Interpretive Summary

Utilization of β-glucan from spent brewer's yeast as thickener in skimmed yogurt

Raikos

β-glucan from brewer's yeast was used for yogurt reformulation. The incorporation of β-glucan into the recipe reduced the fermentation times of the yogurt by 1h. The majority of the properties of the reformulated product remained unaffected. Consumer test revealed that the reformulated yogurt was perceived as different but was still acceptable according to the hedonic judgements. β-glucan can be a valuable ingredient for food reformulation with beneficial economic implications for the dairy industry. The effects of β-glucan from yeast on human health need to be further investigated.

Running head: β-GLUCAN FROM YEAST IN YOGURT

Utilization of β-glucan from spent brewer's yeast as thickener in skimmed yogurt: Physicochemical, textural and structural properties related to sensory perception

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ABSTRACT

Powdered β-glucan extracted from brewer's yeast (Yestimun®) was incorporated into skimmed-milk yogurt at varying concentrations (0.2-0.8% w/w) to investigate its potential application as thickener. The effect of β-glucan fortification on the nutritional profile, microstructure, physicochemical properties, and texture of freshly prepared yogurts was investigated. Sensory evaluation was also conducted and was correlated with instrumental analysis. The addition of Yestimun® significantly reduced the fermentation time of the yogurt mix from 4h to 3h. Scanning electron microscopy revealed that β-glucan particles form small spherical clusters within the yogurt matrix. The majority of the physicochemical properties (syneresis, viscosity, colour, titratable acidity) remained unaffected by the incorporation of Yestimun® in the recipe. Textural properties showed a gradual increment with increasing β-glucan concentration. Hardness, total work done, adhesive force and adhesiveness increased by 19.27%, 23.3%, 21.53% and 20.76% respectively, when using the highest amount of Yestimun® powder. Sensory analysis (n=40) indicated that fortifying yogurt with Yestimun® at 0.8% (w/w/) concentration may affect the overall acceptance ratings, which was attributed to adverse flavor and aftertaste effects. However, the overall liking score of the yogurt (5.0/9.0) shows potential for commercialization of the product.

Keywords: yogurt, beta-glucan, brewer's yeast, thickener

INTRODUCTION

Yogurt is a popular dairy product traditionally made by the lactic acid fermentation of liquid milk. Natural yogurt is considered a healthy food with beneficial effects on human health (Granato et al., 2010; Tripathi and Giri, 2014). Its nutritional value is owed to the nutrients present in milk used as starting material, with cow's milk being the predominant milk type used for yogurt manufacturing across the world (Chandan, 2006). In addition, yogurt contains lactic acid bacteria which are essential for fermentation and is therefore widely accepted as a probiotic product. The FAO/WHO Working Group defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). This definition is widely accepted and adopted by the International Scientific Association for Probiotics and Prebiotics (Hill et al., 2014).

The macronutrient composition of milk and in particular the fat content is a major determinant of the sensory qualities of yogurt. Dietary fats contribute to the flavor, appearance and texture of foods and as a result have a positive impact on consumer liking and acceptance (Folkenberg and Martens, 2003). On the other hand, consumers are becoming increasingly aware of the scientific evidence linking high fat diets and the development of chronic diseases such as obesity, diabetes, cancer and cardiovascular diseases (Astrupn et al., 2008; Willett, 2013). Thus, although creaminess and thickness are desired attributes for yogurt based on consumer preferences, there is an increasing demand for products that have little to no fat present in the formulation.

Recently, dietary fiber is gaining popularity as a food ingredient in various food formulations. The beneficial impact of fiber on human health is attributed to a certain extent to its prebiotic effects and as a result food manufacturers are keen to identify ways to include these non-digestible food ingredients in their products (Lam and Cheung, 2013). A few attempts to introduce dietary fiber in dairy products have been documented and include konjac glucomannan (Dai et al., 2016) date fiber (Hashim et al., 2009), chitosan (Seo et al., 2009), inulin (Balthazar et al., 2015), xylooligosaccharides (Ferrão et al., 2018) and galactooligosaccharides (Balthazar et al., 2015; Belsito et al., 2017). β-glucans are a type of dietary fiber isolated from a variety of natural sources such as oats, yeast, bacteria, algae, barley and mushrooms (Ahmad et al., 2012; Zhu et al., 2015). β-glucans are D-glucose monomers linked through β-glycosidic bonds and their structure, molecular weight and functionality largely depends on the source of origin as well as the method used for extraction and purification (Zhu et al., 2016). Spent brewer’s yeast (*Saccharomyces* *cerevisiae*) is a by-product of beer manufacture produced in huge amounts, which is known to be rich in β-glucans (Aimanianda et al., 2009). The European Food Safety Authority (EFSA) has approved the inclusion of yeast β-glucans as a new ingredient in food formulations and recommends a portion ranging between 50 and 200 mg per serving (EFSA, 2011).

Recent research has indicated the potential of β-glucans from spent brewer's yeast as a thickening, water-holding, or oil-binding agent and emulsion stabilizer for food applications (Thammakiti et al., 2004). Furthermore, a few attempts to modify the properties of food products such as bread and mayonnaise with yeast β-glucans are documented (Silva Araujo et al., 2014; Martins et al., 2015; Worrasinchai et al., 2006). To the best of our knowledge, there is no report in the literature on the use of yeast β-glucans as a functional ingredient in yogurt. The objective of this study was to assess the potential of a commercially available β-glucan powder (Yestimun®) manufactured from spent brewer's yeast as a thickening agent in skimmed yogurt. The effects of β-glucan inclusion on the yogurt gel formation during the fermentation process was monitored. The physicochemical and structural properties of yogurts with varying concentrations of β-glucan were determined and a correlation with the product's sensory perception was attempted.

MATERIALS AND METHODS

Materials

Dried skimmed cow's milk powder (SMP) Marvel ® brand, was obtained from Tesco supermarket (Aberdeen, UK). Insoluble (1/3)-(1/6)-β-glucan powder made from brewer's yeast (Yestimun®) was kindly provided by LEIBER GmbH (Bramsche, Germany). Freeze-dried yogurt starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Goat Nutrition Ltd., Ashford, England) was used to prepare yogurt starter. Phenolphthalein was purchased from Sigma Aldrich (St. Louis, MO). All reagents used were of analytical grade.

Scanning electron microscopy (SEM)

Morphological characterization of Yestimun® powder by scanning electron microscopy was performed according to the method of Limberger-Bayer et al. (2014) with some modifications. Yestimun® powder samples were sprinkled onto the surface of a carbon tape on a 12.5 mm pin stub. The stub surface was gently blown with an air duster to remove unattached β-glucan powder. The samples were then made electrically conductive by coating with a thin layer of gold-palladium using a Quorum Q150 ES sputter coater (Quorum Technologies Ltd, East Sussex, UK). The specimens were then imaged at an accelerating voltage of 10kV using a Zeiss EVO MA10 Scanning Electron Microscope (Carl Zeiss Ltd, Cambridge, UK).

Yogurt mix preparation

Five samples of yogurt were prepared including skimmed yogurt control (SYC) along with yogurts containing varying concentrations of Yestimun® powder (0.2, 0.4, 0.6 and 0.8% w/w). Yogurt mix was made up to 1 kg for each sample using milk powder, filtered water, freshly prepared yogurt starter and Yestimun® powder. Yogurt starter was prepared by dissolving the freeze-dried culture (5 g) in 840 g of water and adding 155 g of SMP (0.5 g lactic culture/100 g milk). The recipe for all samples included 16% (w/w) of dried milk powder and 3% (w/w) of yogurt starter. Yestimun® powder was added to the samples according to the different percentages (0.2-0.8% w/w) and the water content was adjusted accordingly. Yogurt mixes (milk powder, water and Yestimun® powder if applicable) were heated to 80°C for 10 min and then immediately cooled down to a temperature of approximately 45°C. This was followed by the addition of the 30g of yogurt starter to the mixes. Samples were then poured in a sterile container and placed in a yogurt fermenter (Lakeland, Aberdeen, UK) set at 44°C. A portable food and dairy pH meter (Hanna Instruments Ltd, Leighton Buzzard, UK) was used to measure the changes in pH of the samples during fermentation on an hourly basis until a pH of 4.5 was reached. At the end of the fermentation process samples were gently stirred and stored at 4°C overnight until further analysis.

Turbiscan Measurements

The fermentation process of yogurt samples was monitored using a Turbiscan MA2000 (Formulaction, Ramonville St. Agne, France). The apparatus comprises of a detection head equipped with a near-infrared light source (880 nm) which scans the length of the sample, acquiring transmission and backscattering data every 40 μm. Samples were weighed into a cylindrical borosilicate glass tube (25 mm inner diameter and 60 mm high) so that the sample and the vial weighed 40 g to ensure consistency. The light source scanned the sample at 5 min intervals from top to bottom and measured the percentage of light backscattered or transmitted over a 15 hour period at 42 °C. The coagulation process was evaluated using the TSI (Turbiscan Stability Index) parameter calculated by the Turbisoft Lab 2.2 sofware using the following formulae:

$$\mathrm{BS}=\frac{1}{\sqrt{\lambda *}} \qquad (1)$$

$$\lambda^*(\varphi, d) = \frac{2d}{3\varphi(1-g)Qs} \qquad (2)$$

$$\mathrm{TSI}=\sqrt{\frac{\sum_{\mathrm{i}=1}^{\mathrm{n}}(\chi i-\chi BS)^2}{n-1}} \qquad (3)$$

where λ^* is the photon transport mean free path in the analysed dispersion, φ is the volume fraction of particles, d is the mean diameter of particles, g and Qs are the optical parameters given by the Mie theory. χi is the average backscattering for each minute of measurement, χBS is the average χi, and n is the number of scans.

Gross nutrient composition

Energy, moisture, ash, fat, carbohydrates, total sugars and dietary fibre in the samples were determined according to the standard AOAC (1990) official methods. Protein content was determined by combustion according to the Dumas principle and calculation of the crude protein content. Carbohydrates were determined by subtracting the sum of moisture, protein, fat and ash percentages from 100%. Quantification of the β-glucan content (56% w/w) of the Yestimun® powder was calculated using the enzymatic yeast β-glucan kit (Megazyme, Co. Wicklow, Ireland).

Colour Analysis

Colour properties were determined with a Konica Minolta CR1 10 colorimeter (Konica Minolta Solutions Ltd, Basildon, UK) as described by Corradini et al. (2014). The colour parameters L* (lightness), a* (red/greenness), and b*(yellow/blueness) of the yogurt samples were evaluated according to the International Commission on Illumination (CIE) L*a*b* system.

Titratable Acidity (TA) Measurement

A portable food and dairy pH meter (Hanna Instruments Ltd, Leighton Buzzard, UK) was used to measure the changes in pH of the samples during fermentation on an hourly basis. The titratable acidity of the yogurt samples were measured according to the AOAC titration method (AOAC International, 1999) using a Stuart digital burette BT50 (Cole-Palmer, Hanwell London, UK). Briefly, 9.0 g of yogurt sample were diluted with equal parts Milli-Q water and 0.1M NaOH was used for titration using phenolphthalein as indicator. The TA was expressed as percent lactic acid as follows:

Lactic acid % = $V \times 0.009/W \times 100$

where V is the volume of 0.1 M NaOH (mL) and W is the weight of yogurt (g).

Texture Analysis

Texture measurements of yogurt were performed using a CT3 Texture Analyzer (Brookfield Engineering Laboratories Inc., Middleboro, MA) and a cylindrical mesh probe (TA-MP) suitable for yogurt measurements. Data was recorded using Texture Proc CT V1.3 Build 15 software. Yogurt samples (200 g) were tested in 250 ml Corning® polypropylene cone beakers (Sigma Aldrich, St. Louis, MO) using the following compression test setting: target distance 30.0mm, trigger load: 10g, test speed: 1.00 mm/s, return speed: 1.00 mm/s (Dai et al., 2016). The parameters determined were hardness, total work done, adhesive force and adhesiveness.

Apparent viscosity

Apparent viscosity was determined at 4°C using a rotational viscometer (Cole-Palmer Instrument Co., Ltd, London, UK) equipped with an R2 spindle at a speed of 60 rpm (Seo et al., 2009). 200g of each sample were thoroughly mixed in 250 ml Corning® polypropylene cone beakers (height 8 cm, radii of the circular ends 4 cm and 3 cm respectively) and the spindle was lowered to the immersion mark located on the spindle shaft. The readings were recorded at the 15th second of the measurement period as millipascal seconds (mPas.s).

Syneresis

Syneresis was measured using the drainage method utilized by Dai et al., 2016. Approximately 25g of yogurt sample was weighed on a 2V folded filter paper (qualitative, catalogue no. 1202-185, Whatman, Maidstone, UK) and placed on the top of a funnel. Syneresis of whey was measured by gravity at 4°C for 120 min in a volumetric flask and the syneresis value was calculated as the percent weight of whey collected in a flask divided by the initial weight of the yogurt sample.

Microscopic Structures

Microscopic images of the yogurt samples containing Yestimun® powder were captured by confocal laser scanning microscopy (CLSM) according to the method of Skytte et al. (2015) with modifications. Images were obtained with a Carl Zeiss LSM 710 (Carl Zeiss Ltd, Cambridge, UK) inverted confocal microscope. High purity Calcofluor fluorescent stain (Megazyme, Co. Wicklow, Ireland) was used for staining the β-glucan in yogurt (Kivelä et al., 2009). Samples were gently stirred and 20µl/l calcofluor in 100mN sodium carbonate buffer were added at a ratio of 1:1 (v/v). The mixtures were pipetted on to microscope glass slides, sealed with a coverslip and allowed to rest for 5 min at room temperature. Sample micrographs were captured using a 40x oil immersion objective. Calcofluor was excited at 405nm and the emission signal was collected at 467nm. Images were captured at a resolution of 1024x1024 pixels.

Consumer test

A total of 40 consumers (untrained, 10 male and 30 female, 22 aged 18-30 years, 8 aged 31-45 years and 10 aged 46-60) consisting of students and staff of the University of Aberdeen were recruited and instructed on how to perform sensory evaluation. Most panellists (32/40) were regular consumers of dairy products (consume yogurt at least twice a week) and all declared no food allergies or lactose intolerance. The evaluation of the yogurt samples was performed at the Human Nutrition Unit of Rowett Institute and aimed to assess the degree of liking based on specific sensory attributes (Perina et al., 2015). Yogurt samples (20 g) were marked with 3-digit codes and presented in white plastic cups. Order of sampling was randomized for each panellist and water was served between tasting samples. For each sample (control and 0.8% Yestimun® powder), the participants were asked to rate their liking for its appearance, flavour, texture, aftertaste and overall liking on 9-box structured hedonic scales (from 1=”I dislike extremely” to 9=”I like it extremely”) as described by Pimentel et al. (2013).

Statistical analysis

Results are expressed as mean ± standard deviation (SD) of three replicates (each replicate corresponds to a different batch). Statistical analysis of the data was performed using the statistical software SPSS Statistics 24.0 (SPSS Inc., Chicago, IL). Data were analyzed by analysis of variance (ANOVA) and the student’s T-test and significant differences ($p<0.05$) were detected by the *Scheffé’s* post hoc test.

RESULTS AND DISCUSSION

Scanning electron microscopy (SEM)

The morphology of the Yestimun® β-glucan powder was observed by scanning electron microscopy (Fig. 1). The SEM images demonstrated the relatively irregular particle size of beta-glucan particles. The formation of aggregates between β-glucan particles is also observed (Hunter et al., 2002). SEM analysis illustrated the ridge-like nature of the beta-glucan, with the smooth surface and undulated edges being displayed. The native microstructure of β-glucan particles is retained as indicated by the oval to elliptical shape of the granular particles.

Turbiscan Stability Index (TSI) and changes in pH during the fermentation process

Turbiscan analysis monitors the fermentation process in real time and provides insight into the gelation process as a result of yogurt formation. The kinetics of flocculation are calculated based on the backscattering or transmission intensities from the middle of the sample over time. Figure 2 shows the TSI for the yogurt samples recorded over a 15h period, which highlights the two different regimes of the backscattering process. The first part of the curve which shows a gradual incline up to approximately 2h is where the backscattering levels increase due to the onset and evolution of the coagulation process. The highest point on the curve, also known as the gelation point, indicates the formation of the jellified network and is evident by the relatively stable backscattering levels. Figure 2B suggests that yogurt samples fortified with Yestimun® β-glucan powder show a steep increase in TSI compared to the control sample. This indicates that the process of yogurt formation occurs at a faster rate in the presence of β-glucan powder. This finding is in agreement with the pH readings that were recorded hourly during the fermentation of the yogurt samples (Fig. 2A). The pH of samples that contained Yestimun® powder decreased at a faster rate than the control. pH readings during the fermentation process showed that all the samples fortified with beta-glucan reached the end-point (pH 4.5) after exactly 3h regardless the amount of Yestimun® powder, whereas the same levels of acidity were recorded after 4h for the control. This finding has important economic implications for the dairy industry as reduced fermentation times can accelerate processing and hence reduce the cost of production. Production costs may be further reduced if efficient methods for the production of β-glucan from spent brewer's yeast can be developed for the utilization of this by-product of food processing as a valuable functional ingredient in various food formulations. A few studies have demonstrated that the supplementation of fermented dairy products with a range of dietary fibers including β-glucan affect the growth rates and activities of lactic acid bacteria species (Bruno et al., 2002; Donkor et al., 2007; Ozer et al;., 2005). The incorporation of dietary fiber is likely to have a prebiotic effect on the lactic acid bacteria present in the starter culture, which increases their growth kinetics and results in shorter fermentation times (Ramchandran and Shah, 2008). Preliminary analysis carried out in our lab suggests that Yestimun® powder can be utilized as the sole source of nutrients for the growth and reproduction of a few bacterial species present in human gut including lactic acid (data not shown). Thus it is likely that the inclusion of β-glucan from yeast favors the growth kinetics of the fermenting bacteria and as a result lactic acid production is increased leading to a faster pH development. Furthermore, the fortification of yogurt with β-glucan may increase the amount of organic acids such as lactic and propionic acid produced during the fermentation process (Vasiljevic al., 2007). Further investigations are required to elucidate the effect of Yestimun® powder on the viability of lactic acid bacteria species and particularly for *Lactobacillus bulgaricus* and *Streptococcus thermophilus* utilised in this study.

Chemical composition and caloric values

Proximate analysis is utilized in order to determine different macronutrients that are broken down into categories by means of common chemical properties. The proximate analysis and caloric values of the yogurt samples are presented in Table 1. The AOAC method used for the determination of the total dietary fibre is an enzymatic-gravimetric procedure (Dhingra et al., 2012). Noticeable changes were observed with the dietary fibre levels in the samples. Non-surprisingly, there was an increase in the fibre that correlated with the percentage of β-glucan powder added to the samples. There was a decrease in the moisture content which corresponds to the replacement of water from the recipe with varying amounts of Yestimun® powder. The sodium and fat levels were the same for all samples with a reading of <0.1g/100g. Protein and carbohydrate levels remained unaffected whereas there was an increase in caloric values by 2kCal/100g between the control and the sample with the highest β-glucan powder concentration.

Microscopic Structure

The microstructure of protein networks in yogurts defines important physical properties of the yogurt, which affect product quality. Confocal scanning laser microscopy (CSLM) provides valuable information on the architecture of the network formed in structurally complex food matrices and has become a standard imaging technique for fermented dairy products such as yogurt (Skytte et al., 2015). Confocal electron micrographs of the structure of yogurt samples (Fig. 3) were obtained after one week of storage at 4°C. All the yogurt samples showed homogeneous structures containing relatively small serum pores. The fluorescent areas are indicative of the presence of β-glucan in the yogurt. As shown in Fig. 3A-E, the control sample did not display any fluorescent features, whereas increasing the amount of Yestimun® in the recipe resulted in a considerable increase in the number and size of clusters of β-glucan aggregates within the yogurt sample. The shape of the clusters is spherical with a diameter >20μm for samples fortified with 0.4-0.8% Yestimun® powder. The structures observed could be related to some sensory features of the different yogurt formulations. The protein network formation as a result of casein aggregation seems to be unaffected by the incorporation of β-glucan. This is attributed to the low water solubility of the β -glucan from baker's yeast, which has a structure consisting of long linear chains of glucose with β-(1→3) as well as (1→6) linkages (Gardiner, 2000).

Physicochemical properties

Table 3 shows the syneresis, apparent viscosity, titratable acidity and colour of control yogurts and yogurts fortified with β-glucan. The majority of the physicochemical properties of yogurts were not significantly affected by the addition of Yestimun® powder. Syneresis denotes the expulsion of liquid whey from yogurt due to contraction of the gel (Walstra, 1993) and is considered a primary defect often related to consumer acceptability. The phenomenon is typically associated with a loose casein micelle network resulting in a weak gel incapable of retaining water. There was a non-significant ($P>0.05$) decrease in the syneresis percentage with increasing the β-glucan amount incorporated in the recipe. This could be attributed to the distribution of the β-glucan particles within the yogurt matrix combined with their known ability to bind water and possess a high water retention capacity (Dhingra et al., 2012).

Comparatively, other studies (Singh et al. 2015; Vasiljevic al., 2007) suggest that the addition of oat and barley β-glucan resulted in increased syneresis exhibited by the yogurt samples. This effect was attributed to the formation of a weak gel that was incapable of retaining water due to the interference of the long polysaccharide molecules with the casein micelle network during gel formation (Lucey et al., 1998). This disparity in the findings could be owed to the structural differences between soluble and insoluble β-glucan molecules.

Viscosity measures the resistance of a fluid which is being deformed by either shear stress or tensile stress. Yogurt displays a non-Newtonian shear-thinning behaviour, which means that viscosity decreases with an increase in shear rate (Lambo et al., 2005). In this study, the apparent viscosity of the yogurt samples was not significantly affected ($P>0.05$) by the addition of Yestimun® powder. Previous studies have shown that apparent viscosity of yogurt was significantly increased with the addition of inulin (Balthazar et al., 2015). This effect was attributed to the ability of the water soluble polysaccharide to retain water and extensively interact with milk proteins leading to an increase in the molar mass. In this case, the low water solubility of β-glucan and the formation of small clusters within the yogurt matrix, prevented the formation of an extended structure capable of entrapping large amounts of water.

The titratable acidity of the yogurt was also not significantly ($P>0.05$) affected by the addition of β-glucan, with values ranging from 1.60 to 1.73 (Table 2). Similar results were reported for yogurt fortified with oat fiber (Fernández-García et al., 1998), which suggests that the metabolic activity of the lactic acid bacteria and thus the production of organic acids during the fermentation process was not affected by the addition of β-glucan from yeast.

The colour attributes of yogurt samples are also displayed in Table 2. The L* value represents lightness (100) and blackness (0), which is considered the most important determinant of the perceived appearance of yogurt. The a* values represent red-green (positive-negative) and b* values yellow-blue (positive-negative) hues of the yogurt samples (Dai et al., 2016). No significant differences (P>0.05) were detected in the L* parameter of the yogurt which highlighted that the whiteness of the samples were similar, irrespective of beta-glucan addition. The a* (negative) and b* (positive) parameters indicated that all the samples displayed greenish and yellowish hues. The a-values (redness) decreased, whereas the b-values (yellowness) increased with increasing the levels of β-glucan in the yogurt mix. The effects on the a* and b* parameters show that there is a β-glucan concentration-dependent effect. The findings of the present study are similar to previous studies of yogurt fortification with konjac glucomannan (Dai et al., 2016). The increase in the yellowish hue was justified as being the product of Mallard reaction in milk during heating. Hashim et al. (2009) also reported that the yellowness of yogurt depends on the levels of dietary fiber present and as a result yogurt colour is dependent on the colour of the fiber source.

Texture analysis

Textural characteristics of foods are related to consumer liking and determined to a large extent the acceptance of the product. Hardness denotes the force required to attain a given deformation while adhesive force is the force required to “pull” the sample from the probe. Adhesiveness is a good indicator of the sample’s spoonability (viscosity) and relates to the work necessary to overcome the attractive forces between a substance and the surface of other materials. Total work done is dependent on the strength of the internal bonds within a product and is often related to its consistency. The yogurt gels formed with β-glucan levels up to 0.4% were similar or lower compared to the control as determined by all textural parameters. Further addition of Yestimun® powder (>0.6%) resulted in increased textural properties and the effect was concentration-dependent. The greatest difference was observed with the hardness and adhesive force parameters between the control and the 0.8% yogurt sample. Although there was a clear incremental effect at the highest β-glucan concentration for all textural attributes, these were not at a significant level (P>0.05). Similar findings are reported by Singh et al. (2012), who demonstrated that the addition of purified oat β-glucan at levels higher than 0.3% resulted in noticeable differences in the textural characteristics of set-style yogurts. The increased hardness may be attributed to the increased solid non-fat content of the recipe and the decreased moisture levels (Table 1), following the addition of the Yestimun® powder. Adhesiveness was not significantly (P>0.05) different for all the samples compared to the control, which is in line with the apparent viscosity measurements. The increased consistency of the sample containing 0.8% Yestimun® powder as indicated by the total work done parameter may be related to the decreased levels of syneresis exhibited at this concentration of β-glucan.

Sensory evaluation

The development of fermented milk products with the inclusion of novel ingredients is likely to impact on the organoleptic properties of the food. Thus evaluating the sensory properties of the newly developed yogurt is essential for optimizing the recipe and determines product acceptability and consumer liking (Janiaski et al., 2016). A common approach adopted by the dairy industry for new product development is the use of descriptive analysis (Torres et al., 2016). This method employs a panel to identify and quantify the intensity of specific sensory attributes which are considered important for product profiling and acceptability. Descriptive sensory methodologies based on consumer perceptions can be used to obtain a generalized sensory profiling of the product but has certain limitations (Cruz et al., 2013). A large number of participants (>100) is required and secondly more specific sensory attributes can only be assessed by trained panelists. In this study, a consumer test was performed to allow the sensory description of the reformulated product and obtain useful information based on hedonic judgements (Oliveira et al., 2017). The sample with the highest β-glucan concentration (0.8% w/w) was selected for sensory profiling because instrumental analysis indicated that it was more distinctively different to the control than any other sample. Table 4 shows the sensory evaluation scores (non-specific attributes) for the control and 0.8% yogurt sample. The sensory attributes measured were not significantly different ($P>0.05$) between the two samples and results are in accordance with the instrumental analysis. Overall, the control yogurt received the best scores in all attributes evaluated, suggesting that the addition of Yestimun® powder decreased the sensory performance of the yogurt mix. The mean acceptability scores for the characteristics measured for the control yogurt ranged from 6.0 to 6.7 which corresponds to "like slightly" on the 1-9 point hedonic scale. The yogurt sample fortified with the 0.8% beta-glucan had scores ranging from 4.7 to 6.5 which is equivalent to "dislike slightly", "neither like or dislike" and "like slightly". The flavor and the aftertaste of the yogurt fortified with the β-glucan had the lowest scores (<5.0), which may at least partially account for the overall liking score obtained for the product. Sensory analysis revealed no major differences in terms of appearance, which is in agreement with the results obtained from the colour measurements (L^*). The slightly higher texture score of the control, may reflect the increased hardness of the yogurt containing β-glucan, as indicated by the instrumental analysis. Considering that yogurts were prepared using skimmed milk powder and that the highest β-glucan concentration was selected for sensory testing, it is reasonable that any scores higher than 5 are considered acceptable. The relatively small number of participants (40) is the main limitation of the current study. However, the preliminary sensory data obtained can be used for the formulation stages of food matrices which contain β-glucan from spent brewer's yeast.

CONCLUSIONS

The current study demonstrated that the fortification of yogurt with β-glucan can greatly affect gel network formation, resulting in a substantial decrease in the fermentation time. TSI and hourly pH results both confirmed that the fortified yogurt took approximately 1h less to reach the end point pH of 4.5 compared to yogurt control. The results indicated that there were no significant differences between the physicochemical properties assessed, however there was a significant difference between the a* and b* parameters of the yogurt control and yogurts fortified with 0.6% and 0.8% β-glucan. The addition of the β-glucan had an incremental effect on the textural properties which were more detectable at 0.8% concentration. Results obtained from instrumental analysis are in agreement with the sensory analysis. Despite the fact that the addition of Yestimun® powder had an adverse effect on the overall liking of the yogurt, no significant differences were identified between the sensory evaluation scores for the yogurt control and the yogurt fortified with 0.8% β-glucan. Further work is required to investigate the effect of purified β-glucan from yeast on the viability of lactic acid bacteria.

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Conflicts of interest

The authors declares that there are no conflicts of interest.

REFERENCES

Ahmad, A., Anjum, F. M., Zahoor, T., Nawaz, H., and Dilshad, S. M. R. 2012. Beta glucan: a valuable functional ingredient in foods. Crit. Rev. Food Sci. Nutr. 52: 201-212.

Aimanianda, V., Clavaud, C., Simenel, C., Fontaine, T., Delepierre, M., and Latgé, J. - P. 2009. Cell Wall β-(1,6)-Glucan of Saccharomyces cerevisiae. J. Biol. Chem. 284:13401-13412.

AOAC, 1990. Official methods of analysis (15th ed.). Washington DC: Association of Official Analytical Chemists.

Astrup, A., Dyerberg, J., Selelck, M., and Stender, S. 2008. Nutrition transition and its relationship to the development of obesity and related chronic diseases. Obes. Rev. 9: 48-52.

Balthazar, C. F., Conte-Junior, C. A., Moraes, J., Costa, M. P., Raices, R. S. L., Franco, R. M., Cruz, A. G., and Silva, A. C. O. 2016. Physicochemical evaluation of sheep milk yogurts containing different levels of inulin. J. Dairy Sci. 99:4160–4168.

Balthazar, C. F., H. L. A. Silva, R. M. S. Celeguini, R. Santos, G. M. Pastore, C. A. Conte Junior, M. Q. Freitas, L. C. Nogueira, M. C. Silva, and A. G. Cruz. 2015b. Effect of galactooligosaccharide addition on the physical, optical, and sensory acceptance of vanilla ice cream. J. Dairy Sci. 98:4266–4272.

Belsito, P. C., Ferreira, M. V. S., Cappato, L. P., Cavalcanti, R. N., Vidal, V. A. S., Pimentel, T. C., Esmerino, E. A., Balthazar, C. F., Neto, R. P. C., Tavares, M. I. B., Zacarchenco, P. B., Freitas, M. Q., Silva, M. C., Raices, R. S. L., Pastore, G. M., Pollonio, M. A. R., Cruz, A. G., 2017. Manufacture of Requeijão cremoso processed cheese with galactooligosaccharide. Carbohydr. Polym. 174:869-875.

Chandan, R. C., and O'Reil, K. R., 2006. Ingredients for yogurt manufacture. In: Chandran R. C. (Ed.) Manufacturing Yogurt and Fermented Milks, Blackwell Publishing, Ames, Iowa, USA.

Corradini, S. A., S. G. S. Madrona, J. V. Visentainer, E. G. Bonafe, C. B. Carvalho, P. M. Roche, and Prado, I. N. 2014. Sensorial and fatty acid profile of ice cream manufactured with milk of crossbred cows fed palm oil and coconut fat. J. Dairy Sci. 97:6745–6753.

Cruz, A. G., Cadena, R. S., Castro, W. S., Esmerino, E. A., Rodrigues, J. B., Gaze, L., Faria, J. A. F., Freitas, M. Q., Deliza, R., and Bolini, H. M. A. (2013). Consumer perception of probiotic yogurt: Performance of check all that apply (CATA), projective mapping, sorting and intensity scale. Food Res. Int., 54: 601–610.

Dai, S., Corke, H., and Shah, N. P. 2016. Utilization of konjac glucomannan as a fat replacer in low-fat and skimmed yogurt. J. Dairy Sci. 99: 7063–7074.

Dhingra, D., Michael, M., Rajput, H., and Patil, R. T. 2012. Dietary fiber in foods: A review. J. Food Sci. Technol. 49: 255–266.

EFSA Panel on Dietetic Products NaAN. 2011. Scientific opinion on the safety of “yeast β-glucans” as a novel food ingredient. EFSA J., 9: 2137.

FAO/WHO. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Food and Agriculture Organization of the United Nations.

Fernandez-Garcia, E., McGregor, J., and Traylor, S. 1998. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. J. Dairy Sci. 81: 655–663.

Ferrão, L. L., Ferreira, M. V. S., Cavalcanti, R. N., Carvalho, A. F. A., Pimentel, T. C., Silva, R., Esmerino, E. A., Neto, R. P. C., Tavares, M. I. B., Freitas, M. Q., Menezes, J. C. V., Cabral, L. M., Moraes, J., Silva, M. C., Mathias, S. P., Raices, R. S. L., Pastore, G. M., Cruz, A. G, 2018. The xylooligosaccharide addition and sodium reduction in requeijão cremoso processed cheese. Food Res. Int. 107:137-147.

Folkenberg D. M, and Martens M. 2003. Sensory properties of low fat yoghurts. Part B: Hedonic evaluations of plain yoghurts by consumers correlated to fat content, sensory profile and consumer attitudes. Milchwissenschaft-Milk Sci Int. 58:154–157.

Gardiner, T., and Carter G. 2000. β-glucan biological activities: A review (condensed version), GlycoScience 1: 1–2.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F., and Shah, N. P. 2010. Probiotic dairy products as functional foods. Comp. Rev. Food Sci. Food Safety 9: 455-470.

Hashim, I. B., Khalil, A. H., and Afifi, H. S. 2009. Quality characteristics and consumer acceptance of yogurt fortified with date fiber. J. Dairy Sci. 92: 5403–5407.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J. & Salminen, S. 2014. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11(8): 506-514.

Hunter, K.W., Gault, R.A., Berner, R.A. 2002. Preparation of microparticulate β-glucan from Saccharomyces cerevisiae for use in immune potentiation. Lett. Appl. Microbiol. 35: 267– 271.

Janiaski, D. R., Pimentel, T. C. Cruz, A. G., and Prudencio, S. H. 2016. Strawberry-flavored yogurts and whey beverages: What is the sensory profile of the ideal product? J. Dairy Sci. 99: 5273-5283.

Kivelä, R., Nyström, L., Salovaara, H., and Sontag-Strohm, T. (2009). Role of oxidative cleavage and acid hydrolysis of oat beta-glucan in modelled beverage conditions. J. Cereal Sci., 50:190-197.

Lam, K.-L., and Cheung, P. C-K. 2013. Non-digestible long chain beta-glucans as novel prebiotics. Bioact. Carbohydr. Dietary Fibre 2: 45-64.

Lambo, A. M., Oste, R., and Nyman, M. E. G. 2005. Dietary fiber in fermented oat and barley b -glucan rich concentrates. Food Chem. 89: 283–293.

Limberger-Bayer, V. M., De Francisco, A., Chan, A., Oro, T., Ogliari, P. J., Barreto, P. L. M. 2014. Barley β-glucans extraction and partial characterization. Food Chem. 154:84-89.

Lucey, J. A., Munro, P. A., and Singh, H. 1998. Whey separation in acid skim milk gels made with glucono-6-lactone: Effects of heat treatment and gelation temperature. J. Texture Stud. 29: 413–426.

Martins, Z. E., Erben, M., Gallardo, A. E., Silva, R., Barbosa, I. et al. 2015. Original article Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread. Int. J. Food Sci. Technol. 50: 1855–1863.

Oliveira, E. W., Esmerino, E. A., Carr, B. T., Pinto, L. P. F., Silva, H. L. A., Pimentel, T. C., Bolini, H. M. A., Cruz, A. G., and Freitas, M. Q. 2017. Reformulating Minas Frescal cheese using consumers' perceptions: Insights from intensity scales and check-all-that-apply questionnaires. J. Dairy Sci. 100: 6111-6124.

Perina, N. P., Granato, D., Hirota, C., Cruz, A. G., Bogsan, C. S. B., & Oliveira, M. N. (2015). Effect of vegetal-oil emulsion and passion fruit peel-powder on sensory acceptance of functional yogurt. Food Res. Int., 70: 134–141.

Pimentel, T. C., A. G. Cruz, and S. H. Prudencio. 2013. Short communication: Influence of long-chain inulin and Lactobacillus paracasei subspecies paracasei on the sensory profile and acceptance of a traditional yogurt. J. Dairy Sci. 96:6233–6241.

Ramchandran, L., and Shah, N. P. 2008. Effect of Addition of Versagel® on Microbial, Chemical and Physical Properties of Low-Fat Yogurt. J Food Sci. 73:M360:M367

Seo, M. H., Lee, S.-Y., Chang, Y. H., and Kwak, H. S., 2009 Physicochemical, microbial, and sensory properties of yogurt supplemented with nanopowdered chitosan during storage. J. Dairy Sci. 92: 5907–5916.

Singh, M., Kim, S., and Liu, S. X., 2012. Effect of Purified Oat b-Glucan on Fermentation of Set-Style Yogurt Mix. J. Food Sci. 77: E195-E201.

Skytte, J. L., Ghita, O., Whelan, P. F., Andersen, U., Møller, F., Dahl, A. B., and Larsen, R. 2015. Evaluation of Yogurt Microstructure Using Confocal Laser Scanning Microscopy and Image Analysis 80, J. Food Sci. 80:1218–1228.

Thammakiti, S., Suphantharika, M., Phaesuwan, T., and Verduyn, C. 2004. Preparation of spent brewer's yeast b-glucans for potential applications in the food industry. Int. J. Food Sci. Technol. 39: 21–29.

Torres, F. R., Esmerino, E. A., Carr, B. T., Ferrão, L. L., Granato, D., Pimentel, T. C., Bolini, H. M. A., Freitas, M. Q., and Cruz, A. G. 2016. Rapid consumer-based sensory characterization of requeijão cremoso, a spreadable processed cheese: Performance of new statistical approaches to evaluate check-all-that-apply data. J. Dairy Sci. 100: 6100-6110.

Tripathi, M. K., and Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. J. Funct. Foods, 9, 225-241.

Vasiljevic, T., Kealy, T., and Mishra, V. 2007. Effects of β-Glucan Addition to a Probiotic Containing Yogurt. J. Food Sci. 72: C405–C411.

Wahlqvist, M. L. 2005. The new nutrition science: sustainability and development. Public Health Nutr. 8: 766–772.

Walstra, P. 1993. The Syneresis of Curd. In: Fox, P.F. (Ed.), Cheese: Chemistry, Physics and Microbiology: Volume 1 General Aspects. Springer US, Boston, MA, pp. 141–191.

Willett, W. 2013. Nutritional Epidemiology. Oxford, New York, Oxford University Press.

Worrasinchai, S., Suphantharika, M., Pinjai, S., and Jamnong, P. 2006. Β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. Food Hydrocoll. 20: 68–78.

Zhu, F., Du, B., Bian, Z., and Xu, B. 2015. β-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. J. Food Compos. Anal. 41: 165–173.

Zhu, F., Du, B., and Xu, B., 2016. A critical review on production and industrial applications of β-glucans. Food Hydrocoll. 52: 275–288.

Zhu, Q., Li, J., Liu, H., Saito, M., Tatsumi, E., and Yin, L.: 2015. Development of stable water-in-oil emulsions using polyglycerol polyricinoleate and whey protein isolate and the impact on the quality of bittern-tofu. J. Disp. Sci. Technol. 36: 1548–1555.

Figure captions

Figure 1. Scanning electron microscopy (SEM) images for commercially powdered β-glucan.

Figure 2. Effect of β-glucan addition on the fermentation process of yogurts monitored by A. pH and B. the Turbiscan stability index (TSI)

Figure 3. Confocal laser scanning microscopy (CLSM) of yogurt samples stained with the fluorescent dye Calcofluor: A yogurt control, B 0.2%, C 0.4%, D 0.6% and E 0.8% β-glucan. Scale bar is set at 20μm.

Table: 1. Proximate analysis of yogurts containing varying concentrations of β-glucan

Nutritional Analysis	**% (w/w) β-glucan**				
	0	0.2	0.4	0.6	0.8
Energy (kcal/100g)	62	62	63	63	64
Protein (g/100g)	6.51	6.44	6.63	6.29	6.53
Ash (U) (g/100g)	1.31	1.32	1.32	1.32	1.33
Moisture (g/100g)	83.18	83.01	82.72	82.63	82.37
Carbohydrates (g/100g)	9.00	9.02	8.90	9.19	8.93
Total Sugars (g/100g)	7.31	7.13	7.31	7.20	7.30
Sodium (g/100g)	<0.1	<0.1	<0.1	<0.1	<0.1
Dietary Fibre (g/100g)	<0.1	0.20	0.43	0.57	0.84
Fat (g/100g)	<0.1	<0.1	<0.1	<0.1	<0.1

Table 2. Syneresis, apparent viscosity, titratable acidity (TA) and colour of yogurts fortified with β-glucan (means±SD)

β-glucan (g/100g)	Syneresis (%)	Viscosity (Pa.s)	TA (%)	Colour		
				L*	**a***	**b***
0.0	12.06 ± 1.5^{a}	11.61 ± 2.2^{a}	1.73 ± 0.07^{a}	46.00 ± 3.2^{a}	-1.10 ± 0.1^{a}	7.03 ± 0.3^{a}
0.2	12.61 ± 2.2^{a}	10.06 ± 1.3^{a}	1.62 ± 0.09^{a}	45.38 ± 3.5^{a}	-0.98 ± 0.1^{a}	7.08 ± 0.4^{a,c}
0.4	10.89 ± 2.4^{a}	11.18± 1.7^{a}	1.60 ± 0.11^{a}	47.78 ± 0.8^{a}	-0.97 ± 0.1^{a}	7.20 ± 0.3^{a,c}
0.6	10.77 ± 0.9^{a}	11.80 ± 2.1^{a}	1.69 ± 0.12^{a}	45.93 ± 2.4^{a}	-0.68 ± 0.0^{b}	7.76 ±0.3^{b,c}
0.8	10.29 ± 1.9^{a}	10.75 ± 0.6^{a}	1.73 ± 0.17^{a}	45.83 ± 2.0^{a}	-0.60 ± 0.1^{b}	7.65 ± 0.3^{a,c}

Means within columns having different lower case letters are significantly different (P<0.05)

Table 3. Texture attributes of stirred yogurt samples

	(g/100g) β-glucan				
Textural property	0	0.2	0.4	0.6	0.8
Hardness (g)	26.00 ± 5.2^{a}	22.20± 6.4^{a}	23.20 ± 5.0^{a}	27.60 ± 4.6^{a}	31.00± 4.9^{a}
Total Work Done (mJ)	6.00 ± 1.0^{a}	5.36 ± 1.5^{a}	5.66 ± 1.4^{a}	6.66 ± 1.2^{a}	7.40 ± 1.1^{a}
Adhesive Force (g)	26.00 ± 3.7^{a}	25.20 ± 6.5^{a}	26.40 ± 3.2^{a}	28.60 ± 4.0^{a}	31.60 ± 2.8^{a}
Adhesiveness (mJ)	8.38± 1.3^{a}	8.12± 2.3^{a}	8.32 ± 1.0^{a}	9.22 ± 1.5^{a}	10.12 ± 0.9^{a}

Means within rows having different lower case letters are significantly different ($P<0.05$)

Table 4. Non-specific sensory attributes of stirred yogurt samples fortified with β-glucan (means±SD)

	Appearance	Flavour	Texture	Aftertaste	Overall Liking
Control	6.7 ± 1.5[a]	6.2 ± 1.7[a]	6.1 ± 1.6[a]	6.0 ± 1.6[a]	6.2 ± 1.8[a]
0.8% β-glucan	6.5 ± 1.6[a]	4.9 ± 1.9[a]	5.4 ± 1.7[a]	4.7 ± 1.7[a]	5.0 ± 1.8[a]

Means within columns having different lower case letters are significantly different ($P<0.05$)