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YOGUCHEESES - YOGURTS FORTIFIED WITH MELTED CHEESE – MICROSTRUCTURAL, TEXTURAL AND RHEOLOGICAL CHARACTERIZATION

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Dr. Michael Mullan
Editor
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Dear Dr. Mullin

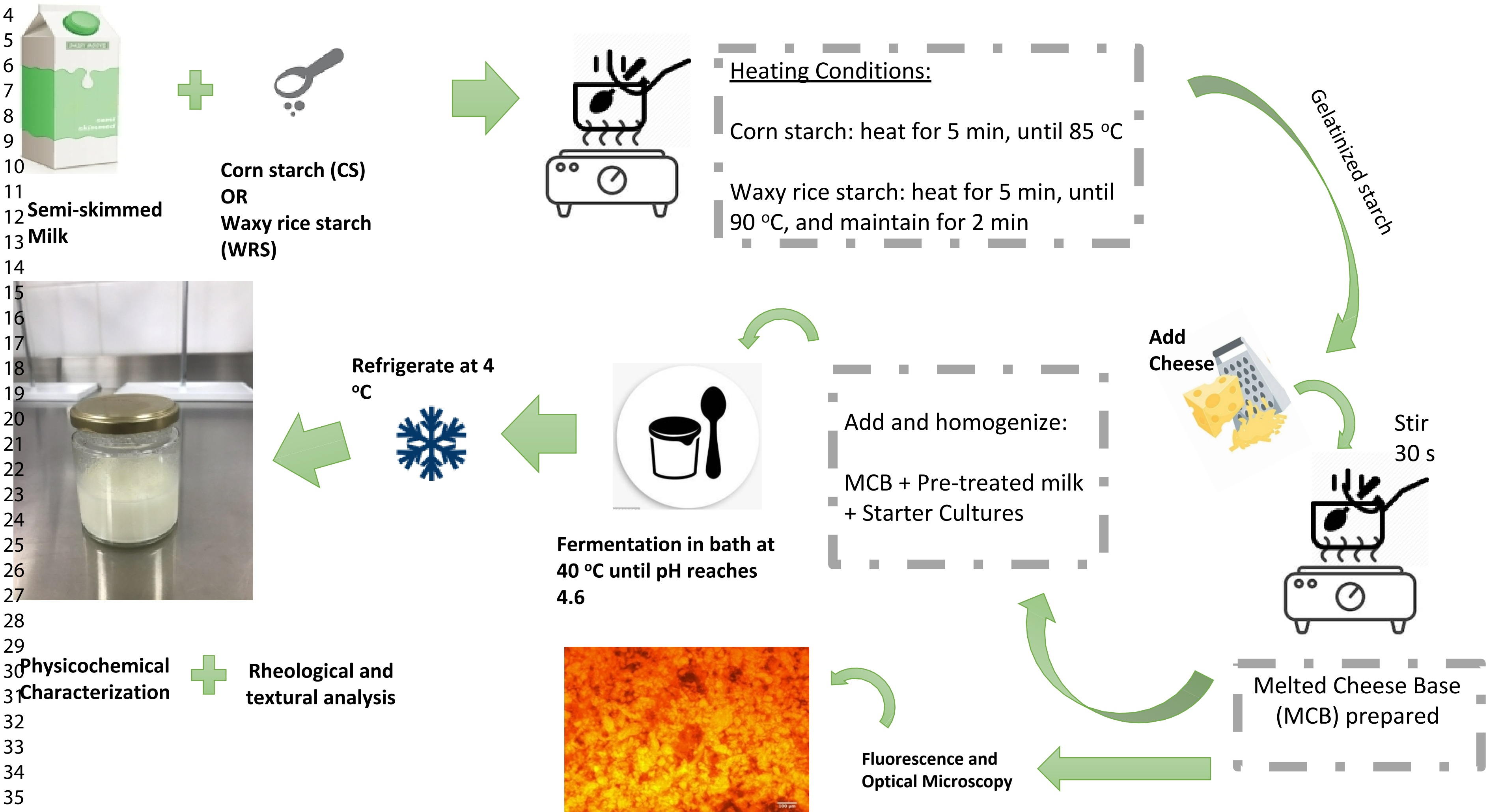
The authors are very pleased to know that, after the first revision, the manuscript **ID IJDT-0028-21.R1** was considered accepted by the reviewers. Nevertheless, changes were required by the editorial office regarding the extent of information on table headings and figures legends. A new version was prepared with those headings and legends considerable expanded, for clarity. The added text is marked with blue color.

We hope the changes introduced fulfil the objectives, otherwise we will be available for any further adjustments.

We thank once again your attention and look forward to the final acceptance of the manuscript.

With my best regards

(Assistant Professor)


Semi-skimmed Milk
Corn starch (CS)
OR
Waxy rice starch (WRS)
Heating Conditions:
Corn starch: heat for 5 min, until 85 °C
Waxy rice starch: heat for 5 min, until 90 °C, and maintain for 2 min
Gelatinized starch
Add Cheese
Stir 30 s
Melted Cheese Base (MCB) prepared
Add and homogenize:
MCB + Pre-treated milk + Starter Cultures
Fermentation in bath at 40 °C until pH reaches 4.6
Refrigerate at 4 °C
Physicochemical Characterization
Rheological and textural analysis
Fluorescence and Optical Microscopy

YOGUCHEESES - YOGURTS FORTIFIED WITH MELTED CHEESE – MICROSTRUCTURAL, TEXTURAL AND RHEOLOGICAL CHARACTERIZATION

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Running headline: Yogucheeses

Abstract

Yogurts are generally enriched with dairy proteins powders. This research developed a cheese-fortified yogurt – *yogucheese*. First, Emmental cheese was dispersed into sub-millimeter particles by melting it in a hot paste of gelatinized corn starch (CS), or waxy rice starch (WRS) in milk, forming a *melted cheese base* (MCB). This MCB was mixed with further milk in different proportions, before addition of starter culture and fermentation. The products were then characterized in terms of macronutrient composition and physical properties. The new yogurts had a firmer gel structure, lower syneresis, and higher viscosity than controls, and were stable for 14 days.

Keywords: cheese-fortified yogurt; microscopy; rheology; textural analysis; syneresis.

Introduction

Yogurt is one of the most common dairy products and its consumption has been increasing in the last years. It is prepared by fermenting cows’, goats´ or other ruminants´ milk using lactic acid-producing bacteria, under a controlled temperature and environmental conditions (Das *et al.* 2019).

Two types of yogurts are available on the market: with a firm gel-like structure (set type), or with a thick liquid consistency (stirred type). In set type yogurt, milk is inoculated with the starter, placed in the final package and subsequently fermented whereas in stirred type yogurt, the fermentation takes place in larger vessels, and then the gels are disrupted by vigorous stirring, before being transferred to the final package (Oraç and Akın 2019).

Yogurt holds a high nutritional and health benefits connotation, such as digestion enhancement, immune system boosting, anticarcinogenic activity, and reduction in serum cholesterol (Nastaj *et al.* 2019; Sarfraz et al. 2019). Besides its nutritional value, the viscosity and rate of syneresis are important indexes of sensory qualities and stability of yogurt products (Fang and Guo 2019). In recent times, the market has valorised products with increased protein content contributing to satiety promotion, with a thicker consistency, and that do not whey-off during storage. Furthermore, another recent trend is the avoidance of chemical additives, and the use of clean label ingredients. Accompanying these market trends and innovations required by industry and consumers, the academia has produced, along the last decades, a large volume of studies on yogurts and acid gels. Nevertheless, the effort is still high nowadays, fostering innovations in product texture and composition enrichment or improvement, as demonstrated by a recent issue of this periodical (volume 74, issue 1, pages 1-257), with several articles on the subject.

In order to attend to the above mentioned features and trends, the formulation of yogurts frequently includes supplementary dairy protein powders and stabilizing hydrocolloids. Among these are native and modified starches, gelatine, or xanthan gum, among others. They can provide structural stabilization, reduce syneresis, and can also have emulsification properties (He *et al.* 2019).

In order to increase the protein content, whey and whey concentrates, caseinates, and skim milk powders are also commonly included (Damin, Alcântara *et al.* 2009). For example, Bong and Moraru (2014) evaluated the addition of micellar casein concentrate to fortify Greek-style yogurts. All samples showed a shear-thinning behaviour associated with a weak-gel structure, with the fortified yogurt samples presenting lower water holding capacity than the controls.

A review study also states that the addition of skimmed milk powder (SMP) produces good quality yogurt, with increased viscosity and gel strength; furthermore, the addition of whey protein favours the final product viscosity, firmness, gel strength (G′), and syneresis (Karam *et al.* 2013).

Lobato-Calleros *et al.* (2014) showed that addition of native or chemically modified starches, from different origins, to reduced-fat yogurts contributed to the formation of more stable milk gels. Najgebauer-Lejko *et al.* (2007) studied the effect of the addition of different starches in set-style yogurts and reported products with lower acidity and higher resistance to whey separation. Goncalvez *et al.* (2005) added gelatin and starch, separately, to stirred yogurt and observed that the addition of these thickeners resulted in a significant increase in viscosity, ropiness, mouthfeel, and creaminess, while also reducing syneresis. Pang *et al.* (2016) concluded that the combination of whey protein isolate and gelling polysaccharides (starch, carrageenan and xanthan/locust bean gum) as gelatin replacers in yogurts induced stronger gels with higher water-holding capacity.

In this work, we aimed to produce protein-fortified yogurts using cheese as ingredient. This cheese can possibly come from surpluses from the industry and retail sector, which would contribute to a sustainable added-value strategy for reducing food waste and encourage a circular economy system. A significant part of surplus cheese is used for the manufacture of processed cheese; however, besides natural cheese these composites also contain additional sodium-containing chemicals, colors and flavors for aroma, taste and texture. Targeting a more natural approach and benefiting from the additional health benefits of yogurt as a fermented product in comparison to processed cheese, our study offers the opportunity to enhance the nutritional profile of yogurt using natural cheese surplus. In addition to the sustainable character of such solution, it must be highlighted that besides protein, the cheese also provides several other nutrients, such as minerals and fat, as well as flavor compounds.

Cheese can be dispersed into sub-millimeter particles by mixing it in a hot paste of gelatinized starch in milk. This *melted cheese base* (MCB) can be an ingredient for the development of novel dairy products incorporating ripened cheese, which is the subject of ongoing work in our group.

We selected grated Emmental cheese and corn starch (CS) or waxy rice starch (WRS) for the preparation of the MCBs. This was mixed with further milk in different proportions, before addition of starter culture and fermentation. The resultant cheese-fortified yogurts (*yogucheeses*) were then characterized in terms of macronutrient composition, pH and titratable acidity, syneresis, textural and rheological properties. To the best of our knowledge this is the first study using cheese surpluses to enrich protein content of yogurt matrices, creating added nutritional value in a sustainable manner.

Materials and Methods

Materials

The materials used were commercial grated Emmental cheese (Milbona, Germany), commercial native corn starch (Maizena, Unilever, Portugal), waxy rice starch (Remyline XS, BENEO GmbH Germany, kindly provided by Nutripar, Portugal), semi-skimmed (1.5 % fat) HTST milk (Vigor, Portugal), and commercial standard yogurt culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Condi, Portugal), which is a lyophilized, direct vat set culture.

Experimental Methods

MCB Preparation

CS or WRS was dispersed in cold milk, at a ratio of 5 g starch per 100 g milk, and the mixture was heated for 5 minutes, with continuous stirring, until 85 °C (for CS), or 90 °C (for WRS). At this point, the gelatinization of the starch was noticeable, and the grated cheese was added, at a ratio of 34 g cheese per 100 g milk. The mixture was then removed from the hotplate and stirred until the cheese was fully dispersed, with no visible, macroscopic pieces. The MCB was left to cool down to room temperature (ca. 21 °C).

Preparation of Yogurt Samples

The milk used to prepare all the following samples was previously heat treated at 90 °C for 10 min and then cooled to 43 °C (Torres *et al.* 2018). In order to enable the preparation of samples with different incorporations of ripened cheese, while using the same MCB and keeping constant the starch concentration, we mixed in different proportions heat treated milk, milk with 3.6 % gelatinized starch, and MCB prepared as above. The amounts are reported in Table 1. The mixtures (100 g) were prepared in small glass flasks (60 mm diameter, 65 mm height). Three different control samples (without MCB addition) were prepared: one containing plain milk (CL), and two containing milk with 2.0 % (w/w) final concentration of either CS (CL_CS) or WRS (CL_WRS).

For the cheese-fortified yogurt samples, three different incorporations were tested, corresponding to adding MCB at 20 % , 40 %, and 60 % (w/w) of overall mixture, (A20, A40, A60). Except for the milk control (CL), all other samples had CS or WRS at a final concentration of 2 % (w/w). Commercial starter was used at the recommended level of 7 g per L of preparation.

Incubation of the yogurt samples was carried out at 40 °C for about 4.5 hours, the time required for the pH to reach 4.6. The samples were then stored at 4 °C.

Light and Fluorescence Optical Microscopy

The microstructure of MCBs prepared with 3.6% (w/w) CS or WRS and 24.5% (w/w) grated Emmental cheese was analyzed using an Olympus BX51 fluorescence optical microscope (Olympus, Japan), with a 10 x objective lens, and the images were captured using an Olympus EP50 camera (Olympus, Japan). Samples were stained with Rhodamine B (1 g/L), in order to visualize starch and protein (fluorescence microscopy); and with Sudan III (1 g/L), for visualizing fat globules (light microscopy). Afterwards, samples were transferred to concave slides and covered with a cover slip. All samples were equilibrated at room temperature for 15 minutes prior to microscopic analysis and all observations were performed in duplicate, with at least four pictures taken per sample. In order to obtain the sizes of protein agglomerates, the software ImageJ (version 1.51 m9) was used.

Physicochemical Analysis

Cheese Protein Content Determination

Total protein of Emmental cheese was determined using a novel method based on the one proposed by (Reichardt and Eckert 1991), but with modifications (Paula Vilela *et al.* 2020). 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 a casein calibration curve, and the protein content in cheese was then calculated. All measurements were done in duplicate. We have shown that this method gives results that are not statistically different from those obtained with the standard Kjeldahl method (Paula Vilela *et al.* 2020).

Cheese Fat Content Determination

Fat content of Emmental cheese was evaluated using the Van Gulik method (ISO 3433 2008). All measurements were done in duplicate.

Time – pH curves and determination of titratable acidity

The pH of selected samples was recorded throughout the fermentation process, using a pH meter (sensION+ PH31, Hach, USA) equipped with a probe Sension+ pH gel combination electrode, with automatic temperature compensation. The same set was used for all other pH measurements. All evaluations were carried out in duplicate.

Titratable acidity (TA) was determined 24 hours after fermentation, following the method from (Bong and Moraru 2014). 9 ± 0.5 g of yogurt sample was diluted in 18 g of deionized water. Then, a titration was carried out using a standard 0.1 M NaOH solution and 0.5 ml phenolphthalein as an indicator. TA, expressed as % of Lactic Acid, was calculated using the equation (Nielsen 2017):

$$TA(\%\ Lactic\ Acid) = \frac{M\ NaOH \times V\ NaOH \times 90.08}{w_{sample}} \times 100\% \quad (1)$$

Where *M* is the molarity of NaOH solution; *V* NaOH is the total volume (L) of NaOH used in the titration; 90.08 $g.mol^{-1}$ is the molecular weight of lactate ; and w_{sample} (g) is the weight of the sample. All measurements were made in duplicate.

Evaluation of syneresis

20 ± 0.5 g of yogurt sample (at 4 ± 1 °C) was placed in 50 mL falcon tubes and centrifuged at 220 x *g* for 15 min, at 4 °C. The clear supernatant was poured off, weighed, and syneresis was expressed as the ratio between the weights of supernatant and original yogurt sample (Lobato-Calleros *et al.*, 2014). These measurements were made in duplicate.

Textural Properties

Texture of the samples was analysed using a TA.TX.plus texture analyser (Stable Micro Systems, Godalming, UK), calibrated with a 30 kg loading cell. The texture profile analysis (TPA) was performed with the samples pre-equilibrated at 22 ± 0.5 °C for 15 min. The tests were carried out keeping the samples in the vials in which they were originally prepared, thus avoiding gel disturbance with transfers. The equipment had a cylinder probe (36 mm diameter and 34 mm height), and the test was carried out with a trigger force of 5 g, speed of 1 mm/s, and a penetration distance of 12 mm, which corresponds to a maximum deformation of about 40% of the sample height. Hardness, adhesiveness, cohesiveness, and springiness of the samples were determined using the Exponent PC software (version 5). All measurements were carried out in duplicate samples.

Rheological Properties

The rheological properties of the yogurt samples were determined by oscillatory amplitude and frequency sweep tests, using a Bholin Gemini rheometer (Malvern Panalytical, Malvern, UK), with a cone and plate geometry probe. The samples, prepared 24 hours before and kept at 4 ºC, were slightly homogenized by mixing with a spoon before each test, then 1 mL aliquots were transferred to the probe. Samples were equilibrated at 7.5 °C before measurements. The tests were performed in duplicate.

An amplitude sweep with a strain range from 0.0001 to 0.1, at the frequency of 1 Hz, was performed, in order to find the linear viscoelastic region (LVR).

Afterwards, a frequency sweep from 0.01 to 10 Hz was applied with a strain of 0.001 , shown to be in the LVR, in order to determine several viscoelastic properties, including the storage modulus (G'), the viscous or loss modulus (G''), and the dynamic viscosity curve (η).

Product Stability Tests

A representative group of samples was selected for a stability study: a control sample, a control sample with starch, and samples with 40% (w/w) MCB incorporation, prepared with either CS or WRS. The samples were maintained at 4 °C and their stability was evaluated over the following two weeks, for visual aspect of the gel and whey separation (syneresis).

Statistical Analysis

ANOVA one-way tests were used for the statistical analysis of the physicochemical, textural and rheological data, 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 (version 26) was used for the statistical analysis of the results.

Results and Discussion

Light and Fluorescence Optical Microscopy of MCBs

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 (MCB) has been under study in our laboratory, with applications in different dairy products. The ripened cheese will provide added nutritional value and flavour to the final products. Simultaneously, this utilization of cheese can contribute to preventing potential food waste and boosting circular economy in the food sector. Figures 1 A–C are microscopic images of MCB samples, prepared with 3.6% (w/w) CS or WRS and 24.5% (w/w) Emmental cheese.

The dye Rhodamine B (Figs. 1A and 1C) stains proteins red and starch yellow, and the dye Sudan III (Fig. 1B and 1D) stains starch brown. In both MCBs, one can observe an intertwined protein-starch matrix, with sporadic starch granules remnants. The cheese protein aggregates have a size range from 5 to 200 μm in diameter. In some of these aggregates, starch layers can be seen covering their surfaces. This surrounding starch points to a strong interaction with the cheese caseins.

The dye Sudan III (Figs. 1B and 1D) stains fat globules in red and the starch is stained brown. The protein rich areas correspond to the white areas. The fat globules were entrapped in the MCB matrix, with sizes ranging from < 1 μm up to 100 μm and are spherical in shape, found in aggregate form. Some smeared red staining was observed in some areas of the matrix, likely due to the fat dispersed by the high temperatures and shearing used in the preparation of the MCBs (Macdougall *et al.* 2019).

We have recently demonstrated (Paula Vilela *et al.* 2020) 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 (α1-4) glucose chains (Bortnowska and Goluch 2018; Considine *et al.* 2010; Noisuwan 2009; Stephen *et al.* 2006). Therefore, we can speculate that the interactions between starch and the protein aggregates can also be dominated by electrostatic and hydrophobic bonds (Considine *et al.* 2010; Sun *et al.* 2016). Future studies are deserved to study specifically this feature.

Macronutrient Composition of the Yogurt Samples

Based on the corresponding compositions of the raw materials, the macronutrient compositions of the yogucheese samples are presented in Table 2. The ingredient with the highest influence on the protein and fat levels is the cheese added, so these were subjected to analyses. For the milk, we considered the supplier's nutritional values. We found the analyses of ingredients, in particular those of cheese, to be more reliable than the analyses of the yogucheeses, as these are more prone to interferences by the starch, particularly in the case of the fat determination.

The protein and fat contents of the Emmental cheese were determined as 31.5 ± 0.01 % (Paula Vilela *et al.* 2020) and 29 ± 0.07 % (w/w), respectively.

In a typical commercial yogurt, protein values most often range from 2.5 to 4 % (w/w), and lipids range from 1.5 to 4% (w/w) (Bullard *et al.* 2018; Faihst *et al.* 2017; Lobato-Calleros *et al.* 2014; Moore *et al.* 2018; Torres *et al.* 2018). In Greek yogurts, these values increase up to 11 % (w/w) for proteins and up to 10 % (w/w) for fat (Bong and Moraru 2014; Moore *et al.* 2018). Some research studies produced yogurts with values up to 5.6 % in proteins by fortifying them with whey proteins microparticulated powder or skim milk powder (Faihst *et al.* 2017; Lobato-Calleros *et al.* 2014; Pang *et al.* 2016; Tamime *et al.* 1996; Torres *et al.* 2018). Hence, the range of protein and lipid values obtained for our cheese-fortified yogurts are at, and above, the ones of full-fat commercial yogurts, even matching the ones of some Greek yogurts in the samples A40. Moreover, current consumer trends in terms of snacking products are also changing, with cheese-containing snacks being one of the biggest opportunities for creative new product developments, business growth, and improved profitability (NewNutrition Business 2020). Cheese offers consumers a high-protein, low-sugar alternative to sweet or carbohydrate-heavy snacks. As seen from table 2, our product can be considerably high in protein and the cheese incorporation can be a key factor in the final consumer acceptance.

Regarding total carbohydrates, the starch incorporation was fixed as 2 %, and the difference between these two values is the lactose content, provided mainly by the milk. The absence of sugar or other sweeteners, or any *artificial* ingredient, should also be highlighted.

An issue can be raised regarding the salt level found in the yogucheeses, as ripened cheeses can have a considerable concentration. In this case, Emmental has a particularly low salt content of only 0.6 % (w/w). Considering the yogucheese samples with the highest cheese incorporation (A60), the cheese would contribute to just 0.1 % salt, which is a fairly low level. If cheeses with higher salt concentrations were used, e.g. a rather high value of 1.9 %, then those samples would carry 0.28 % salt, corresponding to 0.11 g sodium /100 g, a value that still does not raise health concerns. Recall that the World Health Organisation recommends that adults consume less than 2 g of sodium per day as part of a healthy eating pattern.

Time-pH Curves

Figures 2 and 3 show the time-pH profiles during the fermentation period. The two control samples with starch (CL_CS and CL_WRS) start at slightly higher pH values than the plain milk control (CL), but the difference disappears at under 1-hour incubation time. Samples with a low amount of cheese (A20_CS and A20_WRS) start with pH lower than the above controls but reach pH 4.6 at about the same time (240 minutes). The observed fermentation times are within the usual range for yogurts, and similar time-pH profiles can be found in previous works (Bong and Moraru 2014; Singh and Byars 2009; Singh and Kim 2009).

Interestingly, the curves for the samples with higher amount of cheese (A40 and A60) start at considerably lower pH values but show higher resistance to decrease the pH at late fermentation stages. At about 2 hours, these curves cross the control curves, showing higher pH values thereafter. CL and A20 samples reach pH 4.6 at about 4 hours, while A40 and A60 take 30 minutes longer. This behaviour can be explained by the buffering capacity provided by the cheese proteins. If fermentation is stopped at 4 hours, for instance, the samples with more cheese will have a higher pH, a feature that can eventually be valorised sensorially by consumers. These samples also represent an example of discrepancy in the results of pH and titratable acidity (next section).

Syneresis and TA

Table 3 presents the values of syneresis and TA of the various yogucheese samples. Regarding syneresis, a comparison among the yogurt samples of plain milk (CL) and milk plus starch (CL_CS and CL_WRS), suggests that the incorporation of starch gives gels with lower whey retention. This might be due to a less regular protein gel structure in the presence of starch. We note that these values of syneresis are consistent with others reported for yogurts prepared with starch as a thickener (Ares *et al.* 2007). However, the addition of dispersed cheese leads to a significant reduction in syneresis. This effect was particularly strong in the samples with WRS. It is possible that the cheese proteins, and complexes of cheese proteins with starch, lead to free water binding; or that some of the dispersed cheese proteins take part in the continuous acid gel structure. The differences in syneresis between samples with CS and with WRS might originate from differences in the structure of the starch – cheese protein complexes and how they interact, or take part, in the continuous acid gel network. It has been reported that products that contain starch release water mainly due to retrogradation of amylose (Guardeño *et al.* 2012); as WRS has a very low percentage of amylose, this can then be a preponderant factor for the lower syneresis observed in the samples with this starch. Future studies, with a detailed characterization of these yogurt structures are justified.

Regarding TA, it is observed that, the more cheese is incorporated, the higher the TA values. This is an expected result, since cheese itself provides acidity. This can also be seen in Figs.

2 and 3, with lower initial pH values for samples with more cheese. Overall, the TA values obtained are consistent with the ones in literature for other yogurt samples (Bong and Moraru 2014).

Textural Properties

The textural parameters observed for the yogucheese samples are reported in Table 4. The original time – force curves (not shown) indicated that all samples, except CL, resisted fracture under the test conditions.

Coincidently, that control sample of plain milk (CL) has significantly higher hardness ($p < 0.05$) than all the others, except for sample A60_WRS. Addition of starch might interfere with continuity and/or regularity of the acid gel, leading to lower hardness. (John S. Mounsey & O'Riordan, 2008), for instance, showed that the addition of starch to imitation cheeses decreased hardness values. Similarly, (Mounsey and O'Riordan 2001; Ye *et al.* 2009) concluded that the addition of waxy rice starch to a model processed cheese and an imitation cheese also decreased hardness. Although revealing a similar trend, one must be aware of the differences between the products being compared. Other studies (He *et al.* 2019; Tavakolipour *et al.* 2014) have reported that the addition of modified and resistant starches to yogurt samples increased hardness when comparing with control samples. It is important to refer, however, that there are differences in starch type, methodology of yogurt preparation, and sample handling between these studies and the one presented herein.

In our samples, the addition of ripened cheese led to an increase in hardness, more pronounced in samples with WRS. In fact, sample A60_WRS showed a hardness value similar to that of the milk-control. Although it could be speculated that the cheese protein – starch complexes could hinder regular gel formation, it is also possible that those complexes interact with the acid gel, functioning as active fillers, as stated above.

Adhesiveness varies among samples with a trend similar to hardness, albeit being unrelated properties. The springiness of all samples was high and comparable; the lower value of sample A60_CS is not statistically different from the others. Addition of starch increased cohesiveness relative to the milk control, and further addition of cheese did not alter the effect. This is another sign of the active interaction between the acid gel formed by the milk protein and the starch-cheese protein complexes from the MCB.

Rheological Properties

The frequency dependence of the rheological parameters elastic modulus (*G'*), viscous modulus (*G''*) and dynamic viscosity for the yogurt samples made with CS and WRS are presented in Figures 4 to 6 (A and B).

Analysing Figs. 4 and 5 reveals that both *G'* and *G''* have weak frequency dependences, with slight increases, for all samples. This and the fact that the elastic modulus is higher than the viscous modulus (*G'* > *G''*), are characteristics of systems that have a weak gel network (Bong and Moraru 2014; Lobato-Calleros *et al.* 2014; Singh and Byars 2009).

Addition of starch, either CS or WRS, leads to lower *G'* values than that of the milk-control sample (CL). The incorporation of cheese increases *G'*, with samples A40 and A60 surpassing CL, particularly when combined with WRS. The differences between CL_CS and A60_CS, and between CL_WRS and A60_WRS, are statistically significant ($p < 0.05$). These last two observations are in line with those regarding hardness by textural analysis discussed above.

The reason for the rheological parameters *G'* and *G''* being slightly higher for the samples with CS, can be due to the higher amylose content of this starch, compared to WRS. (Mounsey and O'Riordan 2008) found that gels from amylose-containing starch had higher elasticity than from amylose-free starch, attributed to the increased rigidity and decreased swelling power of the amylose-containing starch granules.

As seen in Fig. 6, for all samples, the viscosity decreases with frequency, a characteristic of shear thinning behaviour (Bong and Moraru 2014). Yogurts have been characterized as a pseudoplastic material with this behaviour being credited to weak electrostatic and hydrophobic interactions within the yogurt matrix, which are easily disrupted by shear (Bong and Moraru 2014; Lobato-Calleros *et al.* 2014). CL_CS and CL_WRS have lower viscosity than CL. Regarding the controls, significant differences were found between CL and CL_CS and between CL and CL_WRS, with the difference being higher in this last case. Incorporation of cheese increased viscosity; sample A60_CS showed statistically higher dynamic viscosity ($p < 0.05$) than the control samples CL and CL_CS.

Stability Test

In order to determine the stability of the samples during storage, syneresis was measured after 14 days of refrigerated storage for a group of representative yogucheese samples. The results in Table 5 show that, after 14 days, the syneresis of the samples remained unaffected ($p > 0.05$). As for the visual aspect of the yogucheeses, also no alterations were noticeable, showing a good stability in all cases.

Conclusions

A successful novel dairy product, a cheese-fortified yogurt, was developed. This was possible by melting the cheese in a hot paste of gelatinized starch. This alternative strategy for utilization of cheese can contribute to food surplus minimization, while bringing additional nutrients and specific flavors to these yogucheeses. Ingredients that are not compatible with clean label designation are not used, neither are sweeteners of any kind. The preparation process requires minimum number of ingredients, and it is quite straightforward. The adaptation of manufacturers to this novel variant of yogurt would also be relatively simple. Therefore, yogucheeses can potentially represent a novel line of dairy products, economically and environmentally interesting and rewarding, and in line with the recent consumers´ trends and demands for more sustainable and health-promoting foods.

The incorporation of CS and WRS, combined with the incorporation of ripened cheese, created yogurt samples with a firmer gel structure, lower syneresis, and higher viscosity. All the yogurts were stable throughout a period of 14 days, with no alterations in their visual aspect and syneresis behavior.

Future work will deepen various features of this novel product: more detailed analyses of the microstructures of both MCBs and yogucheeses with confocal and scanning electron microscopies; studies of cultures growth during fermentation and their evolution during product storage, as well as total counts at several critical time points; evaluation of consumers' acceptance through carefully designed sensorial tests (Costa *et al.* 2020) – although preliminary ones were already carried out-, that will include samples with different cheeses, at varying levels of incorporation.

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Declarations of interest: none

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure Captions

Figure 1. Optical microscopy images of melted cheese base (MCB) samples prepared with corn starch (CS) (1A and 1B) or waxy rice starch (WRS) (1C and 1D) and Emmental cheese, stained with Rhodamine B (1A and 1C) and Sudan III (1B and 1D).

Figure 2. Time-pH curve for yogurt samples made with corn starch (CS). ● CL, ▲ CL_CS, ♦ A20_CS, ■ A40_CS, and × A60_CS. Formulations of these samples are as described in Table 1. CL: milk control; CL_CS: milk control with 2% CS; A20_CS: sample with 20 % melted cheese base (MCB); A40_CS: sample with 40 % MCB; A60_CS: sample with 60% MCB.

Figure 3. Time-pH curve for yogurt samples made with waxy rice starch (WRS). ● CL, ▲ CL_WRS, ♦ A20_WRS, ■ A40_WRS, and × A60_WRS. Formulations of these samples are as described in Table 1. CL: milk control; CL_WRS: milk control with 2% WRS; A20_WRS: sample with 20 % melted cheese base (MCB); A40_WRS: sample with 40 % MCB; A60_WRS: sample with 60% MCB.

Figure 4. Elastic modulus (*G'*) for yogurt samples containing corn starch (CS) (4A) or waxy rice starch (WRS) (4B) (n = 4). ♦ CL, ■ CL_CS or CL_WRS, ● A20_CS or A20_WRS, × A40_CS or A40_WRS, and ▲ A60_CS or A60_WRS. Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

Figure 5. Viscous modulus (*G''*) for yogurt samples containing corn starch (CS) (5A) or waxy rice starch (WRS) (5B) (*n* = 4). ◆ CL, ■ CL_CS or CL_WRS, ● A20_CS or A20_WRS, × A40_CS or A40_WRS, and ▲ A60_CS or A60_WRS. Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

Figure 6. Dynamic viscosity for yogurt samples containing corn starch (CS) (6A) or waxy rice starch (WRS) (6B) (*n* = 4). ◆ CL, ■ CL_CS or CL_WRS, ● A20_CS or A20_WRS, × A40_CS or A40_WRS, and ▲ A60_CS or A60_WRS. Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

Table List

***Table 1* –** Mixes for the bases of control and yogucheese samples. MCB is a melted cheese base in milk, with 3.6 % corn starch (CS) or waxy rice starch (WRS), and 24.5 % (w/w) ripened cheese. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

<table>
<tr><th></th><th>Milk (g)</th><th>Milk + 3.6% starch (g)</th><th>MCB (g)</th></tr>
<tr><td>CL</td><td>100</td><td>0</td><td>0</td></tr>
<tr><td>CL_CS</td><td rowspan="2">40</td><td rowspan="2">60</td><td rowspan="2">0</td></tr>
<tr><td>CL_WRS</td></tr>
<tr><td>A20_CS</td><td rowspan="2">40</td><td rowspan="2">40</td><td rowspan="2">20</td></tr>
<tr><td>A20_WRS</td></tr>
<tr><td>A40_CS</td><td rowspan="2">40</td><td rowspan="2">20</td><td rowspan="2">40</td></tr>
<tr><td>A40_WRS</td></tr>
<tr><td>A60_CS</td><td rowspan="2">40</td><td rowspan="2">0</td><td rowspan="2">60</td></tr>
<tr><td>A60_WRS</td></tr>
</table>

Table 2 – Macronutrient composition of yogucheese samples. All values are given as % (w/w). Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

	Proteins	**Lipids**	**Total carbohydrates**
CL	3.40	1.60	4.90
CL_CS **CL_WRS**	3.28	1.54	4.72
A20_CS **A20_WRS**	4.61	2.91	4.77
A40_CS **A40_WRS**	5.81	4.22	4.64
A60_CS **A60_WRS**	7.02	5.53	4.51

Table 3 – Syneresis and titratable acidity (TA) values of the yogurt samples (*n* = 4). Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

	Syneresis (%)	**TA (% Lactic Acid)**
CL	22.9 ± 2.4 [a]	0.82 ± 0.03 [a,b]
CL_CS	37.8 ± 4.8 [b]	0.77 ± 0.01 [a]
CL_WRS	34.8 ± 4.4 [b]	0.75 ± 0.01 [a]
A20_CS	31.6 ± 3.4 [b]	0.87 ± 0.05 [b]
A20_WRS	24.5 ± 3.5 [a]	0.96 ± 0.02 [d]
A40_CS	24.6 ± 2.0 [a]	1.02 ± 0.01 [c,d]
A40_WRS	11.8 ± 1.1 [c]	1.14 ± 0.05 [e]
A60_CS	13.4 ± 0.4 [c]	1.05 ± 0.05 [c]
A60_WRS	1.9 ± 0.8 [d]	1.25 ± 0.06 [f]

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

***Table 4* –** Textural parameters of the yogurt samples (*n* = 3). Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.

Sample	Hardness (g)	Adhesiveness (g)	Springiness (g.s)	Cohesiveness
CL	218.44 ± 19.31 ^{a,c}	-150.06 ± 11.93 ^{a}	0.973 ± 0.002 ^{a}	0.410 ± 0.002 ^{a}
CL_CS	93.93 ± 29.27 ^{b}	-54.19 ± 26.34 ^{a}	0.986 ± 0.012 ^{a}	0.520 ± 0.023 ^{a,c}
CL_WRS	80.84 ± 10.75 ^{b}	-63.59 ± 11.35 ^{a}	0.976 ± 0.026 ^{a}	0.547 ± 0.015 ^{a,c}
A20_CS	90.60 ± 28.87 ^{b}	-50.68 ± 15.46 ^{a}	0.958 ± 0.018 ^{a}	0.521 ± 0.032 ^{a,c}
A20_WRS	90.56 ± 16.30 ^{b}	-70.69 ± 20.70 ^{a}	0.959 ± 0.002 ^{a}	0.544 ± 0.020 ^{a,c}
A40_CS	91.57 ± 16.80 ^{b}	-63.80 ± 28.46 ^{a}	0.957 ± 0.012 ^{a}	0.605 ± 0.010 ^{b,c}
A40_WRS	118.28 ± 7.86 ^{a,b}	-99.18 ± 6.18 ^{a}	0.933 ± 0.004 ^{a}	0.618 ± 0.021 ^{b,c}
A60_CS	123.26 ± 44.96 ^{a,b}	-64.14 ± 36.11 ^{a}	0.760 ± 0.138 ^{a}	0.605 ± 0.122 ^{b,c}
A60_WRS	224.39 ± 3.97 ^{c}	-171.54 ± 48.86 ^{a}	0.936 ± 0.011 ^{a}	0.602 ± 0.018 ^{b,c}

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

647 ***Table 5*** **–** Syneresis values (weight %) for a group of selected yogurt samples, 24 hours and 14 days
648 after fermentation (*n* = 4). Formulations of these samples are as described in Table 1. CL: milk
649 control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A40_CS and A40_WRS:
650 samples with 40 % MCB.

651

Sample	24 hours	14 days
CL	22.89 ± 2.36 [a]	18.11 ± 3.16[a]
CL_CS	37.78 ± 4.82 [b]	34.77 ± 2.88[b]
CL_WRS	34.81 ± 4.39 [b]	35.19 ± 4.82[b]
A40_CS	24.61 ± 2.03 [a]	25.32 ± 1.19[a]
A40_WRS	11.79 ± 1.13 [c]	13.77 ± 1.67[c]

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

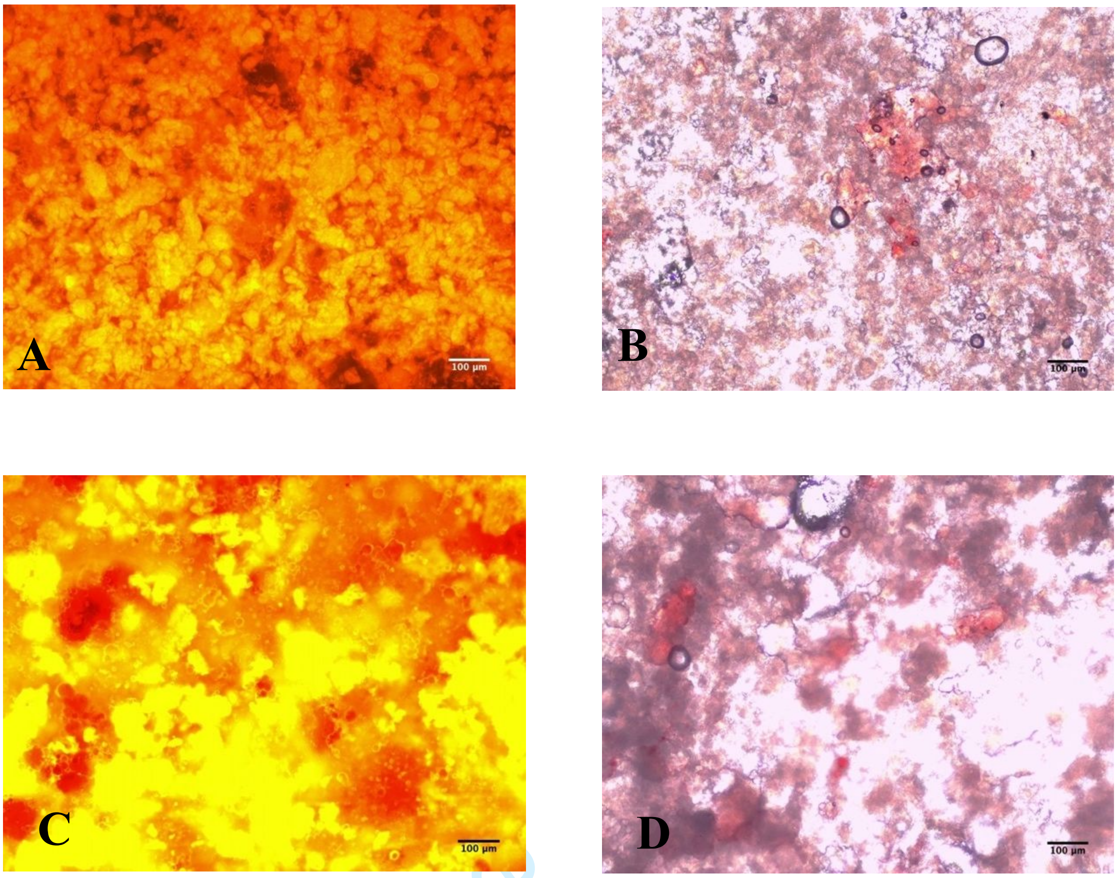
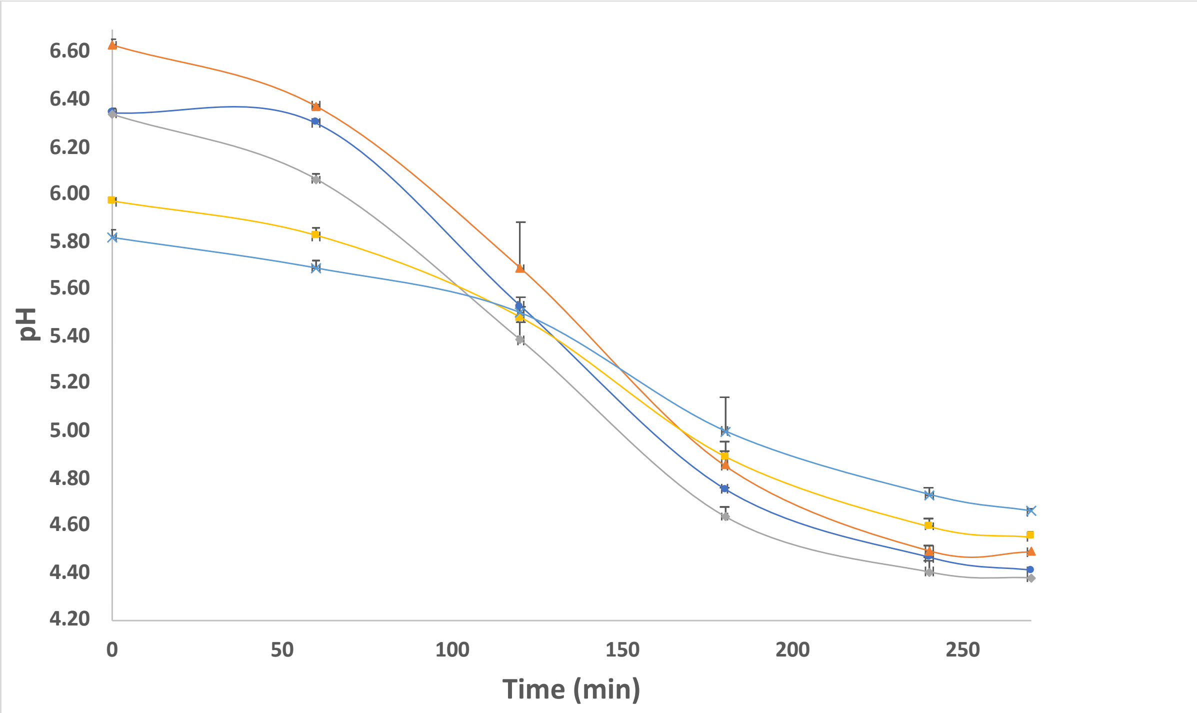


Figure 1. Optical microscopy images of melted cheese base (MCB) samples prepared with corn starch (CS) (1A and 1B) or waxy rice starch (WRS) (1C and 1D) and Emmental cheese, stained with Rhodamine B (1A and 1C) and Sudan III (1B and 1D).



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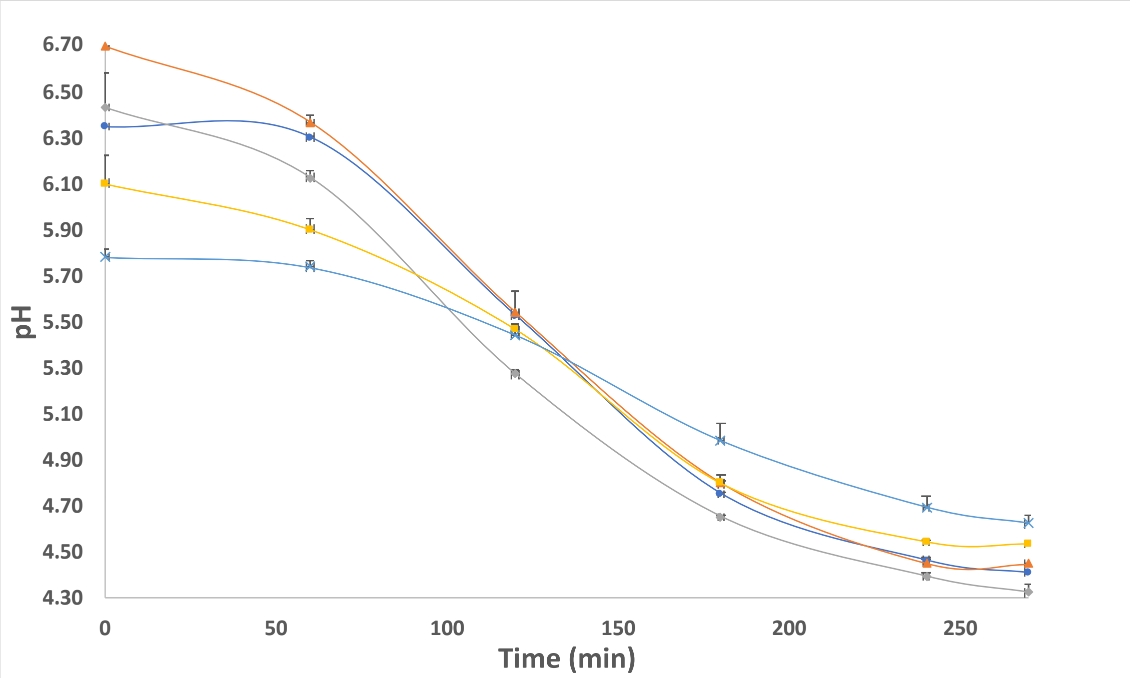


Figure 3. Time-pH curve for yogurt samples made with waxy rice starch (WRS). ● CL, ▲ CL_WRS, ♦ A20_WRS, ■ A40_WRS, and × A60_WRS. Formulations of these samples are as described in Table 1. CL: milk control; CL_WRS: milk control with 2% WRS; A20_WRS: sample with 20 % melted cheese base (MCB); A40_WRS: sample with 40 % MCB; A60_WRS: sample with 60% MCB.

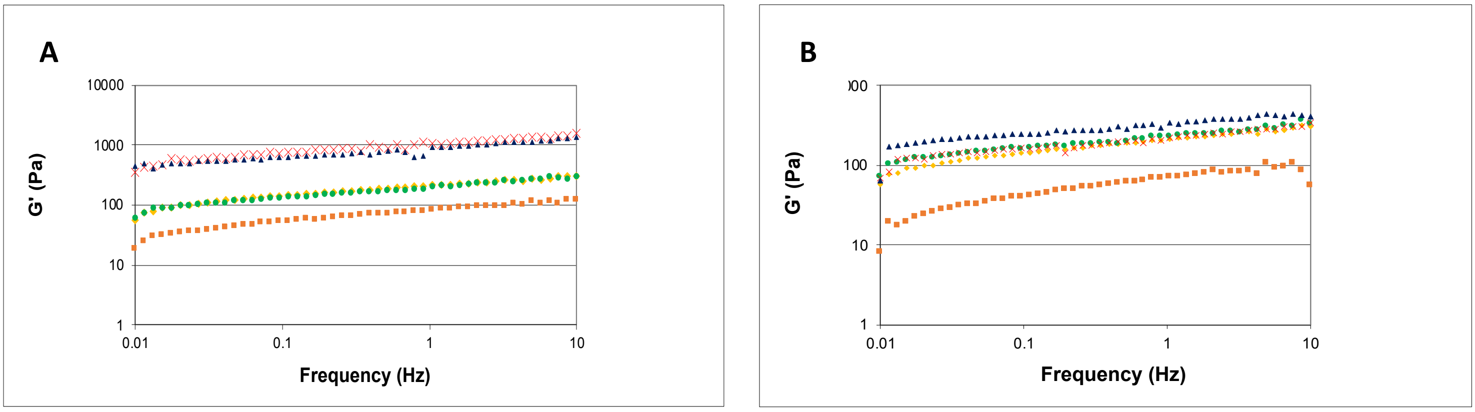
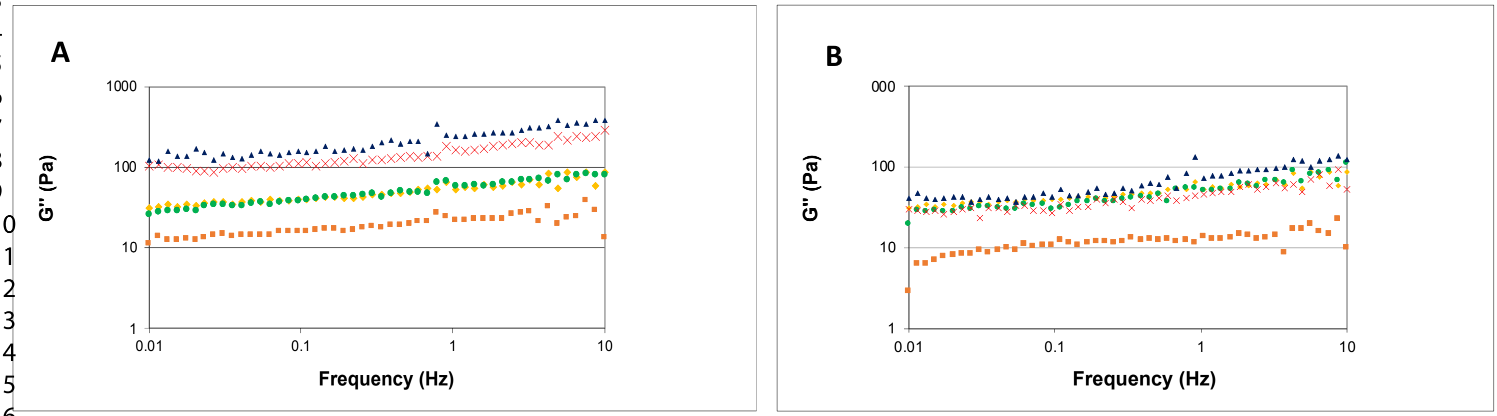


Figure 4. Elastic modulus (*G'*) for yogurt samples containing corn starch (CS) (4A) or waxy rice starch (WRS) (4B) (*n* = 4). ◆ CL, ■ CL_CS or CL_WRS, ● A20_CS or A20_WRS, × A40_CS or A40_WRS, and ▲ A60_CS or A60_WRS. Formulations of these samples are as described in Table 1. CL: milk control; CL_CS and CL_WRS: milk control with 2% CS or WRS; A20_CS and A20_WRS: samples with 20 % melted cheese base (MCB); A40_CS and A40_WRS: samples with 40 % MCB; A60_CS and A60_WRS: samples with 60% MCB.



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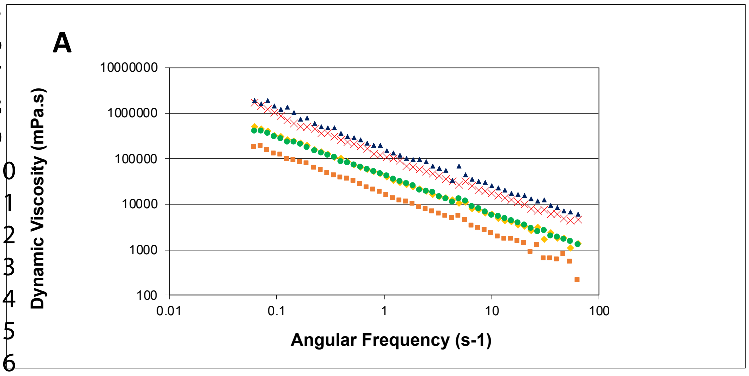

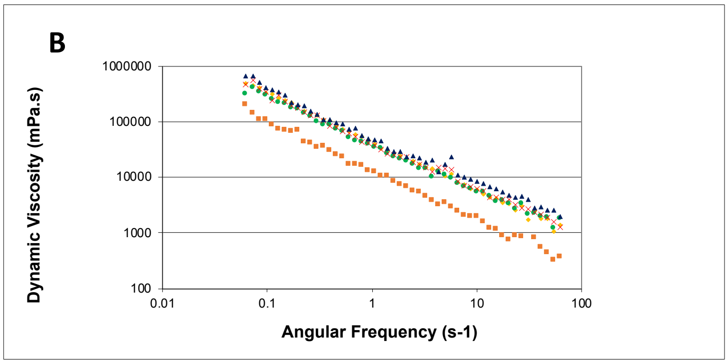

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