive effect on environmental impact [17].

In this study, initially, microbial safety and physicochemical properties of pasteurized milk from Jersey cows reared in the Evrofarma S.A. dairy facilities, northeastern Greece, Thrace, were monitored for a 6-month period, in order to validate the higher protein, fat and calcium content of Jersey milk compared to Holstein Friesian. Subsequently, a new traditional-type Greek yoghurt was produced with pasteurized Jersey milk and investigated for its physicochemical, microbiological and organoleptic properties compared to the commercially available MPC-fortified traditional-type Greek yoghurts made with Holstein Friesian milk. Data supporting the high industrial potential of the new product are presented.

## 2. Materials and Methods

### 2.1. Raw Milk Collection and Pasteurization Process

Two different types of raw milk were collected from approximately 1000 Holstein Friesian cows and 220 Jersey cows reared on the same dairy farm in Mesti, Alexandroupolis, Greece. Both breeds were fed the same corn-silage-based diet. After lactation, the milk was loaded directly onto trucks and stored in separate silos containing either Holstein Friesian or Jersey milk, until pasteurization. Both types of milk were pasteurized by heating at 77 °C for 15 s.

### 2.2. Preparation of Traditional-Type Yoghurts

Traditional-type Greek yoghurt production was performed following standard practice by Evrofarma S.A. Dairy Industry (Didymoteicho, Greece). In detail, the traditional-type Greek yoghurts were prepared using pasteurized milk (by heating at 77 °C for 15 s) from either Jersey or Holstein Friesian cows. After pasteurization, only the Holstein Friesian milk was supplemented with 0.8% (*w/v*) MPC85 (Alinda-Velco SA, Thessaloniki, Greece), in order to achieve the desired total solids content (14% *w/w*) in the final yoghurt products, in accordance with the requirements of Evrofarma S.A. Dairy Industry; fortification of Jersey milk was considered unnecessary. Subsequently, both types of milk were homogenized and heated once again for 5 min at 92 °C. Milk was cooled to 43 °C, and starter cultures containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (YF-3331, Hansen Co., Copenhagen, Denmark) were added into separate containers with either Jersey or Holstein Friesian milk, in accordance with the manufacturer's guidelines, and the fermentation proceeded at 43 °C until pH levels of the yoghurts dropped to 4.0. Finally, after the fermentation process, the yoghurts were stored at 4 °C.

### 2.3. Sampling Procedure

Twenty-four batches of pasteurized milk samples from Jersey or Holstein Friesian cows, provided by the dairy company Evrofarma S.A. (Didymoteicho, Greece), were analysed for a period of 6 months on a weekly basis. Milk samples were collected after pasteurization, sealed into sterile plastic containers, placed in a cooler with freezer ice packs, immediately transported to the laboratory and analysed.

Subsequently, traditional-type Greek yoghurts made with Jersey (product in development) or MPC-fortified Holstein Friesian milk (commercially available product) were provided weekly for a period of 6 weeks, and tests were carried out throughout the safe consumption period of the products (22 days). In the same manner, yoghurt samples were collected the day after production, transported to the laboratory and kept at a refrigerated temperature (4 °C) until analysis was performed.

#### 2.4. Physicochemical Analyses

pH was determined with a pH meter pH-300i (WTW GmbH, Weilheim, Germany), according to the manufacturer's instructions.

Calcium content was determined according to the international standard ISO 12081:2010.

Total acidity was determined by titration with 4 g/L NaOH solution, and phenolphthalein was used as an indicator, according to AOAC 947.05.2000.

Total solids were determined according to the international standard ISO 6731:2010.

Lactose content was determined according to the international standard ISO 22662:2007.

The milk's fat was determined according to the international standard ISO 19662:2018, whereas the yoghurt's fat content was determined according to the international standards ISO 19662:2018 and ISO 3432:2008.

Protein content was determined using a EuroEA Elemental Analyser (EuroVector S.r.l., Pavia, Italy), according to the international standard ISO 14891:2002 (Dumas method). Quantification was carried out using a standard compound (EDTA) (Elemental Microanalysis, Okehampton, UK), and the nitrogen percentage of each sample was multiplied by 6.38.

Salt content was determined according to the international standard ISO 5943:2006.

Moisture content was determined by drying at 105 °C until constant weight (AOAC 925.23.2005).

For the determination of syneresis, 50 g of yoghurt sample was placed in a funnel with Whatman No. 1 filter paper (Whatman Ltd., Maidstone, UK) and left at 4 °C for 5 h. The percentage of syneresis was calculated from the volume of whey separated from the yoghurt mass multiplied by 2 [18].

The water holding capacity (WHC) was determined by centrifugation of a 10 × g yoghurt sample at 4500 × g for 30 min at 10 °C and calculated using the following equation [18]:

$$\text{WHC}(\%) = \left(1 - \frac{W1}{W2}\right) \times 100,$$

where W1 is the weight of whey after centrifugation, and W2 is the weight of the yoghurt sample.

#### Determination of Fatty Acid Methyl Esters (FAMES) in Milk

Fatty acids were initially isolated from milk samples, according to the instructions of the MAK174 kit (Sigma-Aldrich, St. Louis, MO, USA), followed by methyl esterification. The composition of FAMES was analysed with a MASTER GC Fast Gas Chromatography system (DANI Instruments S.p.a, Milan, Italy) equipped with a MASTER AS autosampler (DANI Instruments S.p.a.), a flame ionization detector (FID) and a Supelcowax 10 column (30 m × 0.25 mm i. d., 0.25 µm film thickness, Supelcowax 10, Bellefonte, PA, USA). For analysis, 1 µL of each sample was injected directly into the GC column. The column temperature was set at 50 °C and then increased to 260 °C at a rate of 3 °C/min, where it remained for 5 min. The injector was set to split mode (1:10) at 240 °C. The probe temperature was set at 280 °C, and He (linear velocity 0.40 m/s) was used as a carrier gas. Fatty acids were identified based on the Supelco 37 Component FAME Mix standard (Sigma-Aldrich, St. Louis, MO, USA).

## 2.5. Microbiological Analyses

Pasteurized milk and traditional-type Greek yoghurt samples were tested for total mesophilic counts (TMC) in Plate Count Agar (PCA) (Condalab, Madrid, Spain), according to the international standard ISO 4833-1:2013; coliforms in Violet Red Bile Agar (V.R.B.A.) (Condalab, Madrid, Spain), according to international standard ISO 4832:2006; *Enterobacteriaceae* in Violet Red Bile Glucose Agar (V.R.B.G.A.) (Condalab, Madrid, Spain), according to international standard ISO 21528-2:2017; *Staphylococcus aureus* in Baird-Parker Agar (BP) (Condalab, Madrid, Spain), according to international standard ISO 6888-1:1999; and yeasts/moulds in Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Condalab, Madrid, Spain), according to standard ISO 21527-1:2008. Additionally, the presence of *Salmonella* spp. and *Listeria monocytogenes* was determined, in accordance with the international standards ISO 6579-1:2017 and ISO 11290-1:2017, respectively.

## 2.6. Preliminary Sensory Evaluation of Yoghurts

The sensory evaluation (appearance, texture, flavour and aroma) of the traditional-type Greek yoghurts made with Jersey milk was carried out 2, 14 and 22 days after production. The test was conducted by a mixed panel of 10 adult subjects (5 men and 5 women) who are frequent consumers of dairy products. The subjects were asked to rate the products using a scale from 0 to 5 (0 = unacceptable, 5 = exceptional) using previously approved protocols [19]. The sensory evaluation was a blind, randomized test, and samples were administered at 4 °C in unmarked white containers. For comparison purposes, commercial samples of traditional-type Greek yoghurts made with MPC-fortified Holstein Friesian milk, provided by Evrofarma S.A. Dairy Industry (Didymoteicho, Greece), were also evaluated.

## 2.7. Statistical Analysis

Statistical significance was determined by factorial analysis of variance (ANOVA), followed by Duncan's multiple range test. Statistica version 12.0 was used to compute statistical significance at  $p < 0.05$ , coefficients and ANOVA.

## 3. Results

### 3.1. Milk Analyses

#### 3.1.1. Physicochemical Properties of Milk Samples

According to the results (Table 1), the total acidity, total solids, calcium, fat and protein content were significantly ( $p < 0.05$ ) different between Jersey and Holstein Friesian milk.

**Table 1.** Physicochemical properties of pasteurized Jersey or Holstein Friesian milk samples.

| Physicochemical Property         | Pasteurized Jersey Milk  | Pasteurized Holstein Friesian Milk |
|----------------------------------|--------------------------|------------------------------------|
| pH                               | 6.9 ± 0.1                | 6.9 ± 0.1                          |
| Total acidity                    | 0.14 ± 0.03 <sup>a</sup> | 0.12 ± 0.04 <sup>b</sup>           |
| Calcium content (mg/100 g)       | 142 ± 6 <sup>a</sup>     | 119 ± 4 <sup>b</sup>               |
| Total solids content (g/100 g)   | 13.6 ± 0.5 <sup>a</sup>  | 12.0 ± 0.2 <sup>b</sup>            |
| Lactose content (g/100 g)        | 4.6 ± 0.1                | 4.6 ± 0.1                          |
| Total protein content (g/100 mL) | 4.05 ± 0.07 <sup>a</sup> | 3.35 ± 0.11 <sup>b</sup>           |
| Total fat content (g/100 g)      | 4.4 ± 0.3 <sup>a</sup>   | 3.5 ± 0.1 <sup>b</sup>             |

Data are presented as mean ± SD. Significant differences ( $p < 0.05$ ) between Jersey and Holstein Friesian milk samples are shown with different letters in superscript.

The total acidity of Jersey milk samples (0.14 g lactic acid/100 g) was significantly higher ( $p < 0.05$ ) than Holstein Friesian milk (0.12 g lactic acid/100 g).

The total solids content in Jersey milk samples was also higher ( $p < 0.01$ ) (14.1%) compared to Holstein Friesian samples (12.0%).

Jersey milk had a higher ( $p < 0.01$ ) calcium concentration (19.9%) (141 mg calcium/100 g on average) compared to Holstein Friesian milk (118 mg calcium/100 g).

The fat content of Jersey milk was higher ( $p < 0.01$ ) by 29.41% at 4.4 g/100 g compared to Holstein Friesian milk at 3.5 g/100 g.

Additionally, Jersey milk had a higher ( $p < 0.01$ ) protein concentration by 20.6% (4.05 g/100 mL) compared to Holstein Friesian milk (3.35 g/100 mL).

In contrast, no significant ( $p > 0.05$ ) differences were noted between the pH and lactose content of the two types of milk (6.9 and 4.6 g/100 g, respectively).

Importantly, the fatty acid composition differed between pasteurized Jersey and Holstein Friesian milk samples (Table 2). In particular, Jersey milk had higher levels ( $p < 0.05$ ) of capric (C10:0), stearic (C18:0) and docosanoic acid (C22:0) methyl esters. Conversely, Holstein Friesian milk contained significantly higher levels ( $p < 0.05$ ) of pentadecanoic (C15:0), pentadecenoic (C15:1), heptadecenoic (C17:1), eicosanoic (C20:0), eicosadienoic (C20:2) and eicosapentaenoic acid (C20:5n3) methyl esters.

**Table 2.** Fatty acid composition of pasteurized milk from Jersey and Holstein Friesian cows.

| Fatty Acid                         | Pasteurized Milk Samples  |                           |
|------------------------------------|---------------------------|---------------------------|
|                                    | Jersey (%)                | Holstein Friesian (%)     |
| C <sub>4:0</sub>                   | 3.33 ± 0.63               | 3.79 ± 0.73               |
| C <sub>6:0</sub>                   | 1.51 ± 0.51               | 1.54 ± 0.41               |
| C <sub>8:0</sub>                   | 1.97 ± 0.58               | 1.58 ± 0.39               |
| C <sub>10:0</sub>                  | 3.72 ± 0.52 <sup>a</sup>  | 2.96 ± 0.44 <sup>b</sup>  |
| C <sub>11:0</sub>                  | 1.01 ± 0.28               | 1.15 ± 0.36               |
| C <sub>12:0</sub>                  | 2.79 ± 0.39               | 2.87 ± 0.36               |
| C <sub>14:0</sub>                  | 6.19 ± 0.49               | 5.93 ± 0.77               |
| C <sub>15:0</sub>                  | 0.46 ± 0.11 <sup>a</sup>  | 0.72 ± 0.08 <sup>b</sup>  |
| C <sub>15:1</sub>                  | 0.12 ± 0.03 <sup>a</sup>  | 0.83 ± 0.28 <sup>b</sup>  |
| C <sub>16:0</sub>                  | 24.14 ± 2.63              | 21.35 ± 2.26              |
| C <sub>16:1</sub>                  | 0.66 ± 0.20               | 0.70 ± 0.08               |
| C <sub>17:0</sub>                  | 16.79 ± 2.11              | 19.32 ± 2.23              |
| C <sub>17:1</sub>                  | 0.13 ± 0.03 <sup>a</sup>  | 0.39 ± 0.1 <sup>b</sup>   |
| C <sub>18:0</sub>                  | 16.51 ± 2.41 <sup>a</sup> | 13.00 ± 2.03 <sup>b</sup> |
| C <sub>18:1n9c &amp; 18:1n9t</sub> | 9.67 ± 1.55               | 11.30 ± 1.88              |
| C <sub>18:2n6t</sub>               | 4.39 ± 0.84               | 3.93 ± 0.69               |
| C <sub>18:3n6</sub>                | 2.97 ± 0.71               | 3.38 ± 0.82               |
| C <sub>18:3n3</sub>                | 0.26 ± 0.03               | 0.30 ± 0.06               |
| C <sub>20:0</sub>                  | 0.30 ± 0.07 <sup>a</sup>  | 0.62 ± 0.14 <sup>b</sup>  |
| C <sub>20:2</sub>                  | 1.53 ± 0.37 <sup>a</sup>  | 1.95 ± 0.26 <sup>b</sup>  |
| C <sub>20:5n3</sub>                | 0.44 ± 0.11 <sup>a</sup>  | 0.98 ± 0.28 <sup>b</sup>  |
| C <sub>22:0</sub>                  | 0.28 ± 0.06 <sup>a</sup>  | 0.15 ± 0.05 <sup>b</sup>  |
| C <sub>23:0</sub>                  | 0.96 ± 0.16               | 0.84 ± 0.15               |

Data are presented as mean ± SD. Significant differences ( $p < 0.05$ ) between Jersey and Holstein Friesian milk samples are shown with different letters in superscript. The composition of fatty acid methyl esters was determined by gas chromatography (GC/FID). In total, 24 fatty acid methyl esters of different chain sizes (ranging from 4 to 23 carbon atoms) were detected in both Jersey and Holstein Friesian milk samples.

### 3.1.2. Microbiological Properties of Milk Samples

The results of microbiological analysis on Jersey and Holstein Friesian milk samples were evaluated according to Regulation (EC) 853/2004 of the European Parliament [20].

Regarding the microbiological quality of the pasteurized milk samples, none of the tested spoilage and foodborne pathogenic microorganisms (*Enterobacteriaceae*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp.) were detected, as expected. TMC and coliforms for both types of pasteurized milk were below the limits set by the European Regulation (EC), <3.70 logcfu/mL (or 5000 cfu/mL) and <0.70 logcfu/mL (or 5 cfu/mL), respectively.

### 3.2. Yoghurts

#### 3.2.1. Physicochemical Properties of Yoghurts

Yoghurt samples, made with either Jersey or MPC-fortified Holstein Friesian milk, were analysed at various time points throughout their shelf time period (22 days) for their physicochemical properties. In general, the physicochemical properties of all traditional-type yoghurts (Table 3) ranged within normal levels [21]. pH, total acidity and syneresis were significantly ( $p < 0.05$ ) affected by the storage period, while fat and total protein significantly differed ( $p < 0.05$ ) among the two products. Calcium and salt content did not differ between the two yoghurts and was not affected by the storage period. Finally, water holding capacity was increased ( $p < 0.05$ ) in yoghurts made with Jersey milk and was significantly ( $p < 0.05$ ) affected by the storage period in all cases.

**Table 3.** Physicochemical properties of traditional-type Greek yoghurts during the safe consumption period of the product (22 days).

| Physicochemical Properties                        | Type of Milk      | Yoghurt Storage Time at 4 °C (Days) |                           |                          |
|---|-------------------|-------------------------------------|---------------------------|--------------------------|
|   |                   | 2                                   | 14                        | 22                       |
| pH  | Jersey            | 4.03 ± 0.06 <sup>A</sup>            | 3.95 ± 0.06 <sup>A</sup>  | 3.93 ± 0.07 <sup>B</sup> |
|   | Holstein Friesian | 4.03 ± 0.01 <sup>A</sup>            | 3.98 ± 0.06 <sup>AB</sup> | 3.96 ± 0.05 <sup>B</sup> |
| Total acidity<br>(g lactic acid/100 g of yoghurt) | Jersey            | 1.24 ± 0.07 <sup>A</sup>            | 1.32 ± 0.08 <sup>AB</sup> | 1.36 ± 0.08 <sup>B</sup> |
|   | Holstein Friesian | 1.24 ± 0.09 <sup>A</sup>            | 1.30 ± 0.08 <sup>AB</sup> | 1.34 ± 0.04 <sup>B</sup> |
| Calcium<br>(mg/100 g of yoghurt)                  | Jersey            | 120 ± 5                             | 119 ± 3                   | 119 ± 2                  |
|   | Holstein Friesian | 121 ± 2                             | 123 ± 3                   | 118 ± 1                  |
| Salt<br>(g/100 g of yoghurt)                      | Jersey            | 0.14 ± 0.01                         | 0.15 ± 0.01               | 0.15 ± 0.01              |
|   | Holstein Friesian | 0.14 ± 0.01                         | 0.15 ± 0.01               | 0.14 ± 0.01              |
| Syneresis<br>(%)                                  | Jersey            | 36.2 ± 1.5                          | 35.4 ± 2.6                | 34.7 ± 1.9               |
|   | Holstein Friesian | 38.2 ± 1.5 <sup>A</sup>             | 36.9 ± 1.6 <sup>AB</sup>  | 34.3 ± 1.8 <sup>B</sup>  |
| Water holding capacity<br>(%)                     | Jersey            | 55.9 ± 2.3 <sup>Aa</sup>            | 59.1 ± 1.5 <sup>Ba</sup>  | 58.4 ± 1.3 <sup>Ba</sup> |
|   | Holstein Friesian | 52.7 ± 2.7 <sup>b</sup>             | 54.3 ± 2.5 <sup>b</sup>   | 55.0 ± 1.6 <sup>b</sup>  |
| Moisture content<br>(%)                           | Jersey            | 83.0 ± 1.0                          | 83.4 ± 0.9                | 84.1 ± 1.1               |
|   | Holstein Friesian | 83.3 ± 0.3                          | 83.2 ± 1.1                | 83.7 ± 0.9               |
| Fat<br>(%)  | Jersey            | 5.1 ± 0.5 <sup>a</sup>              | 5.1 ± 0.6 <sup>a</sup>    | 5.1 ± 0.5 <sup>a</sup>   |
|   | Holstein Friesian | 4.6 ± 0.5 <sup>b</sup>              | 4.6 ± 0.6 <sup>b</sup>    | 4.6 ± 0.6 <sup>b</sup>   |
| Protein content<br>(g/100 g of yoghurt)           | Jersey            | 4.01 ± 0.11 <sup>a</sup>            | 4.02 ± 0.08 <sup>a</sup>  | 4.04 ± 0.05 <sup>a</sup> |
|   | Holstein Friesian | 4.28 ± 0.03 <sup>b</sup>            | 4.27 ± 0.11 <sup>b</sup>  | 4.29 ± 0.7 <sup>b</sup>  |

Data are presented as mean ± SD. Significant differences ( $p < 0.05$ ) among traditional-type Greek yoghurts, resulting from varying storage periods, are indicated by different uppercase letters in superscript. Significant differences ( $p < 0.05$ ) between traditional-type Greek yoghurts made with either Jersey or MPC-fortified Holstein Friesian milk, at the same storage time point, are indicated by different lowercase letters in superscript.

In particular, initial pH values averaged 4.03 in both types of traditional-type yoghurt and were significantly decreased ( $p < 0.05$ ) after the third week of storage. Along with the decrease in pH, a significant increase ( $p < 0.05$ ) in the total acidity was observed, which reached up to 1.36 g lactic acid/100 g in the third week of storage for both types of yoghurt.

Calcium and salt content ranged from 118 to 123 mg/100 g and 0.14 to 0.15 mg/100 g, respectively, and, in both cases, no significant difference between the two yoghurt products was observed.

Syneresis percentage determined in the traditional-type yoghurts decreased during the third week of storage (34.7%), but not significantly. A slight increase, up to 58.4%, was observed for water holding capacity during storage, but it was not significant ( $p > 0.5$ ).

Moisture content levels ranged from 82.0% to 84.0% in all yoghurts and did not significantly differ between the two products.

The fat content of the traditional-type yoghurts made with Jersey milk (5.1%) was significantly higher ( $p < 0.05$ ) compared to the corresponding yoghurts (4.6%) made from Holstein Friesian milk.

Finally, the total protein content was higher ( $p < 0.05$ ) in the yoghurts made with MPC-fortified Holstein Friesian milk (4.28 g/100 g) compared to those made with Jersey milk (4.03 g/100 g).

### 3.2.2. Microbiological Quality of Yoghurts

To ensure safety and to assess the prevailing microbial flora of the traditional-type yoghurts, weekly microbiological tests were carried out for a period of 22 days (Table 4).

**Table 4.** Microbiological properties of traditional-type Greek yoghurts during the safe consumption period of the product (22 days).

| Microbiological Properties   | Type of Milk      | Yoghurt Storage Time at 4 °C (Days) |                           |                          |
|--|-------------------|-------------------------------------|---------------------------|--------------------------|
|  |                   | 2                                   | 14                        | 22                       |
| TMC (logcfu/g)   | Jersey            | 8.75 ± 0.06 <sup>A</sup>            | 8.56 ± 0.10 <sup>B</sup>  | 8.33 ± 0.15 <sup>C</sup> |
|  | Holstein Friesian | 8.65 ± 0.22 <sup>A</sup>            | 8.54 ± 0.17 <sup>AB</sup> | 8.38 ± 0.14 <sup>B</sup> |
| <i>Streptococcus thermophilus</i> (logcfu/g)                         | Jersey            | 8.71 ± 0.06 <sup>A</sup>            | 8.53 ± 0.11 <sup>AB</sup> | 8.30 ± 0.15 <sup>B</sup> |
|  | Holstein Friesian | 8.60 ± 0.24 <sup>A</sup>            | 8.49 ± 0.18 <sup>AB</sup> | 8.35 ± 0.14 <sup>B</sup> |
| <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> (logcfu/g) | Jersey            | 7.52 ± 0.32 <sup>A</sup>            | 7.30 ± 0.36 <sup>AB</sup> | 7.06 ± 0.22 <sup>B</sup> |
|  | Holstein Friesian | 7.58 ± 0.13 <sup>A</sup>            | 7.49 ± 0.22 <sup>A</sup>  | 7.20 ± 0.18 <sup>B</sup> |
| Yeasts/molds (cfu/g)   | Jersey            | <100                                | <100                      | <100                     |
|  | Holstein Friesian | <100                                | <100                      | <100                     |
| <i>Staphylococcus aureus</i> (cfu/g)                                 | Jersey            | <100                                | <100                      | <100                     |
|  | Holstein Friesian | <100                                | <100                      | <100                     |
| <i>Listeria monocytogenes</i> (+/−)                                  | Jersey            | -                                   | -                         | -                        |
|  | Holstein Friesian | -                                   | -                         | -                        |
| <i>Salmonella</i> spp. (+/−)   | Jersey            | -                                   | -                         | -                        |
|  | Holstein Friesian | -                                   | -                         | -                        |

Data are presented as mean ± SD. Significant differences ( $p < 0.05$ ) among traditional-type Greek yoghurts (Jersey/Holstein Friesian), resulting from varying storage periods, are indicated by different uppercase letters in superscript. TMC: total mesophilic counts, (+): detected in 25 g of yoghurt, (−): not detected in 25 g of yoghurt.

*L. bulgaricus* levels were significantly ( $p < 0.05$ ) influenced by the storage period. In particular, the initial levels of *L. bulgaricus*, ranging >7.50 logcfu/g, were reduced significantly ( $p < 0.05$ ) down to 7.19 logcfu/g during the third week of storage at 4 °C, for both yoghurts produced either with Jersey milk (7.20 logcfu/g) or with MPC-fortified Holstein Friesian milk.

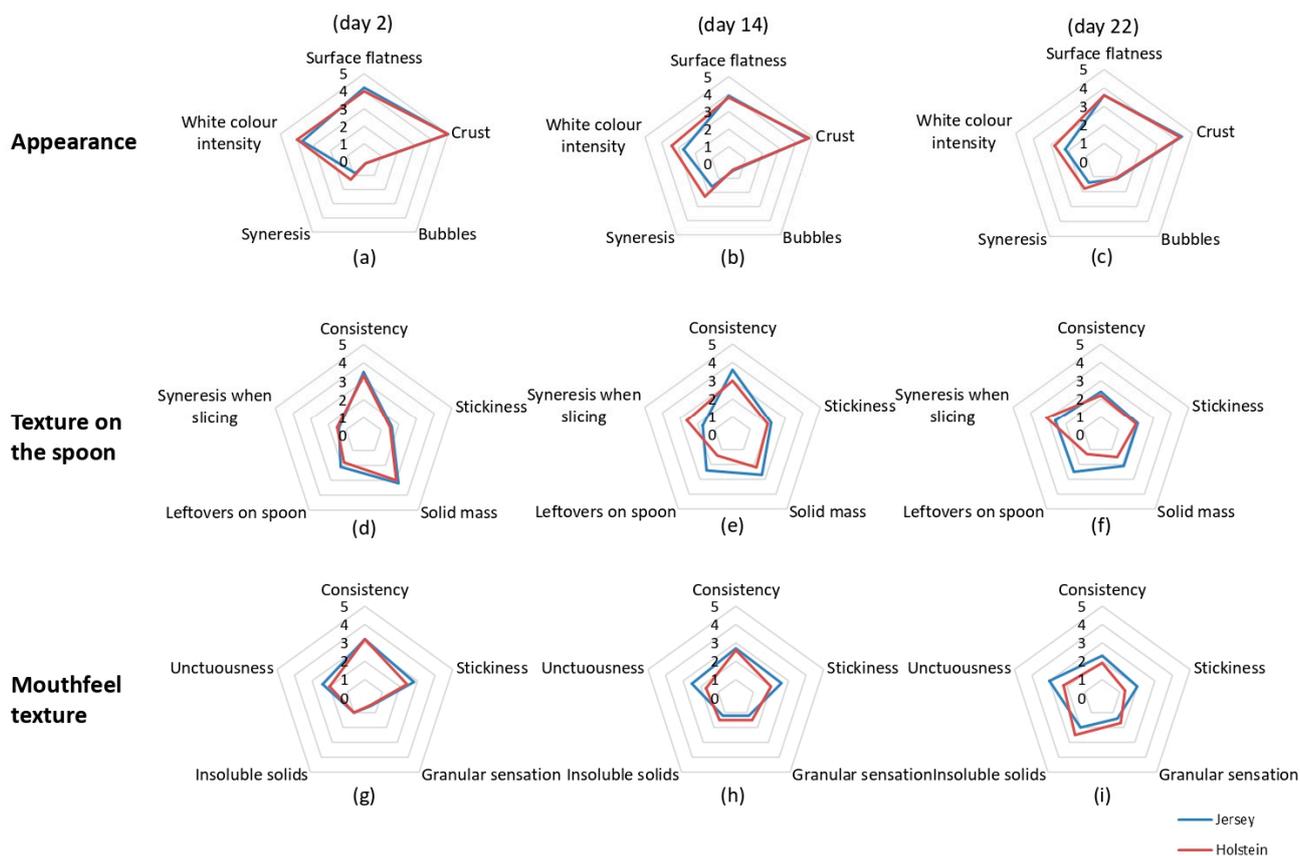
Similarly, the levels of *S. thermophilus* were significantly affected by the storage period ( $p < 0.05$ ) of the products; however, *S. thermophilus* levels remained >8.30 logcfu/g throughout the shelf life of the product.

Finally, it is important to note that in all yoghurts tested, no food spoilage or pathogenic microorganisms (coliforms, yeast/moulds, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp.) were detected throughout the shelf-life period.

### 3.2.3. Preliminary Sensory Evaluation

Traditional-type Greek yoghurts made with Jersey milk were evaluated for their appearance, texture on the spoon and mouthfeel (Figure 1), and they were compared to commercially available traditional-type yoghurts made with MPC-fortified Holstein Friesian milk. According to the results, all product appearance characteristics (flat surface, crust, the presence of bubbles at the edges of the container, syneresis and colour) were significantly ( $p < 0.05$ ) affected by storage at 4 °C. Additionally, syneresis on the surface, as well as the colour of the product, significantly ( $p < 0.05$ ) differed between the two products.

Specifically, at the end of the storage period (day 22), visible crust alterations appeared on the surface of the yoghurt, mainly at the edges of the container, resulting in a less flat product compared to the beginning of storage (day 2). It is also worth noting that at the end of the storage period, an increased ( $p < 0.05$ ) stickiness was observed in products made with Holstein Friesian milk, while a significant change ( $p < 0.05$ ) of colour in yoghurts made with Jersey milk was reported. Nevertheless, the products were still accepted by the panel.



**Figure 1.** Appearance (a–c), texture on the spoon (d–f) and mouthfeel (g–i) of traditional-type Greek yoghurts made with Jersey or Holstein Friesian milk.

The texture characteristics on the spoon, consistency, formation of a solid mass and syneresis when slicing were significantly ( $p < 0.05$ ) affected by the storage duration and differed between the two products. In both products, the increase ( $p < 0.05$ ) in syneresis observed during slicing at the end of the storage period (day 22) combined with the loss of the uniform shape of the yoghurt mass on the spoon resulted in a significant decrease ( $p < 0.05$ ) in product cohesion, compared to the beginning of storage (day 2). However, at day 22, the stickiness of the yoghurt texture increased significantly ( $p < 0.05$ ) for all products, while the traces of yoghurt remaining on the spoon after slicing increased significantly ( $p < 0.05$ ) only for traditional-type yoghurts made from Jersey milk.

Regarding the texture of traditional-type yoghurt upon tasting, the milk used (Jersey or Holstein Friesian) and the storage time of the products affected significantly ( $p < 0.05$ ) the unctuousness, the sticky texture and the presence of insoluble solids. However, product consistency and granule-like sensation were significantly ( $p < 0.05$ ) influenced only by the storage period, while a significant decrease and increase were observed, respectively, at day 22. The increased presence of insoluble solids ( $p < 0.05$ ) in yoghurts made with Holstein Friesian milk on the 22nd day of storage is mainly attributed to the characteristic crust found in traditional-type yoghurt products. When combined with an increase in syneresis and consequent decrease in consistency during storage, a granule-like sensation might be

detected upon tasting the product. Notably, some consumers also reported the presence of clumps in the yoghurts even without the crust, but without adversely affecting the product.

Finally, the flavour, aroma and overall assessment of the traditional-type yoghurts (Table 5) were significantly ( $p < 0.05$ ) influenced only by the duration of shelf life at 4 °C and not by the type of milk used. All products were characterized by a sour taste, indicative of this type of yoghurt [5], which increased in intensity, especially at the end of storage, probably as a result of low pH and increased total acidity. The aroma of the products was described by consumers as natural/traditional, but due to increased syneresis and whey elimination at the end of storage, it was in some cases considered excessive. However, all products were considered acceptable.

**Table 5.** Evaluation of the sensory characteristics of traditional-type Greek yoghurts made from Jersey or Holstein Friesian milk during storage at 4 °C.

| Storage Time (Days) | Milk Type Used | Sensory Characteristic  |                        |                        |
|---------------------|----------------|-------------------------|------------------------|------------------------|
|                     |                | Flavour                 | Aroma                  | Overall Assessment     |
| 2                   | Jersey         | 3.9 ± 0.3 <sup>ac</sup> | 4.0 ± 0.1 <sup>a</sup> | 4.0 ± 0.1 <sup>a</sup> |
|                     | Holstein       | 4.0 ± 0.1 <sup>a</sup>  | 4.0 ± 0.5 <sup>a</sup> | 4.2 ± 0.4 <sup>a</sup> |
| 14                  | Jersey         | 4.1 ± 0.3 <sup>a</sup>  | 3.8 ± 0.4 <sup>a</sup> | 4.1 ± 0.3 <sup>a</sup> |
|                     | Holstein       | 4.0 ± 0.1 <sup>a</sup>  | 3.7 ± 0.5 <sup>a</sup> | 4.1 ± 0.3 <sup>a</sup> |
| 22                  | Jersey         | 3.6 ± 0.5 <sup>bc</sup> | 3.1 ± 0.3 <sup>b</sup> | 3.2 ± 0.4 <sup>b</sup> |
|                     | Holstein       | 3.4 ± 0.5 <sup>b</sup>  | 3.3 ± 0.5 <sup>b</sup> | 3.0 ± 0.1 <sup>b</sup> |

Data are presented as mean ± SD. Significant differences ( $p < 0.05$ ) in the same column are indicated by different letters in superscript.

#### 4. Discussion

As consumer awareness about health-promoting food consumption continues to rise, selecting the appropriate type of milk and dairy products has become a crucial consideration. Milk is considered a basic component of a balanced diet by many individuals, supplying various nutrients that promote human health [22]. Holstein Friesian and Jersey milk are two prevalent types of milk, each with distinct advantages. In general, Holstein Friesian milk is distinguished by its mild taste and high production yield [23], whereas Jersey milk is recognized for its thick texture and creamy colour [24].

While the physicochemical characteristics of milk can be influenced by several factors, such as the age, health and diet of the lactating cow [25,26], it has been observed that milk produced by particular breeds of cows tends to have distinct properties [27,28]. In particular, Jersey cows produce milk with a higher total solids content, including fat and protein, when compared to Holstein Friesian milk [29]. Previous studies have also reported the higher calcium content of Jersey milk [13–15], an important attribute for consumer health with a key role in the dairy industry, as milk and dairy products are widely regarded as its main sources [30]. In accordance with the abovementioned studies, our research confirmed similar findings demonstrating the increased protein, fat and calcium concentration in Jersey compared to Holstein Friesian milk.

The fatty acid composition of milk is another critical factor affecting the nutritional value and sensory properties of dairy products [31]. In general, higher percentages of saturated fatty acids (short, medium and long chain) and lower percentages of unsaturated fats are observed in cow's milk [32]. In our study, saturated fatty acids C10:0, C18:0 and C22:0 were detected in significantly higher levels in Jersey compared to Holstein Friesian milk samples, in agreement with previous studies [33,34]. In contrast, saturated fatty acids C15:0 and C20:0 were determined in higher levels in the Holstein Friesian milk samples. Regarding the unsaturated fatty acids, the monounsaturated fatty acids (MUFA) C15:1 and C17:1 and the polyunsaturated fatty acids (PUFA) C20:2 and C20:5n3 were in higher levels in Holstein Friesian milk samples, and, in all cases, the percentages determined were <2% [15,35,36].

Overall, cow's milk fatty acid composition is influenced by a variety of factors, including the cow's diet, genetics, stage of lactation, processing method and season [37–40]. The type and quality of feed consumed by the cow can significantly impact the fatty acid composition of the milk, with cows fed on fresh pasture or hay producing milk with higher levels of omega-3 fatty acids [41]. Additionally, certain breeds, such as Jersey and Guernsey cows, tend to produce milk with higher levels of fat [42]. The stage of lactation can also impact the fatty acid composition, with milk produced in the early stages being generally higher in fat and protein, while milk produced later in lactation is higher in lactose [43]. Likewise, processing methods, such as homogenization and pasteurization can affect the structure and distribution of fats, affecting the absorption and utilization of fatty acids. In particular, it has been reported that thermal treatment can alter milk's lipid profile, including total lipids, free fatty acids, conjugated linoleic acid and *trans* fatty acids [44–47]. However, according to Xu et al. [44], major alterations in milk's fatty acid profile can be observed only in the case of Ultra-High Temperature (UHT) treated milk, while slighter changes may occur, as well, in pasteurized milk samples [44,48]. Moreover, the season of the year can also affect the fatty acid composition, with cows grazing on fresh pasture in the spring and summer producing milk with higher levels of omega-3 fatty acids, whereas cows fed hay and silage in the winter produce milk with higher levels of omega-6 fatty acids [49]. It has to be noted that even though milk's fatty acid profile is considered a good indicator of nutritional value, in recent years, there has been a strong belief that the type of food matrix is more important for consumer health than the fatty acid composition [50].

Traditional Greek yoghurt is a set-style yoghurt variant with a characteristic crust on its surface, previously described by several studies [5–8,51,52]. Along with the well-known "Greek yoghurt" (strained-type yoghurt), the traditional recipe holds the largest market share in the Greek dairy industry for yoghurt sales [52]. Historically, traditional Greek yoghurt has been produced on a small scale in rural cottage dairy industries using milk from various animals, including ewes, goats and cows, depending on the region's dairy farming practices and the availability of different types of livestock. Nowadays, traditional Greek yoghurt is being produced on a large scale by several Greek dairy industries, appealing to a larger number of consumers. To produce traditional Greek yoghurt industrially, the milk's composition is adjusted to ensure that after being heated, the total solids content is increased by at least 10% compared to the milk's initial total solids content. The heated milk is mechanically dispensed into plastic retail pots of 220 to 300 g and inoculated with starter cultures using a dosimeter pump. The inoculated pots are then incubated in closed rooms with a stable temperature, followed by rapid cooling to prevent post-acidification. Lastly, the pots are sealed with a plastic film and a plastic lid [52]. Regarding the production of traditional Greek yoghurt made with cow's milk, Holstein Friesian milk has been used almost exclusively due to the high dependence of the Greek dairy industry on the Holstein Friesian breed, which numbers approximately 200,000 cows [53]. The Jersey breed has been introduced only recently to Greek dairy farming, and, due to its superior milk attributes, it is regarded a valuable commodity in the production of new, diverse and nutrient-dense dairy products [13].

The total solids content of yoghurt plays a crucial role in determining the firmness and texture of the final product. In general, consumers prefer yoghurts with a higher total solids content, leading to a more appealing thicker and creamier texture [54]. Although most of the physicochemical attributes of the new traditional-type Greek yoghurts made with Jersey milk ranged within the normal levels [5], and no significant differences were observed compared to the yoghurts produced with MPC-fortified Holstein Friesian milk, there were some noticeable variations between the two types of yoghurts. Yoghurts prepared with Jersey milk had a higher fat content, whereas yoghurts produced with Holstein Friesian milk had a higher total protein content. These differences can be explained by the composition of the milk used for yoghurt production, particularly the higher fat content of Jersey milk and the fortification of Holstein Friesian milk with MPC. Notably, fortification of Jersey milk was considered unnecessary.

Moreover, the difference in calcium content, observed in the pasteurized Jersey and Holstein Friesian milk samples, was not recorded in the yoghurts. The observed equivalence in calcium concentration between the two types of yoghurts most likely stems from the addition of MPC to the Holstein Friesian milk, as it contains approximately 2.1 g of calcium per 100 g of protein powder.

The storage duration at 4 °C for 22 days had a significant impact on various attributes, including microbial counts, pH, total acidity and sensory evaluation scores. The initial counts of *S. thermophilus* and *L. bulgaricus* did not differ between the two types of yoghurts and were recorded >8.60 logcfu/g and >7.52 logcfu/g, respectively [19]. However, at the end of the storage duration (22 days), both culture counts were significantly reduced, a result most likely associated with the observed reduction of pH values and the increase of total acidity in all samples [7].

Regarding the sensory properties during storage at 4 °C for 22 days, both types of yoghurt received lower scores for flavour, aroma and overall assessment compared to baseline (day 2), a result mainly due to a sourer taste, a loss of consistency and white colour intensity and a stronger aroma. Similar alterations were also observed in the study of Maragkoudakis et al. [7], in which traditional Greek yoghurts made with different sets of starter cultures, stored at a refrigerated temperature for 14 days, exhibited higher acidity and firmness values on the 14th day of storage compared to initial levels.

In general, the Holstein Friesian breed supplies the vast majority of bovine milk used to manufacture dairy products in Europe [55]. The selection of Holstein Friesian cows relies heavily on their higher milk yield compared to other bovine breeds, estimated to be, on average, >10,000 kg per year [56]. The annual milk production of Jersey cattle, on the contrary, has been estimated to be substantially lower, at around >6000 kg [57]. However, due to the high total solids, protein and fat content, Jersey milk is regarded as more efficient in the production of dairy products, such as cheese and yoghurt. According to previous studies [17], the use of Jersey milk instead of Holstein Friesian milk can result in a reduction in the number of natural resources required to produce the same amount of Cheddar cheese. This estimation was attributed not only to the lower amounts of Jersey milk required for cheese production, but also to the lower overall energy requirements of Jersey cows, which have a significantly lower body weight compared to Holstein Friesian cows. It has been pointed out that the efficiency of Jersey milk in dairy product manufacture also results in significantly lower greenhouse gas emissions. In particular, for the production of 500,000 t of Cheddar cheese, carbon dioxide, methane and nitrous oxide emissions were reduced by 20.5% when Jersey milk was used instead of Holstein-Friesian milk, a result that can help alleviate the problem of global climate change [17].

In the present study, the use of Jersey milk was evaluated as an alternative to MPC-fortified Holstein Friesian milk for the production of a traditional-type Greek yoghurt. Greek yoghurt, strained or traditional-type, has been extremely popular among dairy product consumers, predominantly due to its high protein content and semi-solid texture [5,58,59]. In many cases, fortification of bovine milk with MPC is performed in order to produce the desired effect of increased total solids and protein supplementation [60]. Jersey milk, however, inherently possesses these properties and can be substituted for the fortification of yoghurts with MPC, leading to lower production costs besides the abovementioned technological advantages.

## 5. Conclusions

The findings of this study confirmed that Jersey milk exhibits a significantly higher content of protein, fat and calcium compared to Holstein Friesian milk. Moreover, traditional Greek yoghurts produced using Jersey milk demonstrated similar physicochemical properties to commercially available products. Notably, although the protein content of Jersey milk yoghurt was slightly lower than that of commercially available MPC-fortified Holstein Friesian yoghurts, it was still found to be relatively high (4.03 g/100 g of yoghurt). Sensory evaluation of the products indicated that all yoghurt samples received

similar scores for texture, flavour, aroma and overall assessment, even after a 22-day storage period at 4 °C. These results highlight the great potential of Jersey milk for the production of a nutrient-dense, traditional-type Greek yoghurt, while offering significant technological advantages.

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