

## Article

# Acid Whey from Industrial Greek Strained Yoghurt: Effect of the Kind of Milk and the Way of Straining on Its Composition and Processing by Nanofiltration

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**Abstract:** The acid whey derived during the production of Greek yoghurt, i.e., yoghurt acid whey (YAW), is considered as environmental pollutant due to its low pH and high lactose content. YAW may be obtained by centrifugation or ultrafiltration or filtration through cloth bags (traditional method) of the initial yoghurt, methods that could affect its composition. In the last years, efforts have been made to improve its composition using membrane technology. In the present work, the composition of 35 different YAW samples from Greek Yoghurt dairies was studied. The YAW samples were grouped according to the way of production and the kind of milk in the case of the traditional method. The results showed that both the kind of milk and the way of yoghurt straining affected its composition. Ovine YAW derived from traditional straining was richer in lactic acid and calcium than bovine YAW. Moreover, the composition of bovine YAW varied among the different ways of straining, and this affected its behaviour during membrane processing. Nanofiltration of three representative bovine YAW samples and one ovine YAW sample at their natural pH, i.e., pH 4.5, and at 25 °C removed the lactic acid at a range from 40 to 55%, and the monovalent cations > 60% and retained lactose and galactose at percentages > 95% and 80% respectively.

**Keywords:** acid whey; nanofiltration; ovine whey; bovine whey; lactic acid; lactose



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## 1. Introduction

The consumption of strained yoghurt, known as Greek yoghurt or Greek-style yoghurt, is constantly increasing in Greece and worldwide because of its high nutritional value. In 2004, in the USA, the production of Greek yoghurt corresponded to less than 2% of the total production of various types of yoghurt, while in 2015, it reached 40% of the total production, recording a production of 771,000 tons [1]. However, the increase in Greek yoghurt production has a direct consequence for the production of large amounts of acid whey, herein called yoghurt acid whey (YAW). This is because for the manufacture of this type of yoghurt, 100 parts of milk are converted into 33 parts of yoghurt and 67 parts of YAW, or in other words, 2–3 kg of YAW are generated per 1 kg of Greek yoghurt [1,2]. Greek yoghurt is made worldwide with bovine milk, but in Greece, caprine or ovine milk are also used, while the straining process is carried out by various techniques, such as centrifugation or ultrafiltration or filtration through cloth bags (traditional straining) [3].

YAW is a slightly opaque liquid with a yellow–green colour due to the presence of riboflavin, containing mainly water-soluble components. This co-product may contain 3.33% to 4.99% lactose, 0.17% to 0.68% crude protein, 0.64% to 0.98% ash, 0.64% to 1.40% lactic acid or 5.6% to 7.09% total solids, and its pH ranges from 4.21 to 4.60 [4–7]. Moreover, YAW is rich in calcium, phosphorous, potassium and sodium [6,8]. Because of its high organic composition, the disposal of YAW is an environmental problem. The COD and BOD values for YAW range from 52,400 to 64,400 mg/L and from 45,800 to 50,500 mg/L, respectively [4], but according to a comparative Life Cycle Assessment of the different Greek yoghurt production systems, the environmental profile of this product is not differentiated by the production technology [9]. On the other hand, in the framework of circular economy, research has been conducted for using either raw or powdered YAW as a food ingredient [10,11]. However, the presence of lactic acid and calcium deteriorates the quality of acid whey powders, causing stickiness and caking during spray drying, and hence limits their shelf life [12,13]. Several techniques have been applied to improve the composition of acid whey for better valorisation. It has been shown that electrodialysis of acid whey from fresh cheese under certain conditions can remove 80% of the lactate ions to achieve a similar ratio of lactic acid to lactose as found in sweet whey and, in parallel, can remove 90% of the minerals [14]. Moreover, processing of acid whey from fresh cheese by nanofiltration results in a 30% reduction in lactic acid content and 46–60% reduction in monovalent ions [12]. In addition, nanofiltration of acid whey from cottage cheese at pH 3 removes about 50% of lactic acid while retaining over 90% of lactose [15].

Regarding the composition and processing of YAW, to the best of our knowledge, the literature refers only to bovine YAW without discriminating against the way of its production [4–8]. Therefore, the main objective of the present study was to evaluate the composition of YAW considering the way of its removal, i.e., by centrifugation, ultrafiltration or filtration through cloth bags of the yoghurt gel, as well as the kind of milk. Furthermore, the separation efficiency of lactic acid and other compounds of bovine YAW obtained by the three different methods of straining and those of ovine YAW obtained by the traditional method was assessed by nanofiltration under the same conditions. Thus, this research is divided into two parts: one concerns compositional analyses of individual YAW samples, which were obtained from Greek dairies, and the second part concerns the behaviour in membrane processing, i.e., nanofiltration, of four representative YAW samples (three bovines, and one ovine).

## 2. Materials and Methods

### 2.1. Composition of Yoghurt Acid Whey

#### 2.1.1. Sampling of YAW

Thirty-five different samples of YAW were obtained from ten different Greek Dairies, which produce various Greek yoghurts. Usually, big dairies use bovine milk and perform a mechanical way of straining, i.e., centrifugation or ultrafiltration, to produce strained yoghurt, while small dairies use bovine, caprine and/or ovine milk and perform traditional straining through cloth bags. Thus, according to the kind of milk and the straining method of the initial yoghurt, the YAW samples were classified into five groups as follows: Bovine YAW obtained by centrifugation (samples BYAWce,  $n = 6$ ), by ultrafiltration of yoghurt gel (samples BYAWuf,  $n = 6$ ), by traditional straining of yoghurt gel through cloth bags (samples BYAWcb,  $n = 7$ ), caprine YAW and ovine YAW obtained by traditional straining (samples CYAWcb,  $n = 7$  and samples OYAWcb,  $n = 9$ , respectively).

### 2.1.2. Physicochemical Analyses of YAW Samples

The pH of YAW samples was measured on a pH meter (WTW multi3420, Weilheim Germany), and the acidity, expressed as g of lactic acid per 100 g of YAW, was determined by titration using NaOH N/9 solution and phenolphthalein as the indicator. The total solids, fat and crude protein contents were determined by infrared spectroscopy on a Milkoscan FT120 (Foss, Hilleroed, Denmark). Ash content was determined by the AOAC method [16]. The contents of the main inorganic elements, i.e., calcium, magnesium, potassium and sodium, were determined on a Shimadzu AA-6800 Atomic Absorption Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), equipped with the autosampler Shimadzu ASC-6100 and the software WizAArd v. 2.30 (Shimadzu Corporation, Kyoto, Japan), according to the ISO/IDF standard method [17]. Phosphorus content was determined by molecular absorption spectrometry [18].

The sugars and organic acids contents were determined by the HPLC method on a Perkin Elmer Flexar system (Waltham, MA, USA) coupled with RI and PDA detectors. For the sample preparation, 15 mL of YAW were mixed with 20 mL of tungstic acid (0.7% sodium tungstate dehydrate, 0.01% orthophosphate, 7% sulfuric acid 1N) and the final volume was made to equal 50 mL with ultrapure water. After 15 min, the diluted sample was filtered through Whatman No. 1 filter paper, and then to 1 mL of filtrate 100  $\mu$ L perchloric acid (70%) was added. After an overnight stay at 4 °C, the sample was centrifuged at 12,500 rpm for 60 min at 4 °C. The supernatant was analysed using the ion exchange column Aminex HPX-87H, 300 mm  $\times$  7.8 mm (Biorad Inc., Hercules, CA, USA), and under the conditions described by Karastamatis et al. [7].

The non-denatured whey proteins were qualitatively determined by the RP-HPLC method described by Moatsou et al. [19]. In brief, a Vydac C4 214 TP 5415 column (Columbia, MD, USA) was used, the gradient elution was performed using water-acetonitrile in the presence of trifluoroacetic acid solvents at a flow rate of 1 mL/min, and the eluent was monitored at 214 nm. Prior to analysis, the sample was centrifuged at 3000  $\times$  g for 30 min at 4 °C, and the supernatant was filtered through a syringe filter 0.45  $\mu$ m.

The content of vitamin B12 was determined by the competitive enzyme immunoassay RIDASCREEN®FAST Vitamin B12 kit (R-Biopharm AG, Darmstadt, Germany), using a standard curve. A microplate spectrophotometer Thermo Scientific Multiskan Sky (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for the measurement of absorption at 450 nm.

## 2.2. Membrane Processing of Yoghurt Acid Whey

### 2.2.1. Sampling

Three different lots of bovine YAW, which were generated by ultrafiltration and by centrifugation of yoghurt gel, were collected from two Greek Dairies. Regarding the traditional method of YAW production, i.e., through cloth bags, bovine YAW and ovine YAW were manufactured in triplicate in the Laboratory of Dairy Research. In brief, raw milk (10 kg) was heated at 95 °C for 5 min, it was allowed to cool at about 46 °C, and then it was inoculated with a commercial yoghurt culture (CH-1, Hansen, Skjern, Denmark). Coagulation took place at 45 °C to pH 4.75 (about 3 h), and then the coagulum cooled at 4 °C for 2.5 h. After, it was stirred and poured into a cloth bag to strain at 4 °C for 24 h. The composition of the bovine milk was  $3.68 \pm 0.05$  fat,  $3.29 \pm 0.18$  protein and  $12.33 \pm 0.13$  total solids. The composition of the ovine milk was  $7.31 \pm 0.31$  fat,  $6.31 \pm 0.07$  protein and  $18.36 \pm 0.28$  total solids.

### 2.2.2. Microfiltration and Nanofiltration of YAW

Microfiltration of YAW was performed on a pilot membrane unit MMS SW18 (MMS AG Membrane systems, Urdorf, Switzerland), using one tubular ceramic membrane of 1.4 µm pore size and 0.06 m<sup>2</sup> surface area (TAMI Industries, Z.A. Les Laurons, NYOS CEDEX, France) under constant transmembrane pressure (TMP) 1.94 bar at 25 °C.

Nanofiltration of the microfiltered YAW was performed on the same pilot membrane unit, using one ceramic membrane with nominal molecular weight cut off 250 Da, channel width 10 mm, length 60 mm and surface area 0.01 m<sup>2</sup> (inopor®, Scheßlitz, Germany). TMP was 10 bars, the filtration temperature 23 °C, the duration of processing 3 h and the concentration factor 1.5. Membrane processing of YAW was carried out in triplicate using the different lot of each YAW. After each microfiltration or nanofiltration running, the membrane was cleaned by rinsing in series with water for 20 min, 0.2% alkaline disinfectant solution (P3-hypochloran, ECOLAB) for 20 min, water for 10 min, 1% acid detergent solution (P3-ultrasil 78, ECOLAB) for 25 min, water for 10 min, alkaline solution for 20 min and water for 60 min.

The performance of the separation of the main components of each YAW was evaluated as proposed by Chandrapala et al., 2016 [15]. In brief, the separation efficiency of lactic acid, calcium, phosphorous, magnesium, potassium and sodium of the YAW was evaluated by calculating the sieving coefficient (%) that is defined as follows:  $S (\%) = (CiP/CiF) \times 100$ , where CiP and CiF are the concentration of each component in permeate and feed streams, respectively. The separation efficiency of protein, lactose and galactose was evaluated by calculating the rejection coefficient (%) defined as follows:  $R (\%) = (1 - CiP/CiF) \times 100$ .

### 2.2.3. Analyses of Membrane-Processed YAW

The pH, acidity, gross composition, calcium, magnesium, potassium, sodium, phosphorous, lactose, galactose and lactic acid contents were determined as described previously in Section 2.1.2.

### 2.3. Statistical Analysis

The data obtained were statistically processed with Statgraphics (Centurion XVI Manugistics software, Inc., Rockville, MD, USA). One-way ANOVA was performed, and the difference of the means for each component separately was tested by the least significant difference method at the 95% significance level ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. Physicochemical Composition of Raw YAW

The physicochemical composition of the different groups of YAW is presented in Table 1. It is obvious that the pH of YAW was significantly ( $p < 0.05$ ) affected by the generation method. The pH of mechanically produced YAW, i.e., by ultrafiltration or centrifugation, ranged from  $4.50 \pm 0.06$  to  $4.64 \pm 0.08$ , while the pH of traditionally removed YAW, i.e., filtration through cloth bags, ranged from  $4.11 \pm 0.28$  to  $4.39 \pm 0.22$ . Traditional straining usually takes place in small dairies, in which fermentation of milk is conducted using either fresh yoghurt or commercial yoghurt cultures with low post-acidification activity as the starter culture. Moreover, traditional straining takes more time than centrifugation or ultrafiltration, and this practice causes a further decrease of pH. Therefore, the significantly ( $p < 0.05$ ) lower pH of this type of YAW was attributed to the longer duration of the straining method. The acidity of bovine YAW was from  $0.42 \pm 0.07\%$  to  $0.49 \pm 0.13\%$  and was not significantly ( $p > 0.05$ ) affected by the method of straining. In contrast, the acidity of ovine YAW was significantly higher ( $1.00 \pm 0.27\%$ ), and this was related to its higher lactic acid content (Table 2). Moreover, in a comparative study among

yoghurts made from bovine, caprine and ovine milk, the higher acidity of ovine yoghurt was attributed to its higher protein and mineral content [20].

**Table 1.** pH, acidity (%), gross composition (%), inorganic elements (mg/100 g) and vitamin B12 ( $\mu\text{g/L}$ ) of yoghurt acid whey obtained from various Greek Yoghurt dairies using different straining methods (mean  $\pm$  SD,  $n$  = number of samples).

|                           | BYAWuf ( $n = 6$ )               | BYAWce ( $n = 6$ )              | BYAWcb ( $n = 7$ )              | CYAWcb ( $n = 7$ )                | OYAWcb ( $n = 9$ )                |
|---------------------------|----------------------------------|---------------------------------|---------------------------------|-----------------------------------|-----------------------------------|
| pH                        | 4.50 $\pm$ 0.06 <sup>b,c,*</sup> | 4.64 $\pm$ 0.08 <sup>c</sup>    | 4.39 $\pm$ 0.22 <sup>b</sup>    | 4.38 $\pm$ 0.16 <sup>b</sup>      | 4.11 $\pm$ 0.28 <sup>a</sup>      |
| Acidity                   | 0.49 $\pm$ 0.13 <sup>a</sup>     | 0.42 $\pm$ 0.07 <sup>a</sup>    | 0.49 $\pm$ 0.07 <sup>a</sup>    | 0.60 $\pm$ 0.18 <sup>a</sup>      | 1.00 $\pm$ 0.27 <sup>b</sup>      |
| <i>Gross composition</i>  |                                  |                                 |                                 |                                   |                                   |
| Total solids              | 4.68 $\pm$ 0.72 <sup>a</sup>     | 5.12 $\pm$ 0.82 <sup>a</sup>    | 5.10 $\pm$ 0.42 <sup>a</sup>    | 5.17 $\pm$ 0.53 <sup>a</sup>      | 6.19 $\pm$ 0.73 <sup>b</sup>      |
| Total sugars              | 3.29 $\pm$ 0.23 <sup>a</sup>     | 3.65 $\pm$ 0.53 <sup>a</sup>    | 3.81 $\pm$ 0.43 <sup>a</sup>    | 3.36 $\pm$ 0.50 <sup>a</sup>      | 3.48 $\pm$ 0.59 <sup>a</sup>      |
| Crude Protein             | 0.39 $\pm$ 0.14 <sup>a</sup>     | 0.43 $\pm$ 0.09 <sup>a</sup>    | 0.43 $\pm$ 0.10 <sup>a</sup>    | 0.49 $\pm$ 0.14 <sup>a</sup>      | 0.66 $\pm$ 0.20 <sup>b</sup>      |
| Fat                       | 0.08 $\pm$ 0.01 <sup>a</sup>     | 0.12 $\pm$ 0.06 <sup>a,b</sup>  | 0.07 $\pm$ 0.03 <sup>a</sup>    | 0.09 $\pm$ 0.02 <sup>a</sup>      | 0.16 $\pm$ 0.11 <sup>b</sup>      |
| Ash                       | 0.74 $\pm$ 0.16 <sup>a,b</sup>   | 0.67 $\pm$ 0.10 <sup>a</sup>    | 0.70 $\pm$ 0.07 <sup>a</sup>    | 0.82 $\pm$ 0.10 <sup>b</sup>      | 0.96 $\pm$ 0.08 <sup>c</sup>      |
| <i>Inorganic elements</i> |                                  |                                 |                                 |                                   |                                   |
| Phosphorous               | 70.17 $\pm$ 21.02 <sup>a</sup>   | 66.00 $\pm$ 11.19 <sup>a</sup>  | 70.23 $\pm$ 10.28 <sup>a</sup>  | 73.95 $\pm$ 15.86 <sup>a</sup>    | 92.82 $\pm$ 10.69 <sup>b</sup>    |
| Calcium                   | 63.61 $\pm$ 17.04 <sup>a,b</sup> | 56.52 $\pm$ 8.34 <sup>a</sup>   | 57.65 $\pm$ 8.32 <sup>a,b</sup> | 71.31 $\pm$ 14.00 <sup>b</sup>    | 101.51 $\pm$ 13.95 <sup>c</sup>   |
| Magnesium                 | 11.91 $\pm$ 3.15 <sup>a</sup>    | 11.21 $\pm$ 1.78 <sup>a</sup>   | 10.52 $\pm$ 1.33 <sup>a</sup>   | 17.08 $\pm$ 3.46 <sup>b</sup>     | 21.38 $\pm$ 3.20 <sup>c</sup>     |
| Potassium                 | 193.16 $\pm$ 25.56 <sup>c</sup>  | 146.03 $\pm$ 18.77 <sup>a</sup> | 144.60 $\pm$ 16.85 <sup>a</sup> | 182.51 $\pm$ 35.31 <sup>b,c</sup> | 159.97 $\pm$ 26.95 <sup>a,b</sup> |
| Sodium                    | 82.77 $\pm$ 19.93 <sup>b,c</sup> | 54.11 $\pm$ 5.65 <sup>a,b</sup> | 51.87 $\pm$ 7.57 <sup>a</sup>   | 83.79 $\pm$ 25.56 <sup>c</sup>    | 100.60 $\pm$ 37.50 <sup>c</sup>   |
| Vitamin B12               | 0.93 $\pm$ 0.37 <sup>a</sup>     | 2.70 $\pm$ 0.75 <sup>c</sup>    | 2.02 $\pm$ 0.50 <sup>b</sup>    | 1.93 $\pm$ 0.08 <sup>b</sup>      | 2.90 $\pm$ 0.36 <sup>c</sup>      |

\* Means in the same row with different superscript letters differ significantly ( $p < 0.05$ ). BYAWuf = bovine yoghurt acid whey (YAW) obtained by ultrafiltration; BYAWce = bovine YAW obtained by centrifugation; BYAWcb = bovine YAW obtained by filtration through cloth bags; CYAWcb = caprine YAW obtained by filtration through cloth bags; OYAWcb = ovine YAW obtained by filtration through cloth bags.

**Table 2.** Main sugars and organic acids (g/100 g) of yoghurt acid whey obtained from various Greek Yoghurt dairies using different straining methods (mean  $\pm$  SD,  $n$  = number of samples).

|             | BYAWuf ( $n = 6$ )               | BYAWce ( $n = 6$ )           | BYAWcb ( $n = 7$ )             | CYAWcb ( $n = 7$ )             | OYAWcb ( $n = 9$ )           |
|-------------|----------------------------------|------------------------------|--------------------------------|--------------------------------|------------------------------|
| Lactose     | 2.64 $\pm$ 0.09 <sup>b,c,*</sup> | 3.12 $\pm$ 0.45 <sup>c</sup> | 3.14 $\pm$ 0.42 <sup>c</sup>   | 2.49 $\pm$ 0.48 <sup>a,b</sup> | 2.02 $\pm$ 0.75 <sup>a</sup> |
| Galactose   | 0.64 $\pm$ 0.17 <sup>a,b</sup>   | 0.51 $\pm$ 0.08 <sup>a</sup> | 0.65 $\pm$ 0.09 <sup>a,b</sup> | 0.83 $\pm$ 0.19 <sup>b</sup>   | 1.48 $\pm$ 0.37 <sup>c</sup> |
| Lactic acid | 0.74 $\pm$ 0.22 <sup>a,b</sup>   | 0.61 $\pm$ 0.09 <sup>a</sup> | 0.74 $\pm$ 0.09 <sup>a,b</sup> | 0.91 $\pm$ 0.29 <sup>b</sup>   | 1.37 $\pm$ 0.26 <sup>c</sup> |
| Citric acid | 0.23 $\pm$ 0.04 <sup>a,b</sup>   | 0.18 $\pm$ 0.04 <sup>a</sup> | 0.18 $\pm$ 0.02 <sup>a</sup>   | 0.19 $\pm$ 0.05 <sup>a</sup>   | 0.27 $\pm$ 0.03 <sup>b</sup> |

\* Means in the same row with different superscript letters differ significantly ( $p < 0.05$ ). BYAWuf = bovine yoghurt acid whey (YAW) obtained by ultrafiltration; BYAWce = bovine YAW obtained by centrifugation; BYAWcb = bovine YAW obtained by filtration through cloth bags; CYAWcb = caprine YAW obtained by filtration through cloth bags; OYAWcb = ovine YAW obtained by filtration through cloth bags.

Total solids ranged from  $4.68 \pm 0.72\%$  (in the bovine YAW obtained by ultrafiltration) to  $6.19 \pm 0.73\%$  (in the ovine YAW obtained by traditional straining). The significantly higher total solids content of ovine YAW was probably due to the higher fat, protein and ash contents of this milk compared to bovine and caprine milks [21]. Moreover, the lower total solids content of the bovine YAW derived by ultrafiltration of yoghurt, compared with the total solid contents of the bovine YAWs from centrifugation or traditional filtration of yoghurt, was attributed to the better separation efficiency of the ultrafiltration process. The lower concentration of total solids in YAW from ultrafiltration probably leads to a greater yield in the produced strained yoghurt, since more protein, fat, sugars and ash will have been retained in the yoghurt gel. Moreover, the lower concentration of total sugars might be related to a lower BOD index. The fat and crude protein contents of all YAW samples were  $<0.2\%$  and  $<0.7\%$ , respectively, but they were significantly ( $p < 0.05$ ) higher in the ovine YAW than in the bovine or caprine YAWs. The low fat and crude protein contents were expected since these components are retained in the yoghurt curd.

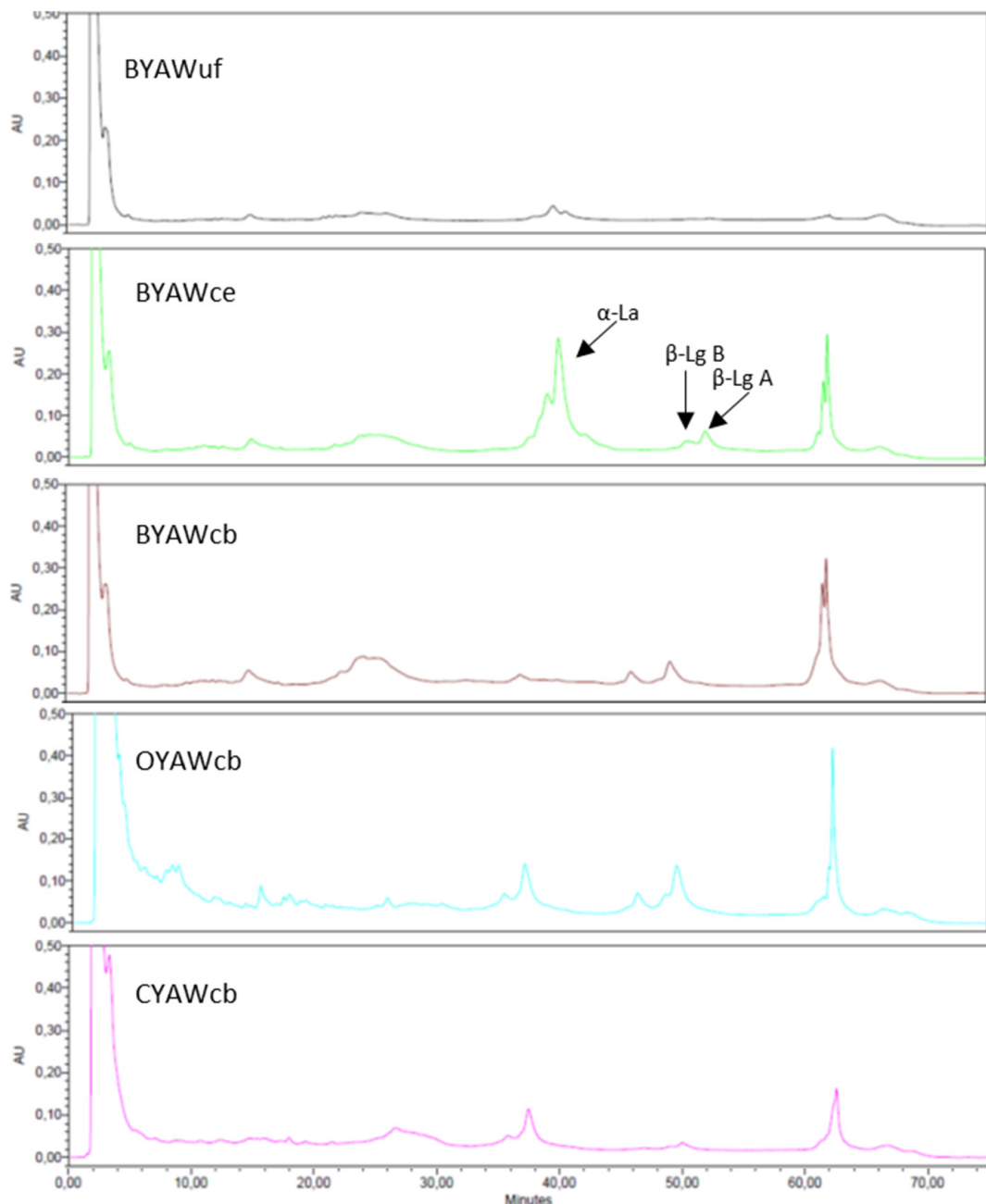


The carbohydrates content was not significantly affected either by the production method, as in the case of bovine YAW, or by the kind of milk, and it ranged from  $3.29 \pm 0.23\%$  to  $3.65 \pm 0.53\%$ . Similarly, the ash content was not significantly affected by the production method, but it was affected by the kind of milk. The ash contents of caprine ( $0.82 \pm 0.1\%$ ) and ovine ( $0.96 \pm 0.08\%$ ) YAWs were significantly ( $p < 0.05$ ) higher than the ash content of bovine YAW ( $0.67 \pm 0.1\%$ – $0.74 \pm 0.16\%$ ). This was probably due to the significantly higher phosphorus, calcium, magnesium and sodium contents of ovine YAW compared to the respective contents of the bovine or caprine YAWs. In general, the obtained results regarding the gross composition agreed with those reported in the literature for YAW [4–7].

Yoghurt is a good source of B12 (cobalamin), since this vitamin is synthesised by bacteria in the rumen of ruminants, absorbed through the gastrointestinal tract and transported via the blood to milk [22]. B12 content ranges from 0.08 to 0.49  $\mu\text{g}/100\text{ g}$  and from 0.07 to 0.10  $\mu\text{g}/100\text{ g}$  in bovine and caprine milk, respectively, while it is about 0.71  $\mu\text{g}/100\text{ g}$  in ovine milk [21]. The concentration of B12 in yoghurt is 0.12–0.60  $\mu\text{g}/100\text{ g}$  [22], but during strained yoghurt production, a part of the B12 content is expected to be lost in the acid whey. The amounts of B12 found in the YAWs of the present study agreed with those reported in the literature, but the B12 content of the bovine YAW was significantly ( $p < 0.05$ ) affected by the production method. Bovine YAW from ultrafiltration of yoghurt gel contained  $0.93 \pm 0.37\text{ }\mu\text{g}/\text{L}$ , while that from centrifugation contained  $2.70 \pm 0.75\text{ }\mu\text{g}/\text{L}$ . The B12 content of caprine and ovine YAWs was  $1.93 \pm 0.08$  and  $2.90 \pm 0.36\text{ }\mu\text{g}/\text{L}$ , respectively. In deproteinised sweet whey from Ricotta cheesemaking, B12 content has been found to be from  $0.11 \pm 0.06$  to  $1.05 \pm 0.33\text{ }\mu\text{g}/100\text{ g}$ , depending on the lactation period [23]. B12 is a biofunctional component of YAW, which may increase the nutritional value of the foods in which YAW is added without prior processing. According to EFSA [24], the adequate intake of cobalamin for adults is set at 4.0  $\mu\text{g}/\text{day}$ .

As expected, lactose showed the highest concentration in all YAW samples (Table 2). Bovine YAW from ultrafiltration contained less lactose ( $2.64 \pm 0.09\%$ ) than bovine YAW from centrifugation ( $3.12 \pm 0.45\%$ ) or traditional straining ( $3.14 \pm 0.42\%$ ) and this probably accounts for the lower total sugars content that this YAW presented (Table 1). On the other hand, concerning the traditional straining, ovine YAW had the lowest lactose content ( $2.02 \pm 0.75\%$ ), and this was in relation to the highest lactic acid content. Apart from lactose, galactose was also present, since this monosaccharide is not metabolised by the yoghurt cultures [25]. Among the organic acids (Table 2), lactic acid, which comes from the fermentation of lactose, was significantly ( $p < 0.05$ ) higher in the ovine YAW obtained by traditional straining. In general, the trend of the lactic acid contents agreed with the trend of acidity (Table 1). The citric acid concentrations were similar to those reported for the corresponding milks, and this was because the yoghurt cultures do not ferment it.

Qualitative analysis of the whey protein content by RP-HPLC is shown in Figure 1. It is obvious that YAW is poor in whey proteins because the heat treatment of milk (about 90–95 °C) denatures them, and hence, they are retained together with casein in the yoghurt gel. Moreover, traditional straining usually takes place in small dairies that apply batch and more severe heat treatment to milk than the big dairies. Hence, the RP-HPLC profile of whey proteins was differentiated by the method that was used for removing the YAW. YAW obtained by the ultrafiltration of yoghurt gel did not contain whey proteins at all, and this can be explained by the fact that these proteins are retained during ultrafiltration [26]. The absence of whey proteins in this type of YAW agreed with its lowest crude protein content. The YAWs obtained from traditional straining contained less residual  $\alpha$ -La and  $\beta$ -Lg than YAW obtained from centrifugation, apart from ovine YAW, which seemed to contain more  $\beta$ -Lg than the other YAWs, probably because of its initial higher content of this protein.



**Figure 1.** Representative RP-HPLC chromatograms of whey proteins in bovine yoghurt acid whey obtained by ultrafiltration (BAWuf), centrifugation (BYAWce) or traditional straining through cloth bags (BYAWcb), and in ovine or caprine yoghurt acid whey obtained by traditional straining (OYAWcb and CYAWcb, respectively).

### 3.2. Membrane Processing of YAW

#### 3.2.1. Composition of YAW After Microfiltration Processing

The composition of microfiltered YAW (MFYAW) that served as feed in the nanofiltration process is shown in Table 3. In general, microfiltration through a membrane with pore size 1.4  $\mu\text{m}$  prior to nanofiltration does not significantly affect the composition of acid whey [15,27], but it is necessary for removing the fat and the microbial and even the somatic cells, which may be present in YAW. Hence, all microfiltered YAWs showed zero fat content. Ovine MFYAW contained significantly ( $p < 0.05$ ) more total solids, total protein, ash, lactose, galactose and lactic acid than bovine MFYAWs. Moreover, it was richer in calcium and phosphorus.

**Table 3.** pH, acidity and composition (%) of microfiltrated yoghurt acid whey obtained from Greek yoghurt production using different straining methods (mean  $\pm$  SD,  $n = 3$ ).

|                            | Bovine YAW                       |                                 |                                | Ovine YAW                       |
|----------------------------|----------------------------------|---------------------------------|--------------------------------|---------------------------------|
|                            | Ultrafiltration                  | Centrifugation                  | Cloth Bags                     | Cloth Bags                      |
| pH                         | 4.47 $\pm$ 0.08 <sup>c,*</sup>   | 4.44 $\pm$ 0.08 <sup>c</sup>    | 4.01 $\pm$ 0.01 <sup>a</sup>   | 4.25 $\pm$ 0.04 <sup>b</sup>    |
| Acidity                    | 0.60 $\pm$ 0.01 <sup>b</sup>     | 0.47 $\pm$ 0.01 <sup>a</sup>    | 0.64 $\pm$ 0.02 <sup>c</sup>   | 0.79 $\pm$ 0.03 <sup>d</sup>    |
| Total solids               | 5.35 $\pm$ 0.05 <sup>b</sup>     | 5.41 $\pm$ 0.01 <sup>b,c</sup>  | 4.68 $\pm$ 0.04 <sup>a</sup>   | 5.61 $\pm$ 0.15 <sup>c</sup>    |
| Fat                        | 0                                | 0                               | 0                              | 0                               |
| Crude protein              | 0.25 $\pm$ 0.02 <sup>a</sup>     | 0.25 $\pm$ 0.03 <sup>a</sup>    | 0.30 $\pm$ 0.01 <sup>b</sup>   | 0.51 $\pm$ 0.02 <sup>c</sup>    |
| Ash                        | 0.85 $\pm$ 0.02 <sup>b</sup>     | 0.71 $\pm$ 0.07 <sup>a</sup>    | 0.74 $\pm$ 0.01 <sup>a</sup>   | 1.00 $\pm$ 0.01 <sup>c</sup>    |
| Lactose                    | 2.72 $\pm$ 0.09 <sup>c</sup>     | 2.86 $\pm$ 0.01 <sup>c</sup>    | 2.28 $\pm$ 0.01 <sup>b</sup>   | 2.05 $\pm$ 0.04 <sup>a</sup>    |
| Galactose                  | 0.77 $\pm$ 0.01 <sup>b</sup>     | 0.52 $\pm$ 0.01 <sup>a</sup>    | 0.85 $\pm$ 0.08 <sup>b</sup>   | 1.33 $\pm$ 0.01 <sup>c</sup>    |
| Lactic acid                | 0.92 $\pm$ 0.01 <sup>b</sup>     | 0.63 $\pm$ 0.07 <sup>a</sup>    | 0.92 $\pm$ 0.04 <sup>b</sup>   | 1.35 $\pm$ 0.01 <sup>c</sup>    |
| <b>Minerals (mg/100 g)</b> |                                  |                                 |                                |                                 |
| Phosphorous                | 87.60 $\pm$ 2.16 <sup>c</sup>    | 68.65 $\pm$ 1.15 <sup>b</sup>   | 51.65 $\pm$ 2.88 <sup>a</sup>  | 91.55 $\pm$ 1.60 <sup>c</sup>   |
| Calcium                    | 120.25 $\pm$ 9.89 <sup>b,c</sup> | 102.60 $\pm$ 13.80 <sup>b</sup> | 73.57 $\pm$ 4.19 <sup>a</sup>  | 142.42 $\pm$ 7.92 <sup>c</sup>  |
| Magnesium                  | 14.00 $\pm$ 0.82 <sup>b</sup>    | 13.33 $\pm$ 0.21 <sup>b</sup>   | 10.35 $\pm$ 0.21 <sup>a</sup>  | 22.40 $\pm$ 2.18 <sup>c</sup>   |
| Potassium                  | 148.53 $\pm$ 0.32 <sup>c</sup>   | 145.49 $\pm$ 2.38 <sup>c</sup>  | 117.59 $\pm$ 2.02 <sup>a</sup> | 135.55 $\pm$ 3.33 <sup>b</sup>  |
| Sodium                     | 57.30 $\pm$ 3.62 <sup>a</sup>    | 57.52 $\pm$ 9.67 <sup>a</sup>   | 97.38 $\pm$ 4.44 <sup>b</sup>  | 105.63 $\pm$ 12.17 <sup>b</sup> |

\* Means with different superscript letters in the same row are significantly different ( $p < 0.05$ ) from each other.

### 3.2.2. Transmission and Retention of YAW Compounds During Nanofiltration

Nanofiltration is a type of membrane process in which separation is achieved through the combination of charge rejection, solubility-diffusion and sieving through micropores (<2 nm). The transmittance of monovalent ions is usually >30% across the membrane, rejection of multivalent ions is >90% and the molecular weight cutoff (MWCO) for neutral compounds is between 150 and 2000 Da. As mentioned before, nanofiltration is applied to acid whey to remove part of the minerals and lactic acid, improving its composition for further production of acid whey powders. During nanofiltration through a membrane with cutoff 250 Da, most of the lactic acid and mineral contents pass through the membrane in permeate, whereas proteins and lactose are retained. However, it is known that permeate flux decreases as the time of processing increases due to the fouling of membrane pores. This fact affects the separation process of a molecule and hence the efficiency of the process.

The results from the nanofiltration of YAW, expressed as the sieving coefficient for the transmission of a molecule or the rejection coefficient for the retention of a molecule, are shown in Table 4. In the case of bovine YAW, the transmission of lactic acid ranged from about 37% to 58%, showing a dependence on the way of removal. Chandrapala et al. [15], who processed acid whey from cream cheese by nanofiltration, showed that lactic acid transmission depended on the pH, membrane type and temperature. They found that at pH 3.0, the sieving coefficient was about 50%, while at pH 4.5, which is the pH of the YAW of the present study, it was 27–29%. Moreover, Casado-Coterillo et al. [28], performing model studies on the separation of lactic acid from acidified Mozzarella cheese whey by nanofiltration through a commercial membrane, achieved lactic acid rejection lower than 37% at pH 3.5. A high level, about 87%, of lactic acid removal has been reported when nanofiltration was combined with diafiltration [29]. Hence, it is obvious that differences in the separation efficiency of lactic acid are due to the different processing conditions. For example, lactic acid rejection as a function of pressure depends on the feed concentration, temperature and flow rate, and the higher the concentration and flow rate, the lower the separation efficiency. On the other hand, the results obtained showed that under the same processing conditions, lactic acid rejection was affected by the properties of YAW. Bovine YAW derived from yoghurt straining by centrifugation showed the highest separation



capacity, while that derived from yoghurt straining by ultrafiltration showed the lowest. However, it was better than those reported for pH 4.5 [14].

**Table 4.** The % sieving coefficients of lactic acid, phosphorous, calcium, magnesium, potassium and sodium and the % rejection coefficients of total protein, lactose and galactose after nanofiltration of microfiltrated (MF) acid whey obtained from Greek yoghurt production using different straining methods (mean  $\pm$  SD,  $n = 3$ ).

|                                   | Bovine MF YAW                   |                                 |                                 | Ovine MF YAW                     |
|-----------------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------------------|
|                                   | Ultrafiltration                 | Centrifugation                  | Cloth Bags                      | Cloth Bags                       |
| <b>Sieving coefficients (%)</b>   |                                 |                                 |                                 |                                  |
| Lactic acid                       | 37.17 $\pm$ 1.83 <sup>a,*</sup> | 58.87 $\pm$ 3.58 <sup>c</sup>   | 55.42 $\pm$ 1.51 <sup>b,c</sup> | 49.26 $\pm$ 4.41 <sup>b</sup>    |
| Phosphorous                       | 23.14 $\pm$ 1.44 <sup>a</sup>   | 45.69 $\pm$ 11.38 <sup>b</sup>  | 19.69 $\pm$ 3.64 <sup>a</sup>   | 32.84 $\pm$ 5.7 <sup>a,b</sup>   |
| Calcium                           | 20.69 $\pm$ 2.13 <sup>a</sup>   | 42.83 $\pm$ 11.41 <sup>b</sup>  | 23.92 $\pm$ 2.75 <sup>a</sup>   | 33.11 $\pm$ 6.19 <sup>a,b</sup>  |
| Magnesium                         | 18.48 $\pm$ 1.48 <sup>a</sup>   | 39.75 $\pm$ 7.74 <sup>b</sup>   | 16.21 $\pm$ 1.53 <sup>a</sup>   | 27.32 $\pm$ 3.70 <sup>a</sup>    |
| Potassium                         | 60.32 $\pm$ 2.88 <sup>a</sup>   | 96.54 $\pm$ 10.83 <sup>b</sup>  | 76.10 $\pm$ 1.24 <sup>a,b</sup> | 85.72 $\pm$ 15.95 <sup>a,b</sup> |
| Sodium                            | 47.10 $\pm$ 6.11 <sup>a</sup>   | 72.89 $\pm$ 15.82 <sup>b</sup>  | 69.04 $\pm$ 11.64 <sup>b</sup>  | 63.79 $\pm$ 0.33 <sup>b</sup>    |
| <b>Rejection coefficients (%)</b> |                                 |                                 |                                 |                                  |
| Crude protein                     | 81.56 $\pm$ 7.02 <sup>a,b</sup> | 79.37 $\pm$ 2.24 <sup>a,b</sup> | 83.38 $\pm$ 1.81 <sup>b</sup>   | 71.45 $\pm$ 6.04 <sup>a</sup>    |
| Lactose                           | 95.92 $\pm$ 4.36 <sup>a</sup>   | 95.51 $\pm$ 2.96 <sup>a</sup>   | 99.67 $\pm$ 0.05 <sup>a</sup>   | 95.29 $\pm$ 0.60 <sup>a</sup>    |
| Galactose                         | 84.34 $\pm$ 6.78 <sup>a</sup>   | 79.22 $\pm$ 6.53 <sup>a</sup>   | 89.11 $\pm$ 0.51 <sup>a</sup>   | 82.85 $\pm$ 3.61 <sup>a</sup>    |

\* Means with different superscript letters in the same row are significantly different ( $p < 0.05$ ) from each other.

Transmission of calcium and magnesium ranged from about 21% to 43% and from about 16% to 40%, respectively. These results are in partial agreement with the values 34–40% and 25–27% reported for the transmission of calcium and magnesium, respectively [16]. Sieving coefficients of potassium and sodium ranged from about 60% to 97% and from 47% to 73%, respectively. Nanofiltration of acid whey from cheese under conditions like the present study has resulted in about 76–79% and 67–70% transmission of potassium and sodium, respectively [15]. In general, removal of the monovalent cations (Na, K) was better than that of divalent cations (Ca, Mg).

Regarding the crude protein retention, the rejection coefficient ranged from about 71% to 88%. However, based on yoghurt technology, the protein content of YAW is more NPN and less true protein, because the high pasteurisation of milk ( $>90$  °C) denatures whey proteins, which are retained along with caseins in the yoghurt curd [7]. This was also shown in Figure 1. Lactose retention was  $>95\%$  no matter the kind of YAW. The high retention was probably due to its higher molecular weight (342 Da) than the nominal pore size of the membrane (250 Da) that was used. In this context, galactose retention was lower, about 79–89%. The lactose rejection was similar to that reported for acid whey from cream cheese [15], while lactose rejection  $> 98\%$  has been reported recently [28,29].

#### 4. Conclusions

The results obtained showed that the composition of acid whey derived from strained yoghurt depends on the method that is used for its removal. More specifically, ultrafiltration of yoghurt gel delivers YAW with the lowest concentration in total solids, while traditional straining delivers YAW with the highest one. Moreover, the severity of the milk heat treatment affects the composition regarding the presence of residual whey proteins. These differences, in their turn, affect the subsequent processing of YAW by membrane technology to separate efficiently the lactic acid and the minerals. Hence, further research is needed to

optimise the nanofiltration conditions for each type of YAW in anticipation of its further processing by spray drying.

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