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Impact of Storage Conditions of Yogurt Dry Ingredients on the Physicochemical Properties of the Final Product

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Abstract: This study investigated the impact of storage conditions of the ingredients for yogurt production on the rheological and physicochemical characteristics of the final fermented product. The novelty is the application of a special mix of milk protein concentrate and sodium caseinate for yogurt production. Separately exposing the protein mix powder and bacteria culture to 20 °C caused considerable changes in the obtained yogurt stiffness and the incubation times required to produce the gel due to a decrease in bacterial count. Minimal changes in bacteria viability were observed after storage at 5 °C. Lower temperature and shorter storage times increased yogurt firmness, viscosity, and storage modulus, resulting in a smoother and more viscous product. A linear correlation was found between yogurt firmness and water activity. Powders stored at lower temperatures and for shorter times produced yogurt with stronger texture and better water binding. Additionally, yogurt obtained from dry ingredients stored under these conditions required shorter incubation times. Storing the starter culture at 5 °C for at least 8 weeks had no significant effects on the physicochemical properties or incubation time requited to produce the final yogurt. This work highlights the importance of storage conditions of yogurt dry ingredients in maintaining the quality of the final product.

Keywords: yogurt; reconstituted; recombined; rheology; texture; water activity; roughness

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1. Introduction

Manufacturing recombined and reconstituted milk and milk products is a technology that appeared at the end of the 20th century [1]. Recombined and reconstituted milk products provide a nutritious and high-quality source of dairy products in the areas where fresh raw milk is not readily available or is in a short supply [2]. Initially, this technology was applied to obtain fluid milk, but it was followed by the production of recombined evaporated milk and sweetened condensed milk. Nowadays, the recombination industry also includes yogurt, butter, and cheese [3].

The basic types of milk powders used in the recombination industry include skim-milk, whole milk, and buttermilk [4]. These products are the main milk powders in the marketplace [5]. However, the introduction of membrane techniques in the dairy industry

has allowed the production of other types of milk powders, containing diverse protein-to-lactose ratios and altered the whey protein to casein ratios, e.g., milk retentate, milk permeate, whey retentate, and whey permeate powders [6,7]. The use of these powders has enabled the production of recombined dairy products, such as concentrated yogurt, which have large protein and small lactose contents.

Numerous investigations were carried out to assess the properties and applications of these powders in the production of dairy products [8–16]. For instance, several researchers recommended the use of these powders to fortify the milk base during yogurt production [17–20]. Dairy powders have a very long shelf life, can be stored at ambient temperatures and can be easily transported [21]. However, since the quality of recombined dairy products is directly related to the composition and the physical, chemical, microbiological and sensory standards of the ingredients used, it is of great importance to conserve the physicochemical and microbiological properties of dairy powders during their storage until their final reconstitution [4].

Freeze-dried (direct-to-vat) thermophilic starter cultures are also required to produce yogurt powders. Freeze-dried cultures can contain higher levels of viability than the dried ones obtained by other drying techniques. They can also be stored in a conventional refrigerator and transported at room temperature [22,23]. These advantages are attributed to the stability of lyophilized cells at room temperature, although their optimal preservation condition is at −20 °C [22]. The major disadvantages of using freeze-dried concentrate cultures are that, compared to the frozen concentrate cultures, they require a longer lag phase during incubation. Some commercial strains do not survive the process well and, compared to other drying techniques, freeze-drying requires higher costs and larger energy consumption [24–27].

The viability of lyophilized starter cultures is influenced by several factors, including the growth medium, freezing rate, drying temperature and composition of the freezing medium. Additionally, the subsequent storage conditions, such as temperature, atmosphere, exposure to light and relative humidity, also have a significant impact on the viability of these cultures [28]. Since the starter culture activities significantly affect the rheological and physicochemical characteristics of acid milk gels [29–32], it is essential to preserve the viability of these cultures during the dry storage of the powder mix, as this will enable the maintenance of the expected rheological and physicochemical characteristics of the final recombined product throughout the storage time.

Understanding how the yogurt powder behaves during storage is important because its shelf life is based on whether the recombined product obtained from the dried mix exhibits any of the physical, chemical or sensory characteristics that are unacceptable for consumption [33]. Therefore, the objective of this study was to examine the impact of yogurt powder formulation storage on the rheological and physicochemical characteristics of the final recombined product. The aim was to create a commercial product with a possibility to store it at standard kitchen conditions (room temperature or in a refrigerator at 5 °C). The novelty is the application of a special mix of milk protein concentrate and sodium caseinate for yogurt production. Preliminary studies have shown that the substitution of dry milk with powdered milk protein mixtures results in yogurt with better rheological properties.

2. Materials and Methods

2.1. Milk Powders

The milk protein concentrate (MPC-85) was supplied by Vitalus Nutrition Inc., Vancouver, BC, Canada. Sodium caseinate (NaCN) was purchased from the American Casein Company, Burlington, NJ, USA. All powders were stored at 5 °C until their use in the experiments. Table 1 presents the composition of these powders as provided by the manufacturers.

Table 1. Composition of milk powders.

Component	MPC-85	NaCN
Protein (%)	85.0	88.0
Lactose (%)	7.0	1.0
Moisture (%)	5.5	6.0
Fat (%)	4.0	1.8
Ash (%)	8.0	4.2

Based on the preliminary studies, the following powder formulation was used to obtain the recombined milk base for yogurt production (Table 2). The selection was based on firmness and taste of the final product. These data will be a subject for a separate publication.

Table 2. Physicochemical composition of the inoculated recombined protein ingredients base obtained by the proposed formulation.

Component	Amount Present in Milk Base
Total solids (%)	13.7
Total protein (%) MPC-85: 81%; NaCN: 19%	10.3
Ratio of casein to whey protein	6:1
Lactose (%)	2.3
Fat (%)	0.5
Ash (%)	1.1
Starter culture (DCU/100 L)	50

2.2. Microbial Cultures

The commercial, concentrated, freeze-dried starter culture (SC) (i.e., direct-to-vat inoculation) YO-MIX™ 215 LYO (Danlac Canada Inc., Calgary, AB, Canada) was used to ferment the recombined milk. The starter was a thermophilic multiple-species culture composed of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus acidophilus* and *Bifidobacterium lactis*. For the production of stirred yogurt, the manufacturer recommends using this culture at a rate of 10–50 direct culture units (DCU) 100 L^{-1} and at an incubation temperature of 42 °C. According to the producer's recommendations, the starter culture was stored at −25 °C. The starter culture was defrosted at room temperature before use.

2.3. Yogurt Manufacturing

Experimental samples were produced in batch mode using the direct recombination technology. Milli-Q water (de-ionized and distilled water) was employed to recombine milk powder. The starter culture was used at a rate of 50 DCU 100 L^{-1}. The mass of 900 g of recombined and inoculated milk was produced per batch of production, but only 500 g of this milk was incubated at 42 ± 1 °C until the desired acidity was reached. This was done to reduce experimental errors caused by mass losses during the recombination process. Figure 1 illustrates the manufacturing procedure in detail.

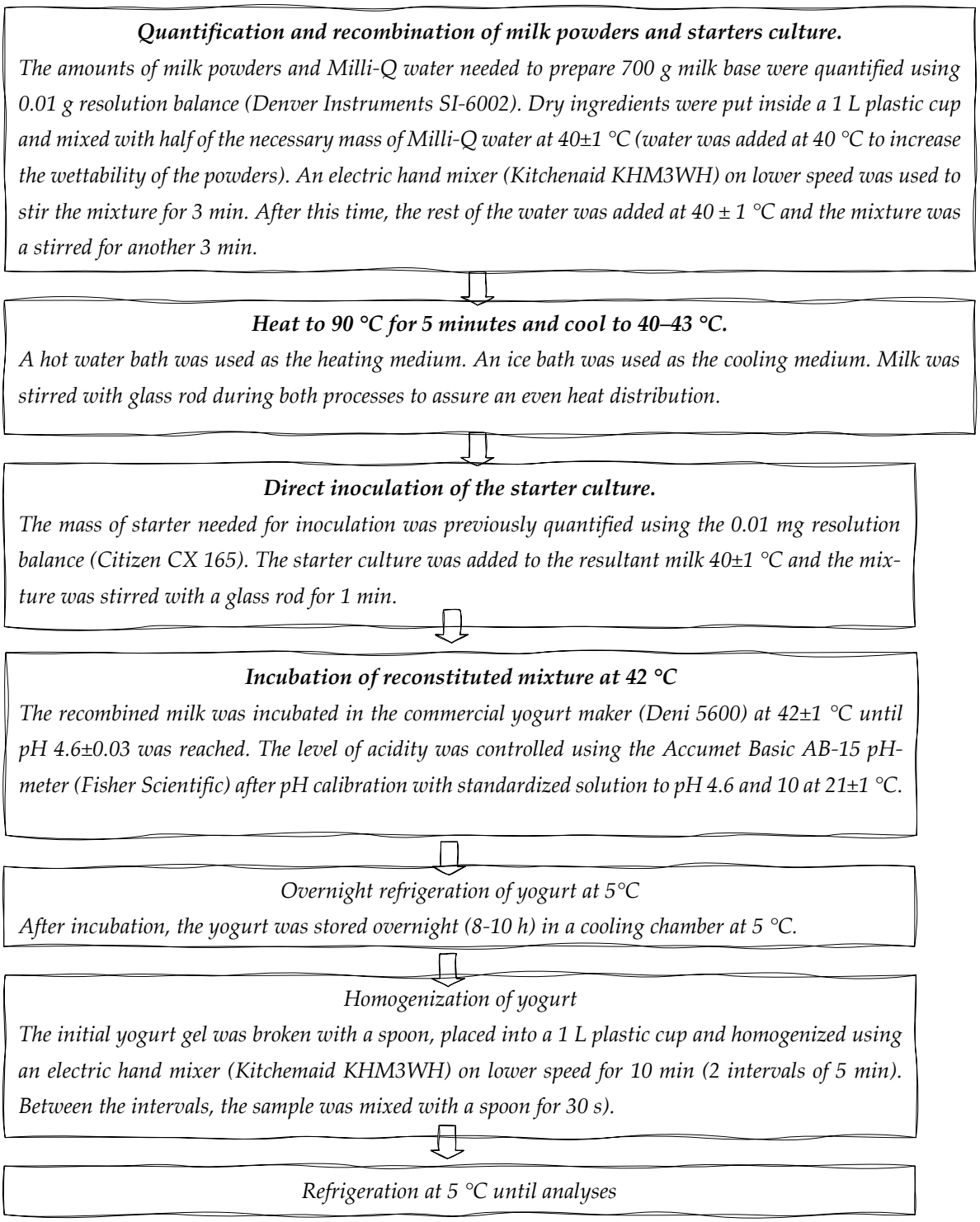

Figure 1. Method of yogurt production.

2.4. Experimental Design

Table 3 shows the different combinations of storage temperature and time for the yogurt dry ingredient formulation used in the study.

Table 3. Combinations of storage temperature and storage time levels considered in the experimental design.

Sample No.	Storage Temperature (°C)	Storage Time (Weeks)
1	5	2
2	20	2
3	5	8
4	20	8

To study how the storage of the yogurt dry ingredients influences the rheological and physicochemical properties of the final yogurt, the amount of milk powders required to produce 700 g of the recombined product was placed into a 1 L plastic cup. The amounts of starter culture required to produce 700 g of yogurt were packed into small polyethylene bags and each bag was put inside a plastic cup containing the milk powders. Plastic cups were covered with plastic lids and stored at 5 °C (inside the cooling chamber) and 20 °C (inside the electric and thermostatically controlled incubator) for 2 or 8 weeks.

2.5. Lactic Bacteria Count

For the lactic bacteria count, MRS agar (*Lactobacillus delbrueckii* subsp. *Lactis),* Rogosa agar *(Lactobacillus acidophilus*), M-17 agar (*Streptococcus thermophilus*) and bifidus blood agar (*Bifidobacterium lactis*) were used (Pol-Aura, Zawroty, Poland) [34,35]. The dry yogurt ingredient formulation (1 g) was mixed with 9.0 mL of peptone water, vortexed for 20 s. Serially diluted samples were made using peptone water. A sample (1 mL) was put on Petri dish and appropriate liquid nutrient agar was poured and mixed with the sample. Dishes were incubated at 35 °C for 48 h. Bacteria colonies were counted and log CFU per gram was calculated.

2.6. Incubation Time Measurements

After storage, the powder formula was recombined and fermented. The incubation time of each sample was registered using a chronometer. The stopwatch was turned on when the samples were put inside the incubator and stopped when the desired level of acidity (pH = 4.60 $\pm$ 0.03) was reached. The resultant yogurts were stored at 5 °C for 8 days before conducting the rheological and physicochemical determinations. Yogurt samples were made in triplicate, producing a total of 12 batches. All measurements were made in triplicate to ensure the accuracy and reproducibility of the results.

2.7. Dynamic Rheological Measurements

Small-amplitude oscillatory rheology (SAOR) tests were carried out using a Paar Physica UDS200 MCR rheometer. The rheometer was set up with a parallel-plate geometry (10 mm plate radius and 1 mm gap setting). All samples were gently stirred with a spoon for 30 s prior to the measurement to mix the potential free whey with the resulting gel. Each sample was loaded into the rheometer and allowed to relax and equilibrate at the measurement temperature (25 $\pm$ 0.1 °C) for 2 min prior to testing. The temperature of the samples inside the rheometer was maintained using a circulating cooling system. All measurements were conducted at 0.01 strain in the linear viscoelastic range previously determined by the strain sweep. The rheological aspects of all samples were evaluated by carrying out stress amplitude sweep tests. The sweeping amplitude from 1.5×10^{-2} to 1.5×10^{-1} mNm at 0.25 Hz was used and 25 measuring points were performed through the sweeping range. The storage (G') and loss (G'') moduli were recorded. Two replications were made for each sample. Finally, the loss tangent (tan δ), i.e., the ratio of G'' to G', was also calculated. Graphs were created using OriginPro70 software (OriginLab, Northampton, MA, USA).

2.8. Ultrasonic Viscosity Measurements

The probe was immersed into the yogurt and the values of viscosity x density (mPas × g/cm^3) were measured. The measurements were made using the ultrasound viscometer Unipan type 505 (UNIPAN, Warsaw, Poland). Six measurements were performed to obtain a single average result.

2.9. Penetration Test of the Yogurt

The firmness of the yogurt was measured with a penetration test using a TAXT2i texturometer (Stable Micro Systems, Haselemere, UK). The maximal force was noted at 15 mm penetration using a 35 mm-diameter aluminium plunger at a speed of 1 mm/s. Measurements were made in six repetitions, and the results are presented as the arithmetic mean.

2.10. Water Activity

Water activity (aw) was measured using an AWMD-10 water activity meter (NAGY, Gäufelden, Germany) with an accuracy of ± 0.001 aw unit. Before the measurement, the device was calibrated according to the special humidity standard (95% HR). Measurements were made at 20 °C.

2.11. Dried Yogurt Surface Topography

Yogurts were dried at 45 °C for 24 h using an air circulation laboratory dryer (SUP-200G Wamed, Warsaw, Poland). The surface was investigated using the optical profilometer GT Contour Surface Metrology (Veeco, Tucson, AZ, USA). The surface topography was characterized in the range from the sub-nanometer to 10 mm. The surface roughness was calculated using the Vision64 (Veeco, Tucon, AZ, USA) computer program.

2.12. Statistical Analysis

Multivariate Pearson correlation was performed using Microsoft Excel 2019. Tukey's test was applied to determine HSD values at $p < 0.05$ (Statistica PL 13.3).

3. Results and Discussion

3.1. Bacteria Count

Table 4 shows the bacteria count in log CFU per gram of yogurt dry ingredient mixture influenced by different storage times and temperatures. Storage at 5 °C statistically did not caused any decrease in bacteria count for all investigated species (±0.06–00.7 log). Storage of yogurt dry ingredients at 20 °C caused a 0.64–0.85 log decrease in LA bacteria count. The most significant decrease in bacteria count was noted after 8 weeks of storage at 20 °C, decreasing from 8.2 to 7.56 log for *Streptococcus thermophiles*, from 8.54 to 7.85 log for *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus acidophilus*, and from 8.11 to 7.26 log for *Bifidobacterium lactis* (Table 4).

Mani-López et al. [36] found that counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* decreased from 1.8 to 3.5 log during storage for 35 days at 5 °C, although the obtained yogurts maintained counts $\geq 10^7$ cfu/mL for 3 weeks. Damin et al. [37] also found that the average initial microbial count for each of the activated cultures was ~10^7 CFU/mL. Bacteria counts 24 h after fermentation were log 8.52, 7.96 and 9.15 for LB, LA, and BL, respectively. LA counts decreased 2.34 log after 28 days of storage and maintained minimum requirements for beneficial properties for 3 weeks of storage. The *S. thermophilus* counts were superior to those of the other strains, and they remained stable throughout the storage period. Similarly, in our research, the lowest lactic bacteria count in yogurt obtained from powdered ingredients stored at 20 °C for 8 weeks was still 6.9 log CFU/mL after 3 weeks of yogurt storage (data not shown). The initial count of lactic bacteria in dry yogurt ingredient mixture has a significant influence on the bacteria count in the final product.

Table 4. Bacteria counts (log CFU/g) in yogurt dry ingredient mixture after storage at 5 °C and 20 °C for different periods of time.

Storage temperature (°C)	5			20		
Storage time (weeks)	0	2	8	0	2	8
***Streptococcus thermophilus* (logCFU/g)**	8.2 [a] (0.06)	8.19 [a] (0.05)	8.16 [a] (0.02)	8.2 [a] (0.06)	7.98 [a] (0.03)	7.56 [b] (0.08)
***Lactobacillus delbrueckii* subsp. *lactis* *Lactobacillus acidophilus* (logCFU/g)**	8.54 [a] (0.05)	8.49 [a] (0.04)	8.47 [a] (0.03)	8.54 [a] (0.05)	8.07 [b] (0.02)	7.85 [c] (0.03)
***Bifidobacterium lactis* (logCFU/g)**	8.11 [a] (0.05)	8.09 [a] (0.04)	8.05 [a] (0.03)	8.11 [a] (0.05)	7.85 [b] (0.03)	7.26 [c] (0.07)

Standard deviation is shown in brackets; means within a row followed by various letters (a–c) are significantly different ($p < 0.05$).

3.2. Rheological Properties

Figures 2 and 3 illustrate the effects of the storage of yogurt dry ingredients on the rheological properties of the obtained yogurt. Small-amplitude oscillatory rheology with the measurement of storage modulus (G′), loss modulus (G″) and loss tangent (LT) can be used to characterize the rheological properties of yogurts without damaging the gel matrix. The storage modulus and loss modulus show, accordingly, an elastic and viscous element in viscoelastic properties of yogurt. The loss tangent is a quotient loss modulus and storage modulus. Low values of loss tangent show that the material is much more elastic than viscous [38]. The rheological properties of the obtained yogurts were significantly affected by the storage of yogurt dry ingredients; the differences were statistically significant at the level of $p < 0.05$ (Table 4). As storage times and temperatures increased, the G′ of the yogurt samples decreased and the tan δ increased. Yogurt samples were less elastic with a higher input of viscous properties [29].

As follows from Figures 2 and 3, to obtain yogurt with better rheological properties, yogurt ingredients must be stored at low temperatures. Higher temperature influenced the viability of starter cultures (Table 4), resulting in lower lactic acid production rates during the incubation of the recombined milk bases. Consequently, longer incubation times were required to acidify the medium up to pH 4.6. The integrity of casein micelles in milk is governed by a localized equilibrium between the hydrophobic interactions and the electrostatic repulsions. During fermentation, as the pH of milk decreases, the colloidal calcium phosphate (CCP) within the casein micelles is solubilized, particularly at pH levels below 6.0. This process is completed at approximately pH 5.0, causing partial rearrangement of the internal structure of the casein micelle. When CCP is dissolved within the casein particles, there is an increase in the electrostatic repulsion between the exposed phosphoserine residues. This weakens the casein–casein interactions and probably contributes to a slight decrease in the G′ values. As the milk pH approaches its isoelectric point (i.e., pH < 5.0), the electrostatic repulsion decreases, which promotes the casein–casein attractions driven by the increased hydrophobic interactions. These factors promote bond formation/strength and thus increase gel stiffness, contributing to an increase in the G′ values [29].

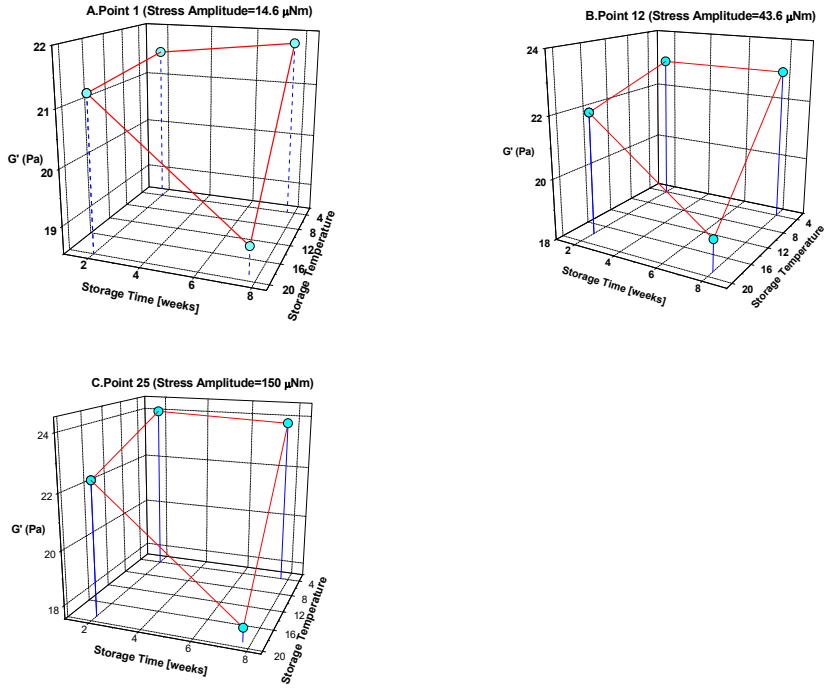

Figure 2. Effects of storage temperature and time (in weeks), applied to yogurt dry ingredients, on the storage modulus (G′) of yogurt made from stored powders. Results show storage modulus at three selected points of the applied stress amplitude range (**A**–**C**).

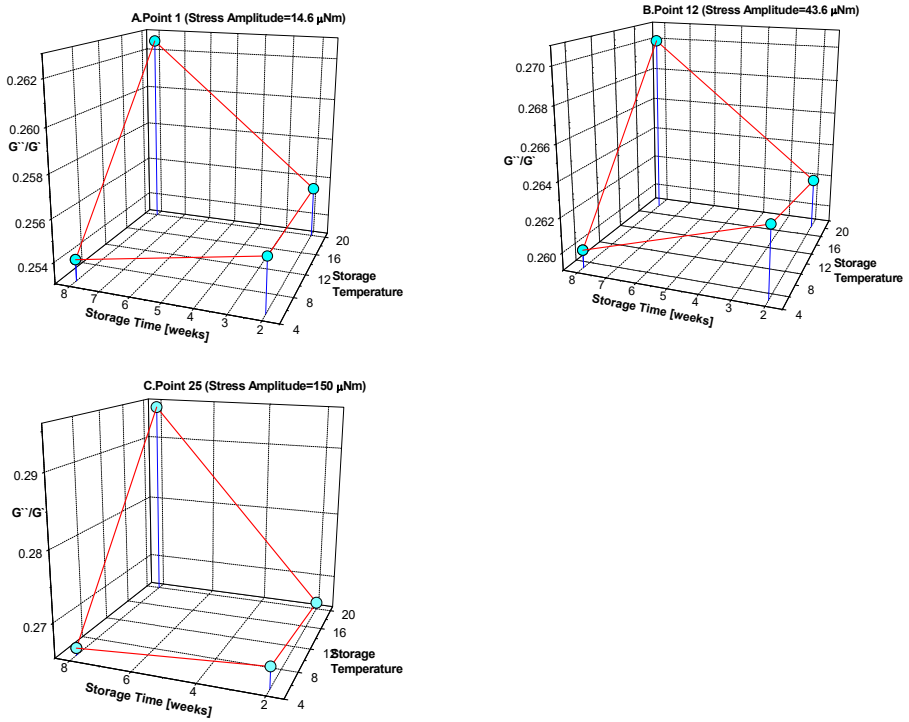

Figure 3. Effects of storage temperature and time (in weeks), applied to yogurt dry ingredients, on the loss modulus (G″) of yogurt made from stored powders. Results show storage modulus at three selected points of the applied stress amplitude range (**A**–**C**).

The CCP solubilization in milk during acidification is a slow process and may require a slightly lower pH for complete dissolution of CCP under fast acidification conditions (i.e., high inoculation rates can be less efficient in solubilizing CCP, as there would be less

time at any particular pH value during milk acidification). When CCP dissolves at a lower pH, caseins can be less susceptible to excessive rearrangements. This is due to the fact that at lower pH values, there will be lower electrostatic repulsion and higher hydrophobic interactions between casein particles, which promotes stiffer gel networks with higher G′ values [29]. For this reason, higher levels of viability of the starter culture resulted in acid milk gels with higher G′ and lower tan δ values.

Table 5 shows all collected data of the physical properties of yogurt obtained from dry ingredients stored at different conditions.

Table 5. Influence of yogurt dry ingredient storage time and temperature on physical properties of produced yogurt.

Sample No.	Firmness (N)	Surf. Roughness R_q (nm)	Ultra. Viscosity (mPas·gcm^{-3})	Storage Mod. at 43.6 µNm (Pa)	Tan Delta at 43.6 µNm	Water Activity
1	0.209 *^{a} (0.02)	412 ^{a} (14)	369 ^{a} (18)	22.8 ^{a} (0.04)	0.261 ^{a} (0.004)	0.944 ^{d} (0.003)
2	0.199 ^{b} (0.03)	452 ^{b} (16)	326 ^{b} (9)	21.9 ^{b} (0.03)	0.262 ^{a} (0.008)	0.951 ^{c} (0.002)
3	0.184 ^{c} (0.03)	589 ^{c} (12)	294 ^{c} (11)	22.7 ^{a} (0.03)	0.255 ^{a} (0.004)	0.956 ^{b} (0.002)
4	0.170 ^{d} (0.06)	764 ^{d} (31)	234 ^{b} (18)	18.4 ^{c} (0.05)	0.270 ^{b} (0.006)	0.967 ^{a} (0.004)

* staS, standard deviation is shown in brackets; means within a column followed by various letter (a–d) are significantly different ($p < 0.05$).

Based on the results presented in Table 5, multivariate Pearson correlation was made. The coefficients of correlation are shown in Table 6.

The dynamic rheological properties, ultrasound viscosity as well as yogurt firmness increased for the yogurt dry ingredients stored at a lower temperature for a shorter period of time (Table 5). The highest values of yogurt firmness (0.209 N), ultrasound viscosity (369 mPas·gcm^{-3}), and storage modulus (22.8 Pa), and the lowest value of tan delta (0.261) were observed in yogurt obtained from dry ingredients stored for 2 weeks at 5 °C. Interestingly, similar yogurt firmness values were observed by Gharibzahedi et al. [39], applying the penetration test method. There was a strong correlation between the firmness of yogurt and ultrasonic viscosity (R^2 = 0.98) (Table 6). Both methods involve puncture of the sample. A lower correlation coefficient value was noted for correlation between firmness and storage modulus (R^2 = 0.73), which is measured at low strain, without fracture of the sample. A strong negative correlation was found between the dry yogurt topography and wet yogurt ultrasound viscosity and storage modulus (R^2 = 0.98 and 0.84, accordingly) (Table 6). Time and temperature storage of yogurt dry ingredients influenced the microstructure of the final product and its viscosity. The formulation stored at a lower temperature and for a shorter time resulted in a more viscous and more elastic product with smaller surface roughness. More rapid drops in pH can lock the protein into a more dispersed structure with a smoother surface and greater density of possibly stronger strands [40]. The smoothest surface of dry yogurt (412 nm) was observed in yogurts derived from dry ingredients stored for 2 weeks at 5 °C. Furthermore, this yogurt also had the best rheological properties (Table 5). A linear correlation was also found between all measured rheological properties and water activity of yogurts (R^2 = 0.86) (Table 6). Yogurts with a stronger and more elastic texture were characterized by smaller water activity. Brodziak et al. [41] investigated changes in the physicochemical properties of yogurt with the added whey protein concentrate (WPC). In most investigated cases, water activity decreased as the WPC content and yogurt firmness increased, regardless of the strain type. Agoda-Tandjawa et al. [42] made water activity measurements as a tool for predicting structural and mechanical properties during dewatering and ageing of sludge. Strengthening the

structure and improving the rheological properties resulted in a decrease in water activity. The stronger texture bonds more water molecules, resulting in a smaller vapour pressure in the space above the yogurt. The yogurt dry ingredients stored at a lower temperature for a shorter period of time produced yogurt with a stronger texture and better binding of water.

Table 6. Multivariate Pearson coefficients for correlation between all measured properties of yogurt produced from dry ingredients stored at different times and temperatures.

	Firmness (N)	**Surf. Roughness R_q (nm)**	**Ultra. Viscosity (mPas·gcm^{-3})**	**Storage Mod. at 43.6 µNm (Pa)**	**Tan Delta at 43.6 µNm**	**Water Activity**
Firmness (N)	1					
Surf. Roughness R_q (nm)	−0.96897	1				
Ultra. viscosity (mPas · gcm^{-3})	0.981541	−0.97781	1			
Storage mod. at 43.6 µNm (Pa)	0.734451	−0.84357	0.84474	1		
Tan delta at 43.6 µNm	−0.36523	0.536141	−0.52787	−0.9	1	
Water activity	−0.97483	0.979971	−0.99928	−0.86402	0.55872	1

3.3. Incubation Time

Changes in the storage time and temperature of yogurt dry ingredients also influenced the yogurt incubation time to the value of pH 4.6 (Table 7). Storage time statistically increased incubation time, but these values were in a standard time range: 7.2–8.6 h. Only for yogurt dry ingredients stored at 20 °C for 8 weeks was a sudden increase in incubation time observed—15.6 h.

Yogurts obtained from the powders stored at lower temperatures needed shorter incubation times. For all bacteria, there was a linear correlation between bacteria counts and incubation time (besides a sample with 15.6 h) (charts are not shown, $R^2 > 0.9$). Storing the starter culture at 5 °C between 2 and 8 weeks did not have significant effects ($p > 0.05$) on the incubation time required to produce the yogurt (8.1 and 8.4 h, accordingly) (Table 7). The storage time of the starter culture at 5 °C also did not statistically affect its viability (8.54–8.11 log) (Table 4). These results are consistent with the findings of Kumar and Gandhi [43], who stated that the freeze-dried concentrated starter cultures are stable at 5 °C without a loss of activity. Furthermore, Saxelin et al. [44] studied the survival of eight different freeze-dried species of lactic acid bacteria during the storage and found that most cultures could be stored for one year at 5 °C without any significant loss in viability. However, it is important to point out that the storage stability of freeze-dried starter cultures is intimately related to the types of cryoprotectants added to the initial culture before freeze-drying. The author concluded that the sucrose-based cryoprotectants induced excellent stability under challenging conditions, including storage at high temperatures and exposure to acid and bile [45].

Table 7. Effects of yogurt dry ingredient storage temperature and time on the incubation times of yogurt to pH 4.6.

Storage temperature (°C)	5			20		
Storage time (weeks)	0	2	8	0	2	8
Incubation Time (hours)	7.2 ^{c} (0.2)	8.1 ^{b} (0.4)	8.4 ^{b} (0.3)	7.2 ^{c} (0.2)	8.6 ^{b} (0.4)	15.6 ^{a} (3.3)

Standard deviation is shown in brackets; means followed by various letter (a–c) are significantly different ($p < 0.05$).

The results show (Table 4) that the prolonged storage at high temperatures caused a detrimental effect on the activity of the freeze-dried starter cultures present in the yogurt dry formula. The largest decrease in bacteria count was observed for the samples stored for 8 weeks at 20 °C, with the highest drop noted for *Bifidobacterium lactis* (0.85 log). This is consistent with the findings reported by other researchers. Achour et al. [46] studied the survival rates of freeze-dried *Lactococcus* starter cultures and demonstrated that temperature had a destructive effect on the survival rates. They reported that the average half-life of a strain maintained at 25 °C was approximately 7 days, compared to about 43 days when stored at 4 °C. Additionally, Bruno and Shah [47] carried out a study dealing with the viability of two freeze-dried strains of *Bifidobacterium* during prolonged storage at various temperatures and concluded that the strains stored at 20 °C exhibited the greatest decline in the viability of bacteria, whereas those stored at −18 °C exhibited the least. For long-term storage, the inactivation of freeze-dried starter cultures depends significantly on the storage conditions. The inactivation is correspondingly related to the storage temperature and moisture content. This is mainly due to the state of dried starter cultures. In general, components of biological materials in the dehydrated states form amorphous structures, with the typical characteristics known as the rubbery (amorphous liquid) and glassy (amorphous solid) states. The most important parameter describing the glassy state is the glass transition temperature (T_g), below which materials exhibit extremely great viscosity, giving them solid-like properties [48]. During storage, the T_g of the dried sample is an important factor affecting the viability of cultures. Thus, the moisture content becomes a key variable. When the dried starter cultures are filled in the moisture-permissible packages, the relative humidity of the storage environments will have an additional effect besides the end moisture after drying [48].

All starter cultures present in the experimental samples were conserved in the non-moisture-permissible packages (small polyethylene sealed bags) at the same relative humidity but at different temperatures. Therefore, it is believed that the dried cultures stored at different temperatures presented different amorphous states, which resulted in different storage stabilities. For instance, the cultures stored at 20 °C for 8 weeks showed the least viability. On the other hand, the O_2 and light are other critical factors influencing the stability of cultures. The main cause of the deterioration of freeze-dried starter cultures is related to the membrane lipid oxidation [28]. Research on the lipid composition of the cell membrane using gas chromatography showed that the unsaturated/saturated fatty acid index of the cell membrane changes during storage [49]. These changes largely affect the passive permeability of the membrane and contribute to cellular death [50]. In addition, rehydration of the cultures is another critical step that can influence the level of viability of freeze-dried microorganisms. The medium itself as well as its molarity and rehydration conditions can drastically affect the rate of recovery [38]. However, as all the starter cultures present in the experimental samples were packed and rehydrated in the same way, the influence of O_2, light, and rehydration cannot be used to explain the rheological differences obtained for the different recombined samples.

Generally, as the level of viability of starter cultures decreased (Table 4), the incubation times required for the production of acid milk gels increased (Table 7) and the final gel obtained had a higher liquid-like behaviour (lower G′ and higher tan δ) (Figures 2 and 3,

Table 5). These results are consistent with the findings by Jumah et al. [30], Sodini et al. [31], Lee and Lucey [29] and Wu et al. [32]. The overall result of the lower viability level of starter cultures was the formation of weaker gels that were more prone to rearrangements. Large-scale rearrangements, related to dynamics and relaxation of the protein–protein bonds, increased the instability of the gel network and reduced its ability to entrap all of the serum phase [46].

Consequently, to reduce the incubation times needed to produce yogurt, the freeze-dried starter culture must be packed in a non-moisture-, non-oxygen- and non-light-permissible package and stored at low temperatures [43,48]. Additionally, the storage stability of the freeze-dried cultures could also be improved by considering the use of freeze-dried cultures containing different types of protective agents, including polyols, polysaccharides, disaccharides, amino acids, proteins, vitamins or salts [50].

4. Conclusions

Exposing the yogurt dry ingredients to ambient temperature (20 °C) for long periods of time caused considerable changes in the incubation times required to produce yogurt and its rheological properties. The storage modulus, ultrasound viscosity, and firmness of yogurt increased as the yogurt dry ingredients were stored at a lower temperature for a shorter period of time. A more dispersed structure with a smoother surface was formed. Strong correlations were found between the yogurt rheological properties and the water activity, and between the dry yogurt topography and wet yogurt ultrasound viscosity and storage modulus. The yogurt dry ingredients stored at a lower temperature for a shorter period of time produced more elastic yogurt with a stronger texture and better binding of water. Yogurts obtained from yogurt dry ingredients stored at lower temperatures and for a shorter period of time required shorter incubation times. Alterations of these two factors within the storage were associated with a loss of activity of the starter cultures present in the samples exposed to high temperatures. Two main recommendations were given to decrease the inactivation rates of starter cultures throughout storage: (i) conserve the dried product at low temperatures, and (ii) consider the use of different packages and cryoprotectants to shelter starter cultures from inactivation. Further research is needed in order to select the appropriate cryoprotectants and packages required to extend the storage stability of the employed starter culture blend. This additional work is indispensable for the attempts to offer yogurt dry ingredients that can be recombined and fermented using the standard parameters and can result in satisfactory rheological properties of the final product.

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