A Starch-Milk Paste Enables the Incorporation of Ripened Cheese in Novel Fresh Cheese

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SUMMARY

Research background: Fresh cheese varieties represent an important share of the whole cheese market. Although with great variability in terms of composition and method of preparation, fresh cheese varieties are bland in flavor and their production originates whey drainage. On the other hand, the cheese market is also responsible for a significant amount of food waste. These motivated the development of a novel fresh cheese incorporating ripened cheese, which can then represent a valorization of ripened cheese surpluses.

Experimental approach. A variable amount of ripened cheese was dispersed in a paste of gelatinized starch (normal corn or waxy rice) in milk, producing melted cheese bases. These cheese bases were diluted with milk, sometimes enriched with skim milk powder, and then renneted. The resultant fresh cheese was characterized for macronutrients content, and physical properties. Sensory analyses of samples incorporating mature Cheddar, goats’, or ewes’ cheese were carried out.

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Results and conclusions. Gel formation of the initial mixture was hindered above 8% \((m/m)\) incorporation of ripened cheese, which could be overcome by the addition of skim milk powder. These observations are corroborated by the hardness values from texture analysis tests. Evaluation of syneresis of different samples enabled to conclude that addition of 2% \((m/m)\) starch and of 2.8% \((m/m)\) skim milk powder contribute to reduce its magnitude by half. Sensory analysis with a consumer panel indicated a preference for a more consistent texture of the fresh cheese, and for the Cheddar flavor.

Novelty and scientific contribution. A novel fresh cheese variety incorporating dispersed ripened cheese was prepared. The proposed method is versatile and quite straightforward and does not use polyphosphate salts or originate whey wastage. The fresh cheese physical and sensorial properties can be manipulated by the amounts and types of starch, ripened cheese, and skim milk powder added; such tailoring of fresh cheese properties widens product portfolio capacity for a larger number of consumer groups. The ripened cheese added can come from non-sellable pieces and unsold stocks from the retail sector, contributing to a reduction of food waste.

Key words: novel fresh cheese, cheese surpluses valorisation, physicochemical analysis, sensory analysis

INTRODUCTION

According to FAO, food waste is a global issue with a significant carbon footprint - as high as 3.3 billion tons of carbon dioxide per year released to the atmosphere. Moreover, the global volume of food waste is estimated at 1.6 billion tons of “primary product equivalents” (1). In recent years, waste valorisation coming from the food chain has gained much attention. In 2015, the European Commission presented ways to enhance the transition to a circular economy through recycling and reuse (2), and most recently adopted the new Circular Economy Action Plan (2020), Europe’s new agenda for sustainable growth, which introduces different measures fostering sustainable consumption. Cheese consumption in the European Union (and other parts of the World) has been increasing, with a yearly consumption per capita in 2019 close to 15 kg (3). Of this, a significant share comes from different varieties of fresh cheese \((e.g.\ \textit{fromage blanc, crème fraîche, cottage cheese, quark, ques fresco, etc.})\), and from spreadable processed cheese. These products are appreciated for a variety of factors, such as freshness, lightness, versatility to be mixed with a variety of other foods or even beverages, \textit{etc.} As they do not undertake a ripening stage, their flavour is generally bland.
The production process of fresh cheese is relatively simple and fast: after pasteurization and cooling, the milk, or milk-cream mixture, is standardized, and the coagulation takes place. The coagulation can be acid- and/or rennet-induced. Along with the coagulating factors, other ingredients, such as preservatives and emulsifiers, can be introduced (4). In order to remove the whey from the curd, draining or centrifugation of the coagulum can be carried out. In order to avoid the removal of whey at this stage, a more recent technology can be applied, namely ultrafiltration of the starting milk, which separates the smaller lactose, water, minerals, and vitamins molecules, from the larger proteins and fat globules. This results in a milk product concentrated in protein and lower in lactose content (5,6), but this process also generates a stream of nutritionally valuable liquid (7).

The current research study aimed at the development of a novel renneted fresh cheese product incorporating (ripened) cheese. The latter may come from the surpluses of the dairy industry and/or the retail sector, thus representing a needed mitigation of food waste, since food waste in the dairy sector for Europe can be over 10%, and for North America and Oceania over 20% (8). The ripened cheese can provide additional nutritional value to the fresh cheese, particularly added protein, lipids and minerals. It will also bring a multitude of flavour and aroma compounds, as well as varying textures.

Ripened cheese can be dispersed into submillimetre particles when mixed in a hot paste of gelatinized starch, being herein designated a melted cheese base. Such dispersions are used, with variants, in the preparation of some simple cheese sauces (9-11). This technique for dispersing matured cheese is an interesting alternative to the use of emulsifying salts, namely polyphosphates, generally employed in processed cheese (10,11). Polyphosphates can be health deleterious, at least for certain groups, such as bone disease patients (12), besides imparting a metallic tone to the processed cheese (13).

Furthermore, the process here proposed dispenses the step of cutting the coagulum and removal of whey, which can be time consuming and costly, and that produces abundant whey wastage. In this process, the milk whey is incorporated in the final product, contributing with minerals and proteins of high biological value.

Therefore, the aim of this study was the development and testing of a new fresh cheese incorporating ripened cheese, dispersed in a paste of gelatinized starch. The nature of the ripened cheese and the type of starch (corn or waxy rice starch) were studied.
MATERIALS AND METHODS

Materials

For the samples preparation, the milk used was semi-skimmed (1.5 % (m/m) fat) HTST milk (Vigor, Portugal). The starches employed were regular corn (Maizena, Unilever) and waxy rice (Remyline XS, BENEÖ GmbH, Mannheim, Germany, kindly provided by Nutripar, Porto, Portugal). The type of cheese were Emmental (Milbona, Bissingen, Germany), goats’ (Queso de Cabra, Lácteas García Baquero S.A., Alcázar de San Juan (Ciudad Real), Spain), ewes’ (Queijo Serra Valmadeiros, Indulac - Indústrias Lácteas S.A., Oliveira de Azeméis, Portugal), and mature Cheddar (Valley Spire, Dale Farm Ltd., Belfast, UK). Skim milk powder was from Regilait (Nestlé S.A, Vevey, Switzerland). The rennet was a liquid preparation of Rhizomucor miehei enzyme (Britex, Lusocoalho, Montes da Senhora, Portugal).

Preparation of melted cheese base

For a typical preparation, corn starch was dispersed in cold milk, at a ratio of 5 g per 100 g milk, in a small non-adherent pan, and the mixture was heated on a hotplate for approximately 5 minutes, with continuous hand stirring, until reaching 85 °C. Temperature was controlled with a kitchen electronic thermometer (LACOR, Bergara, Spain). At this point, the gelatinization of the starch was noticeable and 20 g of grated or finely cut cheese was added to the milk-starch slurry. The mixture was removed from the hotplate and hand stirred for approximately 30 seconds, until the cheese was fully dispersed, with no visible macroscopic pieces. This melted cheese base was left to cool down to room temperature (approx. 21 °C).

When using waxy rice starch, the procedure was similar, but the milk-starch mixture was heated to 90 °C and this temperature maintained for 2 minutes, in order to achieve complete starch gelatinization, before cheese addition.

Light and fluorescence optical microscopy

For analysing the microstructure of the melted cheese bases prepared with grated Emmental cheese and starch, an Olympus BX51 (Olympus, Tokyo, Japan) optical microscope was used, with a 10 x objective lens, and the images were captured using a Power Shot G10 Canon digital camera (Canon, Tokyo, Japan). In order to visualize starch and protein, samples were stained with Rhodamine B (1 g/L) (14,16), and for visualizing fat, with Sudan III (1 g/L) (16). Afterwards, aliquots were transferred to concave slides and covered with a cover slip. Samples were equilibrated at room temperature for 15 minutes prior to microscopic analysis, using the fluorescence mode with
Rhodamine B and the normal light mode with Sudan III samples. All observations were performed in duplicate, with at least four pictures taken per sample. Software ImageJ (17) was used to evaluate the sizes of the protein agglomerates.

**Samples preparation**

Four different control mixtures (without cheese) using milk and the two types of starch were prepared, namely, just HTST milk (sample CT), milk with 2 % (m/m) CS (CT.CS.1) or 2 % (m/m) waxy rice starch (CT.WRS.1), milk with 4 % (m/m) starch (CT.CS.2 and CT.WRS.2), and milk with 2 % (m/m) starch and 2.8 % (m/m) skim milk powder (CT.CS.3 and CT.WRS.3). In order to prepare these samples, milk, milk with 4 % (m/m) gelatinized starch, and milk with 5.7 % (m/m) skim milk powder, were used and mixed in different proportions, according to Table 1. The latter was prepared by adding 6 g skim milk powder to 100 g fluid milk, and the solution left 24 hours at 4 °C, for the powder to fully rehydrate.

For samples containing ripened cheese, we further used a melted cheese base incorporating 4% (m/m) corn starch and 16 % (m/m) of Emmental cheese. Then, samples with an amount of cheese varying between 3.2 and 8.0 % (m/m) (samples EM.CS.1 to 5) were prepared (Table 1). One of the samples (EM.CS.5) included also skim milk powder at 2.9 % (m/m). Similar samples were prepared with waxy rice starch (EM.WRS.1 to 5) (Table 1).

Two other sets of samples were prepared with ewes’ cheese, with either corn starch (EW.CS.1 to 5), or waxy rice starch (samples EW.WRS.1 to 5) (Table 1).

The renneting of all samples was done by adding 240 μL liquid rennet per 100 g of mixture, pre-equilibrated in a thermostated waterbath (TW20, JULABO GmbH, Seelbach, Germany). The temperature was maintained at 35 °C for 15 minutes (until coagulation was completed). The flasks with the samples were then transferred to 4 °C.

Several batches of all samples were prepared, in order to prepare the following tests.

**Determination of total solids**

The determination of the total solids was carried out for a representative group of samples, namely for the ewes’ and Emmental ripened cheese, and for fresh cheese samples EW.CS.4 and EM.CS.4. Aluminium dishes were pre-dried (105 °C, 1 h) and weighed. 3-5 g of sample were placed in the dishes and the weight recorded. The samples were dried in an oven to constant weight (Drying Oven d-6450, Heraeus Thermo Fisher Scientific, Waltham, MA, USA), for 24 h, at 105 °C. The
samples were cooled in the desiccator and the total solids was determined following the AOAC Official Method 925.23 (18), using Eq. 1:

\[
\text{% Total solids} = \frac{\text{dry weight}}{\text{wet weight}} \times 100
\]

Two batches of each sample type were subjected to analysis and, in each case, duplicate measurements were taken. Results are averages ± SD.

**Determination of protein content**

The total protein was determined for the Emmental and ewes’ cheese in duplicate samples, using a non-conventional method. It is based on the one proposed by Reichardt & Eckert (19), but with modifications (19). A standard casein solution was prepared by adding 0.405 g of bovine casein, (Sigma-Aldrich, St. Louis, MO, USA) in 50 mL of deionized water, followed by heating to 40 °C and gently stirring. The absorbance at 280 nm of this solution and several dilutions were determined (Spectronic Helios Gamma UV-Vis Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) and a calibration curve of protein concentration was therefore obtained.

For the determination of the protein content of cheese, about 1.33 g of cheese cut in small pieces was placed in 30 mL of 0.1 M NaOH and left overnight. The following day, the mixture was placed in a waterbath (TW20, JULABO GmbH, Seelbach, Germany) at 40 °C, for 10 min and mixed well. After cooling down, it was centrifuged (Universal 320R, Andreas Hettich GmbH, Germany, centrifuge) at 4000 x g, 4 °C, for 10 min. The top layer of fat was removed, the underlying supernatant was collected, and its volume evaluated. The absorbance at 280 nm was measured by diluting 80 μL of the supernatant with 920 μL of 0.1 M NaOH. The concentration was calculated from the casein curve, and the protein content in cheese then calculated. The determinations were done in duplicate samples of cheese and the averages ± SD were calculated.

We have shown that this method gives results that are not statistically different from those obtained with the standard Kjeldahl method (20).

**Determination of fat content of ripened cheese**

The fat content of the different types of ripened cheese was measured by the Van Gulik method (21). The cheese was grated or cut in small pieces, and precisely 3 g were placed in the lower part of the Van Gulik butyrometer (cheese butyrometer 0 – 40 %, Gerber Instruments, Effretikon, Switzerland). 15 ml of sulfuric acid 62 %, d =1,522 ± 0.005, according to Van Gulik, were added, the
butyrometer was tapped and put in a waterbath at 65 °C until the cheese was fully digested. Then, 1 ml of isoamyl alcohol was added and the contents were mixed thoroughly. Additional sulfuric acid was added, up to the point that the liquid reached the 35 % mark of the butyrometer scale. The butyrometer was tapped and the contents were mixed thoroughly once again. The samples were centrifuged in a Gerber centrifuge (NormMilk centrifuge, International PVI, Milan, Italy), for 6 min, at 1200 rpm and 65 °C. After the centrifugation, the reading of the fat content was done directly on the butyrometer. The determinations were done in duplicate and the average ± SD were calculated.

Determination of syneresis

Control samples and fresh cheese samples EM.CS.2, EM.CS.4, EW.WRS.2 and EW.WRS.4 were centrifuged at 1,500 x g for 15 min, at 20 °C (22), and the mass of the pellet and supernatant (separated whey) were measured. The syneresis was calculated as seen in Eq. 2 (23):

\[
\% \text{ Syneresis} = \frac{\text{Amount of whey separated}}{\text{Amount of sample added}} \times 100
\]

Two batches of each sample type were subjected to analysis and, in each case, duplicate measurements were taken. Results are averages ± SD.

Texture profile analysis

For the texture profile analysis, the renneted samples rested at 4 °C for 24 hours, and then they were equilibrated at 15 °C. A Stable Micro Systems TA.XT Plus Texture Analyzer (Surrey, UK) with a cylindrical probe of 36 mm diameter was used. The fresh cheese samples were tested in the glass flasks of approximately 6 cm-diameter in which they were renneted, without any transfer to avoid gel breakage. A typical texture profile analysis cycle was used, with a trigger force of 5 g. The probe test speed was 1 mm/s and the maximum deformation was set at 12 mm, which corresponded to 33-40 % of sample height. The waiting time between the two compressions was 5 seconds (24,25). The measurements were done in samples of three distinct batches, as this is a destructive test. The average ± SD of each sample type was calculated.

Sensory analysis

The sensory analysis was carried out by a consumer panel of 40 people from the University community, with ages between 15 and 45. Fresh cheese (600 mL each, prepared in 1 L glass flasks) incorporating 6.4 % of four different (ripened) types of cheese were prepared: goats’ cheese (sensorial
sample S.GT), ewes’ (S.EW), and two with Cheddar cheese, without and with skim milk powder at 3 % (sensorial samples S.CH and S.CH.SMP). The starch selected for this purpose was the waxy rice, as preliminary trials showed that it led to fresh cheese with a smoother texture and no perceptible starch taste. Also, albeit the physicochemical studies here reported used Emmental cheese, the sensorial tests employed the stronger flavoured Cheddar, and included a goats’ cheese. After resting overnight at 4 °C, the fresh cheese samples were equilibrated in a waterbath (TW20, JULABO GmbH, Seelbach, Germany) at 8 °C, and maintained at this temperature throughout the session. The panellists were served with a spoonful of each sample in a small plastic cup, upon arrival. The samples were identified by randomized codes. The panellists were asked to give ratings on a 9-point hedonic scale for each product, regarding the following attributes: appearance, odour, texture, flavour, and overall evaluation of the product. They were asked in addition to evaluate the intensity of the flavour of each sample, as too weak, ideal or too strong.

**Statistical analysis**

The statistical analysis of these results was made using an ANOVA one-way test, with the application of the Tuckey test for pairwise comparisons between particular samples. The normality of the data, as well as the homogeneity of variances was verified, and the SPSS software (26) was used for the statistical analysis of the results.

**RESULTS AND DISCUSSION**

**Microstructure of melted cheese base**

Ripened cheese can be dispersed into sub-millimetre particles by mixing it in a hot paste of gelatinized starch in milk. This *melted cheese base* has been under study in our laboratory, in particular its applications. It can be an ingredient for the development of different food products incorporating ripened cheese, which will provide added nutritional value, texture and flavour to the final product, while preventing potential food waste. Figs. 1a-d are microscopic images of melted cheese bases prepared with corn starch (1a and 1c) and waxy rice starch (1b and 1d) using Emmental cheese.

We used light microscopy, together with particular dyes, to analyse the microstructure of melted cheese bases. The dye Rhodamine B (Figs. 1a and 1b) stains proteins orange and starch yellow (14). We can see clearly starch layers surrounding dispersed cheese protein aggregates. Furthermore, some remnants of starch granules are also visible. The protein aggregates have a broad
size range, with a larger length varying from 70 to 900 \( \mu \)m. The surrounding starch points to a strong interaction with the cheese protein. The fact that the cheese protein matrix is not completely dissociated might be due to the protein matrix breaking at the weaker points of interaction among strands, or to the protein matrix breaking at those points susceptible to stronger interactions with the gelatinized starch.

The dye Sudan III (Figs. 1c and 1d) stains fat in red. We see that the fat forms globules entrapped in the cheese matrix (25), with sizes ranging from \( \sim 1 \) \( \mu \)m up to 70 \( \mu \)m in corn starch samples, and up to 50 \( \mu \)m in waxy rice starch samples, which is in accordance with reported fat globules sizes in cheese (28,29). Furthermore, the fat globules are spherical in shape. Same zones show a diffuse staining from dispersed fat in the matrix, resultant from the high temperatures and the shearing employed in the preparation of melted cheese bases (30).

We have recently demonstrated (20) that the matrix of this same Emmental cheese is primarily held by a combination of hydrophobic and electrostatic interactions, including hydrogen bonds. Starch presents a high density of hydrogen bonds, but it also has a hydrophobic character, provided by the segments of double helices formed by (\( \alpha \)1-4) glucose chains (31-33). Therefore, we can speculate that the interactions between starch and the protein aggregates can also be dominated by electrostatic and hydrophobic bonds (31,35). These mutual interactions are the foundation for the cheese-dispersing capability of gelatinized starch. Future studies are deserved to study specifically this feature in more depth.

**Key variables in fresh cheese preparation**

In this research, a novel renneted fresh cheese was developed based on melted cheese bases. Supplementary Material provides a photo of one such sample (Fig. S1). Fresh cheese is generally produced by renneting milk, or a mixture of milk and cream, and then draining the curd, in order to obtain higher concentrations of protein and fat. Alternatively, a few dairy industries start by concentrating the milk by ultrafiltration before renneting. In this latter case, the coagulation can be carried out already in the final package, and, in order to achieve a longer shelf life, the product can be further subjected to heat treatment. In either process, though the product has an increased protein (and fat) content, the flavour is bland compared to ripened cheese. Our technique enables the inclusion of ripened cheese - which could eventually become food waste - into the matrix of the novel fresh cheese, providing additional high value protein, fat, salts, texture and flavour to the product. The production and consumption of fresh cheese varieties have been facing a steady and continuous increase over the past years and constitute a major proportion of the cheese consumed in many
countries, including Portugal, where the traditional form of fresh cheese is designated *queijo fresco*. Fresh cheese varieties may vary between a softer creamy texture to a more continuous gel suitable for cutting. Extreme softness, brittleness or graininess, and lower yields are some of the drawbacks pointed out in conventional fresh cheese manufacture. Our technique enables the achievement of a more consistent and continuous curd at higher yields. It should also be noted that the quality and nutritional balance of fresh dairy products, including fresh cheese varieties, the versatility of their consumption (home cooking and processing use) and longer shelf-life strategies have been pointed out as important features to contribute to increasing EU net exports of fresh dairy products by 2030 (36). The novel fresh cheese developed herein contributes to this product competitiveness where an improved nutritional profile (corresponding to the evolving consumers demands), a higher and much appreciated sensorial quality, an improved versatility of consumption, and a cost-effective production adaptability leading to the reduction of waste and higher yields, are all contributing features, and in the medium-long term they lead to higher *per capita* consumption.

From a technological point of view, differences were observed in the production of conventional and melted cheese base-related renneted fresh cheese; the addition of fungal rennet to plain semi-skimmed milk resulted, after 20 minutes, in a soft gel that, when cut and slightly squeezed, surfaced whey, whereas the renneting of preparations with MCBs was completed in approximately 15 minutes, a shorter time relative to the above control. No surfacing of whey was observed when these gels were cut.

Preliminary trials were carried out varying the amount of cheese and that of starch in the final mixture. Incorporation of cheese above a final mass concentration of approximately 8 % (*m/m*) resulted in a too soft, almost fluid, texture of the fresh cheese. The conclusion being that the incorporation of dispersed ripened cheese inhibited gel formation. In fact, in the melted cheese base, the sub-millimetre cheese particles are coated with starch, as revealed by microscopy. It is thus understandable that these particles act as inert fillers, hindering gel formation by the renneted milk caseins. Therefore, the microstructure of the fresh cheese is envisaged as a typical fresh rennet gel (14,37) that incorporates dissolved starch fragments and dispersed starch-coated ripened cheese particles.

Having an amount of starch above 2 % in the final mixture, although leading to stronger gels, can also lead to a perceptible starch taste in the product, particularly when corn starch is used. Therefore, based on these observations, standard fresh cheese samples had a fixed final starch concentration of 2 % and an 8 % limit for ripened cheese incorporation.
Total solids in ripened cheese and in fresh cheese samples

The determination of dry matter content (total solids) was carried out for two types of ripened cheese and two representative fresh cheese samples incorporating 6.4 % (m/m) of those same cheese varieties. These samples were selected for having identical content of ripened cheese as the ones in sensorial analysis. Duplicate measurements were carried out and the average ± SD are presented in Table 2. As the total solids of the Emmental and ewes’ cheese were similar, the two fresh renneted types of cheese also had identical results, as expected. ANOVA-one way test was applied between the pairs of samples Emmental / Ewe’s cheese and EM.CS.4 / EW.CS.4, showing that the samples of each pair were not significantly different (p < 0.05) in this regard. Regarding the fresh cheese samples, we note that the level of total solids is within typical values of several varieties of conventional fresh cheese varieties, such as cottage cheese, ymer, or fromage blanc (38, 39).

Total protein in ripened cheese

Using our modified method of Reichardt & Eckert (19), with cheese dispersed in hydroxide solution, followed by evaluation of the protein in the fat-free solution by UV absorbance, the protein content of Emmental cheese was 29.8 ± 0.25 % (w/w) and that of ewes’ cheese was 21.3 ± 0.42 % (w/w). These results are very close to the values indicated in the product labels, namely 28 % and 23 %, respectively. This shows that the method employed is quite precise, and it can be used in alternative to the lengthy Kjeldahl one. In fact, previous work in our laboratory has shown a good agreement between the two methods (20).

Total fat in ripened cheese

The Van Gulik method applied to Emmental, ewes’, and goats’ ripened cheese indicated fat contents (± SD) of 29 ± 0.07 % (m/m), 27 ± 0.71 %, and 35 ± 0.00 %, respectively. These values are also close to the ones indicated on the products labels (28 %, 30 %, and 35 %, respectively).

The Van Gulik method was also carried out for samples of the fresh renneted cheeses. However, probably due to interference of the starch, the method was not successful.

Macronutrient composition of fresh cheese samples

Table 3 presents the macronutrient composition of fresh cheese samples incorporating EM and EW cheese. The results were determined based on the corresponding compositions of the raw materials - milk, cheese, and starches. We considered that the validation of the macronutrient composition of the starting materials – particularly the ripened cheese, as described above, is...
sufficient for a reliable estimate of the corresponding composition of the fresh renneted cheese. These have a more complex matrix, with some analytical methods, in particular fat determination, being more prone to interferences.

The above-mentioned experimental results for total solids of samples EM.CS.4 and EW.CS.4 (15.1 % for both) can be compared with the sum of macronutrients in these tables, 15.8 % and 15.2 %, respectively. Albeit these last numbers do not incorporate the (minor) ash contents, the proximity of results supports our methodology.

The composition of this fresh renneted cheese can be compared to those of commercial products. A well-known commercial spreadable processed cheese (Philadelphia, Kraft Foods) has a protein content around 5.4 % for the original version, and 7.4 % for the light version (40). A review of different brands of fromage blanc in the French market points to an average protein content of 5.7 % (39). In terms of fat content, the Original Philadelphia has 21 %, and the light version 11 % (40), while the average of fromage blanc is 7.8 % (39). Our samples have similar protein content, but the formulation can be easily adapted for higher protein, by simply increasing the amount of ripened cheese and/or skim milk powder (see below). In terms of fat, our fresh cheese has significantly lower content than the Philadelphia (or other brands) cream cheese, and also lower than the standard fromage blanc, which can be envisaged as a health-promoting feature. These observations are also in agreement with a recent study of six commercial cream cheeses (41).

An issue can be raised regarding the salt level on the fresh cheese, as ripened cheese generally have a considerable concentration. As the only ingredient contributing significantly to salt level is the ripened cheese, attending that all varieties used have salt levels below 1.9 g/100 g, then it imparts in the fresh cheese, at the incorporations used, a salt level below 0.15 g/100 g, which is fairly low. We point out that there is no salting step in the preparation procedure.

The control samples of just milk had a pH of 6.4, and those of milk plus starch of 6.6. The samples with ripened cheese had, as expected, slightly lower pH, with values reaching 5.8 for those with the highest incorporation. These values are higher than those of common fresh cheese varieties, which, in most cases, are within 4.5 – 5.0 (38); as our samples did not undergo an acidification step, either by fermentation or acid addition.

Level of syneresis

The determination was done for a meaningful set of samples, in duplicate, and the average ± SD of the measurements is presented in Table 4. The control sample CT, containing only renneted milk, shows the highest syneresis values. Incorporation of corn starch or waxy rice starch (CT.CS.1-3 and CT.WRS.1-3) lead to a decrease in syneresis, with the difference to the control CT being
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statistically significant (p < 0.05) at the 4 % (w/w) concentration. Corn starch and waxy rice starch, at equal concentrations, showed no significant differences among them (p > 0.05), therefore, we can conclude that starch addition contributes to water retention in the gel and lower syneresis.

Addition of 2.8 % (m/m) skim milk powder also seemed to contribute to an additional reduction in syneresis, when samples with the same amount of either corn starch or waxy rice starch (2 %) are compared, although with no statistical significance (p > 0.05). This observation deserves further studies using a higher concentration of skim milk powder.

The addition of ripened cheese to fresh cheese samples prepared with corn starch lead to higher syneresis values (though not statistically significant), and in samples with waxy rice starch the addition of ripened cheese affected syneresis minimally, comparing with corresponding controls. In both cases, increasing from 4.8 % to 8 % cheese incorporation did not change syneresis significantly (p > 0.05). Therefore, one can conclude that the presence of starch is the factor with the higher impact on syneresis, as seen also in previous studies (42,43).

Textural analysis

TPA measurements were done in duplicate samples and the average ± SD of the two measurements is presented in Table 5. The parameters selected were hardness, adhesiveness, springiness, and cohesiveness.

The force – deformation curves (not shown) revealed that all samples fractured near or at the hardness level (where the maximum force was applied), except sample CT.WRS.3, which did not fracture. The majority of the cheese samples fractured between 55 and 100 g applied force. The fact that CT.WRS.3 did not fracture resides in the particular resistance of this gel, provided by both the gelatinized waxy starch and the additional rennetable casein from skim milk powder.

Regarding hardness, for control samples, addition of 2% starch (CT.CS.1 or CT.WRS.1) lead to slightly lower values than the one of the milk control (CT), but 4 % starch (CT.CS.2 or CT.WRS.2) reverted the trend; however, the differences are not statistically significant (p > 0.05).

In control samples added with skim milk powder (CT.CS.3 and CT.WRS.3), the one prepared with waxy rice starch showed a significant difference (p < 0.05) with the other control samples. As said above, skim milk powder increases the protein content, which creates a higher concentration of rennetable, gel-forming casein molecules, resulting in a more rigid structure.

Among the samples with ripened cheese, a common trend is observed, namely, increasing the amount of cheese results in lower values for hardness. The dispersed ripened cheese acts as an inert filler, therefore hindering the formation of a more continuous and homogeneous gel network, thus explaining the impact on hardness. But, as with the control samples without cheese, addition of
skim milk powder increased considerably the hardness of the gel, particularly in the presence of waxy rice starch.

The initial slope of the force – deformation curve gives an apparent modulus of elasticity of the gels (44). We have evaluated such slopes for a representative number of samples (data not shown). While the control CT sample had a slope of 20 N/cm, in the controls with starch (CT.CS.1 and CT.WRS.1) the number decreased to 10-12 N/cm, that is, resulted in a softer gel. However, inclusion of skim milk powder (CT.CS.3 and CT.WRS.3) results in an almost 3-fold higher value (36-39 N/cm). Therefore, the inclusion of skim milk powder makes the gel significantly stiffer. Samples incorporating ripened cheese have lower elasticity than CT, indifferently of having corn starch or waxy rice starch. These results are in alignment with those previously reported for hardness.

Regarding adhesiveness, control samples with starch, either corn starch or waxy rice starch, lead to increased (negative) values. On the other hand, addition of SMP decreased adhesiveness of control samples. Samples incorporating ripened cheese showed lower adhesiveness than corresponding controls, and without a significant variation with the level of cheese incorporation. We note that the relatively high values of standard deviation reported for adhesiveness in same samples is a common feature of this analysis, particularly with heterogeneous matrixes, as it is the case (45,46). But one can conclude that starch is the ingredient with the main impact on this property.

As for the cohesiveness values, controls and samples with ripened cheese showed similar values among them, albeit the statistical analysis showed differences in a few cases. For instance, CT and CT.WRS.2, and CT and CT.WRS.1, have statistically significant differences (p < 0.05), indicating that samples with 2 % and 4 % waxy rice starch are more cohesive than the basic milk control.

All samples showed springiness values close to 1, which indicates that they sprung back almost entirely after the first compression, albeit the above-mentioned gel breakage.

**Sensory analysis**

A total number of 40 participants were included in the sensory analysis of the fresh cheese samples, predominantly young people, reflecting a university campus: 40 % were below 24 years-old, 37 % between 25 and 34, 10 % between 35 and 45, and 13 % were above 45.

The panel of participants reported a frequent consumption of dairy products, including cheese (83 % of the participants), milk (75 %), and yoghurt (65 %), with a slightly higher preference for cheese, which makes them a good consumer panel for this product.

Regarding the different modes of consumption of fresh cheese, 63 % of the consumers of the panel slice it over bread or toast, 55 % consume it in salads, and 43 % might also eat it by itself. The
attributes most enjoyed in fresh cheese were reported to be freshness (75 %), followed by texture (55 %), and flavour (48 %). 30 % of the participants are also interested in its low caloric content.

For the purpose of these sensorial tests, we selected the same ripened ewe’s cheese used in the above studies but substituted the (cow’s) Emmental for Cheddar cheese (CH), for its stronger flavour, and also included a goat’s cheese (GT). All three were used at 6.4 % (m/m) in the fresh cheese samples. A fourth sample, made with Cheddar’s, included also skim milk powder for a firmer texture.

The fresh cheese tested were rated for their appearance, odour, texture, flavour, and overall evaluation in a 9-point hedonic scale (in which 1 - dislike extremely, 2 - dislike very much, 3 - dislike moderately, 4 - dislike slightly, 5 - neither like or dislike, 6 - like slightly, 7 - like moderately, 8 - like very much, 9 - like extremely). The participants showed preference for the sample with Cheddar cheese and added with skim milk powder (S.CH.SMP), in all the attributes (Fig. 2a). This fresh cheese also had the highest overall evaluation, with an average of 6.3 points, followed by sample S.GT, with a rating of 5.5.

Regarding the intensity of flavour (Fig. 2b), 50 % of the participants replied that sample S.CH.SMP was just right, and 45 % had the same opinion for sample S.CH. 50 % found sample S.EWE having a too strong flavour, and 42.5 % found sample S.GT having a weak flavour. In fact, ewe’s cheese frequently has an intense flavour, to which many consumers are not familiarized, and/or not expect to find in a fresh cheese. The goat’s cheese used had a milder flavour intensity than either the Cheddar or the ewe’s cheese.

An additional comment made by 10 participants was that, regarding texture, the samples were more similar to yoghurt or quark cheese, rather than to a characteristic Portuguese fresh cheese, which has a denser texture. The relevance given to this property justifies that the sample with highest rating was the one with skim milk powder (S.CH.SMP), which leads to a product with a considerably more consistent and cohesive texture. A recent work points as well to a correlation between results of textural parameters and sensorial evaluation for acid coagulated fresh cheeses (47).

CONCLUSIONS

Ripened cheese can be dispersed in hot gelatinized starch in milk and the resulting slurry (melted cheese base) can be an ingredient for a variety of food products. This represents a valorisation strategy for ripened cheese that could eventually become food waste. In this work, the melted cheese base was diluted with further fluid milk and then renneted, in order to obtain novel fresh cheese. That ingredient not only adds protein and fat to the fresh cheese, but it also adds minerals and flavour. A step of whey drainage is not included and the overall process is extremely
simple. The fresh cheese developed have a valuable and balanced nutritional content, and a texture similar to many commercial fresh cheese types, or spreadable processed cheese. Furthermore, no emulsifying salts or any non-natural ingredient are used, enabling the classification of “clean label” and alignment with the sustainable development goals.

Gel formation of the initial mixture is hindered above a certain incorporation of ripened cheese, but this can be overcome by the addition of skim milk powder (or rennetable casein) to the preparation. Starch and skim milk powder both reduce syneresis of the renneted gel. Starch seems to decrease gel hardness, but addition of skim milk powder has a strong opposite effect. The sensory attributes of the product, such as texture and flavour, can be modulated by varying the amount and type of ripened cheese, and of extra casein (from skim milk powder or other). According to a consumer panel, the preference was higher for a more solid texture and with the flavour of a traditional cow’s cheese.

Therefore, the viability and versatility of this novel fresh cheese is here demonstrated. Further work can focus on minor adjustments of composition and in adjusting production to the pilot scale level.

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CONFLICT OF INTEREST
The authors declare not to have any conflict of interest.

AUTHORS’ CONTRIBUTION
E. Palyvou-Gianna carried out most of the experimental work, but also participated in work design, data analysis and writing of the manuscript. T. Paula-Vilela gave support to experimental work and was involved in data analysis and writing. A. M. Gomes shared the experimental design and the supervision of all tasks. J. P. Ferreira was the main supervisor of all project tasks and the project coordinator.

SUPPLEMENTARY MATERIAL
All supplementary material is available at: www.ftb.com.hr


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https://doi.org/10.1016/j.idairyj.2020.104939

Legends to Figures

Fig. 1. Optical microscopy images of melted cheese bases (MCBs) prepared with corn starch (CS) and with waxy rice starch (WRS), stained with Rhodamine B (a) and b), respectively; and stained with Sudan III (c) and d), respectively.

Fig. 2. Panel evaluation of: a) different attributes and overall acceptability, and b) flavour intensity of the different fresh cheese samples. S.GT, S.EW, S.CH are fresh cheese samples incorporating goats’, ewes’, or Cheddar cheese; S.CH.SMP also incorporates skimmed milk powder (SMP).
Figure 1
Figure 2

a)

![Pentagon chart showing appearance, odor, flavor, texture, and overall evaluation for different samples (S.GT, S.CH, S.EW, S.CH.SMP)].

b)

![Bar chart comparing samples (S.GT, S.CH, S.EW, S.CH.SMP) for intensity of flavor: too weak, the right intensity, too strong].
Table 1. Mixing of different preparations in the formulation of control and fresh cheese samples. Percentages are \( (m/m) \).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milk (g)</th>
<th>Milk + 4 % Starch (g)</th>
<th>Milk + 5.7 % SMP (g)</th>
<th>MCB 16 % Cheese (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CT.CS.1</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CT.WRS.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT.CS.2</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CT.WRS.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT.CS.3</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>CT.WRS.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM.CS.1</td>
<td>50</td>
<td>30</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>EM.WRS.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM.CS.2</td>
<td>50</td>
<td>20</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>EM.WRS.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM.CS.3</td>
<td>50</td>
<td>10</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>EM.WRS.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM.CS.4</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>EM.WRS.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM.CS.5</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>EM.WRS.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EW.CS.1</td>
<td>EW.WRS.1</td>
<td>EW.CS.2</td>
<td>EW.WRS.2</td>
</tr>
<tr>
<td>-----</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>30</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

CT: milk control; CT.CS and CT.WRS: milk control with 2% corn starch (CS) or waxy rice starch (WRS); EM.CS.1 to 5 and EM.WRS.1 to 5: fresh cheese samples with varying amounts of Emmental cheese (EM), with CS or WRS; EW.CS.1 to 5 and EW.WRS.1 to 5: fresh cheese samples with varying amounts of ewes’ cheese (EW); some samples also have skimmed milk powder (SMP).
Table 2. Dry matter content ($m/m$) of two ripened cheeses and two fresh cheese samples ($n=4$).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total solids ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emmental cheese</td>
<td>61.6 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ewes’ cheese</td>
<td>57.8 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EM.CS.4</td>
<td>15.1 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EW.CS.4</td>
<td>15.1 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Samples with the same superscript letter, within the same column, do not present statistical differences among them, according to the Tuckey test.

EM.CS.4 and EW.CS.4: fresh cheese samples containing either Emmental cheese (EM) or ewes’ cheese (EW), and corn starch (CS).
Table 3. Macronutrient composition of the fresh cheese samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proteins (g/100 g)</th>
<th>Lipids (g/100 g)</th>
<th>Carbohydrates (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM.CS.1</td>
<td>4.2</td>
<td>2.4</td>
<td>6.9</td>
</tr>
<tr>
<td>EM.CS.2</td>
<td>4.6</td>
<td>2.9</td>
<td>6.9</td>
</tr>
<tr>
<td>EM.CS.3</td>
<td>5.0</td>
<td>3.3</td>
<td>6.9</td>
</tr>
<tr>
<td>EM.CS.4</td>
<td>5.4</td>
<td>3.8</td>
<td>6.6</td>
</tr>
<tr>
<td>EW.CS.1</td>
<td>3.9</td>
<td>2.4</td>
<td>6.9</td>
</tr>
<tr>
<td>EW.CS.2</td>
<td>4.2</td>
<td>2.8</td>
<td>6.9</td>
</tr>
<tr>
<td>EW.CS.3</td>
<td>4.5</td>
<td>3.2</td>
<td>6.9</td>
</tr>
<tr>
<td>EW.CS.4</td>
<td>4.8</td>
<td>3.6</td>
<td>6.8</td>
</tr>
<tr>
<td>EW.CS.5</td>
<td>5.4</td>
<td>3.6</td>
<td>7.8</td>
</tr>
<tr>
<td>EM.WRS.1</td>
<td>4.2</td>
<td>2.4</td>
<td>6.8</td>
</tr>
<tr>
<td>EM.WRS.2</td>
<td>4.6</td>
<td>2.9</td>
<td>6.8</td>
</tr>
<tr>
<td>EM.WRS.3</td>
<td>5.0</td>
<td>3.3</td>
<td>6.7</td>
</tr>
<tr>
<td>EM.WRS.4</td>
<td>5.4</td>
<td>3.8</td>
<td>6.7</td>
</tr>
<tr>
<td>EM.WRS.5</td>
<td>6.1</td>
<td>3.8</td>
<td>7.6</td>
</tr>
<tr>
<td>EW.WRS.1</td>
<td>3.9</td>
<td>2.4</td>
<td>6.8</td>
</tr>
<tr>
<td>EW.WRS.2</td>
<td>4.2</td>
<td>2.4</td>
<td>6.8</td>
</tr>
<tr>
<td>EW.WRS.3</td>
<td>4.5</td>
<td>2.8</td>
<td>6.7</td>
</tr>
<tr>
<td>EW.WRS.4</td>
<td>4.8</td>
<td>3.2</td>
<td>6.7</td>
</tr>
<tr>
<td>EW.WRS.5</td>
<td>5.4</td>
<td>3.6</td>
<td>7.6</td>
</tr>
</tbody>
</table>
EM.CS1 to 5 and EM.WRS1 to 5: fresh cheese samples with varying amounts of Emmental cheese (EM), with corn starch (CS) or waxy rice starch (WRS); EW.CS1 to 5 and EW.WRS1 to 5: fresh cheese samples with varying amounts of ewes’ cheese (EW); samples with number 5 also have skimmed milk powder (SMP).

Table 4. Average values for syneresis of fresh cheese samples. All percentages are \((m/m)\) \((n = 4)\).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Starch (%)</th>
<th>Ripened Cheese (%)</th>
<th>SMP (%)</th>
<th>Syneresis ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>((48 ± 2.8)^a)</td>
</tr>
<tr>
<td>CT.CS.1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>((23 ± 0.1)^{a,c})</td>
</tr>
<tr>
<td>CT.CS.2</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>((11 ± 2.1)^{b,c})</td>
</tr>
<tr>
<td>CT.CS.3</td>
<td>2</td>
<td>-</td>
<td>2.8</td>
<td>((21 ± 3.6)^{a,c})</td>
</tr>
<tr>
<td>CT.WRS.1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>((36 ± 0.1)^{a,c})</td>
</tr>
<tr>
<td>CT.WRS.2</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>((10 ± 0.3)^{a,c})</td>
</tr>
<tr>
<td>CT.WRS.3</td>
<td>2</td>
<td>-</td>
<td>2.8</td>
<td>((22 ± 1.8)^{a,c})</td>
</tr>
<tr>
<td>EW.CS.2</td>
<td>2</td>
<td>4.8</td>
<td>-</td>
<td>((37 ± 0.1)^{a,c})</td>
</tr>
<tr>
<td>EW.CS.4</td>
<td>2</td>
<td>8</td>
<td>-</td>
<td>((39 ± 1.3)^{a,c})</td>
</tr>
<tr>
<td>EW.WRS.2</td>
<td>2</td>
<td>4.8</td>
<td>-</td>
<td>((42 ± 0.0)^{a})</td>
</tr>
<tr>
<td>EW.WRS.4</td>
<td>2</td>
<td>8</td>
<td>-</td>
<td>((40 ± 0.2)^{a,c})</td>
</tr>
</tbody>
</table>

Samples with the same superscript letter, within the same column, do not present statistical differences among them, according to the Tuckey test.

CT: milk control; CT.CS.1 and 2 and CT.WRS.1 and 2: milk control with corn starch (CS) or waxy rice starch (WRS); CT.CS.3 and CT.WRS.3 also have skimmed milk powder (SMP); EW.CS.2 and 4 and EW.WRS.2 and 4: fresh cheese samples with Emmental (EM) or ewes’ (EW) cheese.
Table 5. Results of textural analysis for fresh cheese samples ($n = 3$).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (g)</th>
<th>Adhesiveness (g)</th>
<th>Springiness (g.s)</th>
<th>Cohesiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>$139.71 \pm 11.18^{d,f}$</td>
<td>$-31.13 \pm 7.43^{a}$</td>
<td>$0.97 \pm 0.01^{a}$</td>
<td>$0.47 \pm 0.03^{a}$</td>
</tr>
<tr>
<td>CT.CS.1</td>
<td>$98.08 \pm 3.30^{c,e}$</td>
<td>$-63.63 \pm 3.17^{a}$</td>
<td>$0.95 \pm 0.01^{a}$</td>
<td>$0.51 \pm 0.00^{a,b}$</td>
</tr>
<tr>
<td>CT.CS.2</td>
<td>$166.51 \pm 12.34^{b,f}$</td>
<td>$-190.52 \pm 16.56^{b,c}$</td>
<td>$0.93 \pm 0.01^{b}$</td>
<td>$0.50 \pm 0.01^{a,b}$</td>
</tr>
<tr>
<td>CT.CS.3</td>
<td>$146.78 \pm 16.9^{a,f}$</td>
<td>$-110.59 \pm 21.30^{a}$</td>
<td>$0.95 \pm 0.00^{a}$</td>
<td>$0.48 \pm 0.02^{a,b}$</td>
</tr>
<tr>
<td>CT.WRS.1</td>
<td>$104.92 \pm 15.81^{a,d,e}$</td>
<td>$-65.21 \pm 12.05^{a}$</td>
<td>$0.95 \pm 0.02^{a}$</td>
<td>$0.62 \pm 0.0^{c}$</td>
</tr>
<tr>
<td>CT.WRS.2</td>
<td>$147.66 \pm 76.8^{a,f}$</td>
<td>$-258.99 \pm 151.91^{b}$</td>
<td>$0.91 \pm 0.03^{b}$</td>
<td>$0.5 \pm 0.00^{b}$</td>
</tr>
<tr>
<td>CT.WRS.3</td>
<td>$227.30 \pm 27.58^{a,b}$</td>
<td>$-209.75 \pm 1.75^{a}$</td>
<td>$0.94 \pm 0.00^{a,b}$</td>
<td>$0.52 \pm 0.00^{a}$</td>
</tr>
<tr>
<td>EM.CS.1</td>
<td>$94.05 \pm 7.02^{c,d,e}$</td>
<td>$-63.1 \pm 8.70^{a,c,e}$</td>
<td>$0.96 \pm 0.00^{a,b}$</td>
<td>$0.48 \pm 0.00^{a,b}$</td>
</tr>
<tr>
<td>EM.CS.2</td>
<td>$77.38 \pm 6.14^{c,d,e}$</td>
<td>$-50.9 \pm 11.8^{a,c,e}$</td>
<td>$0.96 \pm 0.00^{a,b}$</td>
<td>$0.51 \pm 0.00^{a,b}$</td>
</tr>
<tr>
<td>EM.CS.3</td>
<td>$67.61 \pm 0.39^{d,e}$</td>
<td>$-43.24 \pm 3.6^{a,c}$</td>
<td>$0.96 \pm 0.00^{a,b}$</td>
<td>$0.53 \pm 0.00^{a,b}$</td>
</tr>
<tr>
<td>EM.CS.4</td>
<td>$54.74 \pm 1.45^{e}$</td>
<td>$-26.69 \pm 1.5^{a,c}$</td>
<td>$0.97 \pm 0.00^{a,b}$</td>
<td>$0.61 \pm 0.00^{a,b}$</td>
</tr>
<tr>
<td>EM.CS.5</td>
<td>$99.11 \pm 4.76^{c,d,e}$</td>
<td>$-69.84 \pm 12^{a,c,d}$</td>
<td>$0.95 \pm 0.00^{a,b}$</td>
<td>$0.52 \pm 0.00^{a,b}$</td>
</tr>
<tr>
<td>EW.CS.1</td>
<td>$84.43 \pm 2.97^{c,d,e}$</td>
<td>$-49.82 \pm 7.75^{a,c,e}$</td>
<td>$0.96 \pm 0.01^{a,b}$</td>
<td>$0.52 \pm 0.04^{a,b}$</td>
</tr>
<tr>
<td>EW.CS.2</td>
<td>$79.53 \pm 4.79^{c,d,e}$</td>
<td>$-54.41 \pm 2.98^{a,c,e}$</td>
<td>$0.96 \pm 0.00^{a,b}$</td>
<td>$0.51 \pm 0.03^{a,b}$</td>
</tr>
<tr>
<td>EW.CS.3</td>
<td>$63.2 \pm 8.84^{d,e}$</td>
<td>$-31.54 \pm 11.24^{a,c}$</td>
<td>$0.97 \pm 0.00^{a,b}$</td>
<td>$0.56 \pm 0.01^{a,b}$</td>
</tr>
<tr>
<td>EW.CS.4</td>
<td>$57.33 \pm 3.27^{e}$</td>
<td>$-24.55 \pm 9.29^{a,c}$</td>
<td>$0.97 \pm 0.01^{a,b}$</td>
<td>$0.58 \pm 0.03^{a,b}$</td>
</tr>
<tr>
<td>EW.CS.5</td>
<td>$118.52 \pm 2.03^{a,d,e,f}$</td>
<td>$-84.59 \pm 12.28^{a,c,d}$</td>
<td>$0.97 \pm 0.02^{a,b}$</td>
<td>$0.46 \pm 0.01^{a,b}$</td>
</tr>
<tr>
<td>EW.WRS.1</td>
<td>$107.61 \pm 10.03^{a,c,d,e,f}$</td>
<td>$-92.5 \pm 134.58^{a,c,d}$</td>
<td>$0.94 \pm 0.00^{a,b}$</td>
<td>$0.53 \pm 0.02^{a,b}$</td>
</tr>
<tr>
<td>EW.WRS.2</td>
<td>$97.63 \pm 6.52^{c,d,e}$</td>
<td>$-77.25 \pm 115.76^{a,c,d}$</td>
<td>$0.94 \pm 0.01^{a,b}$</td>
<td>$0.53 \pm 0.01^{a,b}$</td>
</tr>
<tr>
<td>EW.WRS.3</td>
<td>$85.66 \pm 4.53^{c,d,e}$</td>
<td>$-63.89 \pm 99.92^{a,c,e}$</td>
<td>$0.95 \pm 0.00^{a,b}$</td>
<td>$0.54 \pm 0.02^{a,b}$</td>
</tr>
<tr>
<td>EW.WRS.4</td>
<td>$72.81 \pm 3.57^{c,d,e}$</td>
<td>$-47.09 \pm 79.53^{a,c,e}$</td>
<td>$0.95 \pm 0.00^{a,b}$</td>
<td>$0.57 \pm 0.00^{a,b,c}$</td>
</tr>
<tr>
<td>EW.WRS.5</td>
<td>$183.04 \pm 2.8^{f}$</td>
<td>$-184.89 \pm 31.33^{a,d,e}$</td>
<td>$0.94 \pm 0.00^{a,b}$</td>
<td>$0.47 \pm 0.00^{a,b}$</td>
</tr>
</tbody>
</table>
Samples with the same superscript letter, within the same column, have no statistical differences among them, according to the Tuckey test.

CT: milk control; CT.CS and CT.WRS: milk control with 2% corn starch (CS) or waxy rice starch (WRS); EM.CS.1 to 5 and EM.WRS.1 to 5: fresh cheese samples with varying amounts of Emmental cheese (EM), with CS or WRS; EW.CS.1 to 5 and EW.WRS.1 to 5: fresh cheese samples with varying amounts of ewes’ cheese (EW); some samples also have skimmed milk powder (SMP).

Fig. S1: Image of a fresh cheese sample, incorporating Emmental cheese and skimmed milk powder (sample EM.CS.5)