Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Microbiology, Nutrition and Dietetics

VALORISATION OF BREWERS' SPENT GRAIN IN A SOY YOGURT PRODUCT

Master´s thesis

Beatrice Sella

Sustainable Agriculture and Food Security

Supervisor: Iveta Klojdová, Ph.D
Co-supervisor: Isabella Taglieri, Ph.D

Disclaimer

This master's thesis is submitted as a requirement for the Master´s degree in Sustainable Agriculture and Food Security at the Czech University of Life Sciences Prague and the Master´s degree in Biosafety and Food Quality at the University of Pisa. The submission is made concurrently to both universities under the double degree program agreement.While the substantive content of this thesis remains consistent for both submissions, there are variations in the title page and the roles of the supervisor and co-supervisor, adhering to the respective guidelines of each university.The dual submission of this thesis is a reflection of the academic collaboration and educational innovation fostered by the double degree program, aiming to broaden the academic and cultural perspectives of its participants.

Declaration

I hereby declare that I have authored this master's thesis carrying the name **Valorisation of brewers' spent grain in a soy yogurt product** independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

Prague 13.02.2024 ______________________________

Abstract

Brewery’s spent grain (BSG) is the most abundant by-product generated from the beer-brewing process.

BSG is rich in fibre and protein and, to date, the main use for the elimination of this by-product has been as an animal feed. However, because of its high nutritional value (such as the content of bioactive phenolic compounds with antioxidant activity), BSG is of interest for application and fortification of human food products, particularly in view of its low cost and availability in large amounts.

The study examines soy yogurt and aims to evaluate the impact of spent grain on soy yogurt compared to a common BSG-free soy yogurt. The effect of spent grains addition (at different levels) on the physico-chemical properties (particle size distribution, syneresis, texture, and rheological behaviour) of the final product has been investigated. Changes in the aforementioned physico-chemical properties during a storage period of 4 weeks have been monitored on a weekly basis.

In fact, the incorporation of BSG in soy yogurt benefited the production line due to its impact in increasing the fermentation rate and supported the Lactic Acid Bacteria (LAB) growth. BSG-added yogurt allowed the LAB growth and increased the pH which can be a benefit for industry to use less sweetener to cover the acidity. The presence of high dietary fibre in BSG contributed to a lower syneresis, a higher shear stress and maintenance of viscosity at certain levels of addition during the storage period. Furthermore, the addition of BSG at certain levels strengthened the survival level of LAB during the storage. A maximum substitution of 2% BSG is suggested to be more suitable in yogurt products due to its ability to preserve the flow behaviour and syneresis in addition to other physical properties. In general, syneresis decreases with increasing BSG levels. The results demonstrate BSG's capacity to bind water, maintaining yogurt's flow characteristics and consistency.

INDEX

CHAPTER 1

Introduction

The valorization and the addition of brewery spent grain (BSG) in food products tends to improve the nutritional value of BSG-added food products[1]. BSG contains valuable compounds including phenolic compounds, amino acids, lipid and fatty acids, and dietary fiber which are responsible for the biological activity of BSG including antioxidant activity, antimicrobial activity, DNA protective and antimutagenic properties, anti-inflammatory and colon health maintenance properties[2]. Therefore, the potential of BSG as a functional food component and a nutraceutical ingredient has been increasingly evaluated.

Moreover, the presence of prebiotic components and the high dietary fibre content of BSG enhance its potential as a prebiotic in yoghurt as a functional food. The utilization of BSG in yogurt production will add beneficial market value for industry because BSG is a low-cost material. Besides the nutritional value of BSG, its potential as an ingredient in yogurt production is supported by the abundance of BSG production. Almost 2 billion hectolitres of beer are produced[3] which generates 40 million tons of BSG annually worldwide.

The incidence of malnutrition has unintentionally increased in developing countries due to continuing population growth and insufficient protein availability. Research[4] is being done to find alternate sources of protein from legume seeds in order to meet the protein demands of developing countries, where animal protein is also expensive and severely insufficient.

---

[1] Naibaho & Korzeniowska, 2021 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage. *Food Hydrocolloids*, *125*, 107412.

[2] ibidem.

[3] Conway, 2019 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage. *Food Hydrocolloids*, *125*, 107412.

[4]Osundahuns, O. F., Amosu, D., & Ifesan, B. O. T. (2007). *Quality Evaluation and Acceptability of Soy-yoghurt with Different Colours and Fruit Flavours*. American Journal of Food Technology.

1.1 The most important steps of beer-brewing process

The beer-brewing process starts with the production of the wort, a sugar-rich solution that will be used in the subsequent fermentation stage to produce ethanol.

To prepare the wort, water and milled barley malt are combined in a mash tun at a temperature that gradually rises from 37° to 78°C. This process breaks down the malt starch into fermentable (maltose and maltotriose) and non-fermentable (dextrins) sugars. During this phase, barley malt proteins are also partially broken down into polypeptides and amino acids. Brewer's spent grain (BSG), also known as the insoluble, undegraded portion of the barley malt grain, is obtained in combination with the wort at the conclusion of this procedure. BSG is produced as a by-product of this process and is filtered through the BSG bed that forms at the bottom of the mash tun before being delivered to the fermentation tank. BSG makes up about 85% of all the by-products produced during the brewing of beer[5].

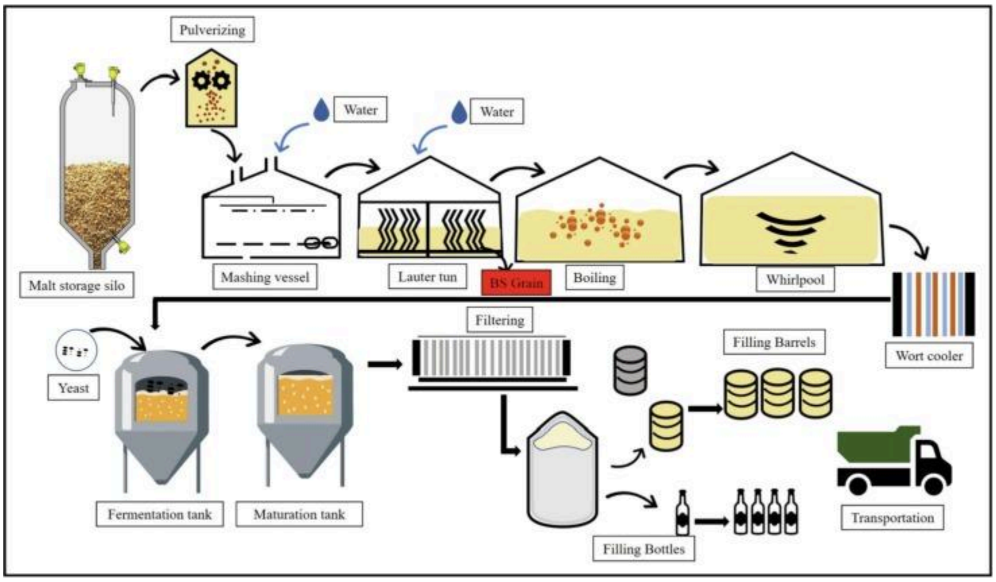


Fig. 1: Schematic representation of different phases involved in beer production[6].

---

[5] Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.
[6] Pabbathi, N. P. P., Velidandi, A., Pogula, S., Gandam, P. K., Baadhe, R. R., Sharma, M., Gupta, V. K. (2022). *Brewer's spent grains-based biorefineries: A critical review.* Fuel, 317, 123435.

1.2 What is Brewery Spent Grain

BSG is constituted by the husks[7] that cover the original barley malt grain in the mixture with part of the pericarp and seed coat layers that are obtained as residual solid material after the wort elaboration step. More or less starchy endosperm and the walls of empty aleurone cells may remain after mashing, depending on the efficiency of the process. Low levels of starch will be present, and depending on the brewing regime used, some hop residues added during mashing may be present. With regard to inter-brewery variation, in particular, BSG is a heterogeneous material. This is due to a number of factors, such as the cereal variety, time of harvesting, type of hops added, the malting and mashing regime, and whether adjuncts were employed during brewing.

Other cereals, such as corn, rice, wheat, rye, or sorghum, may occasionally be added to the mixture of barley malt and other ingredients for the wort elaboration, depending on the type of beer that will be made. In these situations, the BSG is used to separate the insoluble portion of the grains as a distinct fraction following the mashing process. Therefore, BSG can be made from barley malt alone or from a combination of adjuncts (other cereal grains) and barley malt. According to estimates, the yearly global output of BSG is around 38.6×10^6 t. Given that BSG is only found in the barley husks and that not all of the barley grown is used to make beer, this is a sizable sum but compared with the total amount of by-products generated from the most important agricultural crops, the amount of BSG produced annually is much lower [8] [9] [10] [11].

---

[7] Ibidem.

[8] USDA, *World Agricultural Production Archives*. United States Department of Agriculture,Washington, DC (2012), cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013

[9] Zhang Y, Ghaly AE and Li B, *Availability and physical properties of residues from major agricultural crops for energy conversion through thermochemical processes* cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[10] De Mori C and Minella E, *Aspectos econˆomicos e conjunturais da cultura da cevada*. Embrapa Trigo Documentos Online 139. (2012) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[11] Kim S and Dale BE, *Global potential bioethanol production from wasted crops and crop residues.* Biomass Bioenergy 26:361–375 (2004) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

1.2.1 Chemical composition

From a chemical point of view BSG is rich in sugars, proteins and minerals and this composition varies due to some factors which include the variety of the barley used in the process as well as its harvest time and the conditions under which it was cultivated, the conditions used for malting and mashing and the amount and type of the adjuncts added in the mixture with the barley malt for the wort elaboration.

BSG contains a variety of mineral elements, among which silicon, phosphorus and calcium are the most abundant. Silicon is also the most abundant mineral element in other cereals such as rice, oat and wheat straw, but the content of phosphorus and calcium in BSG is particularly higher than those commonly reported for these cereals[12].

However, cellulose, hemicellulose, and lignin make up the majority of BSG's composition, while proteins can make up as much as 30%[13]. Hemicellulose, which primarily consists of arabinoxylan (AX), is the main component of BSG and can be present at levels as high as 40% on a dry weight basis.

Sugars like xylose, arabinose, and glucose make up the hemicellulose and cellulose fractions in plants. On a dry weight basis, sugars make up over half of the BSG makeup, making them a highly significant component. In addition to sugars, lignin and proteins are also present in BSG in substantial proportions. About 30% of the total protein composition is made up of essential amino acids, the majority of which is lysine (14.3%), followed by leucine (6.12%), phenylalanine (4.64%), isoleucine (3.31%), threonine (0.71%), and tryptophan (0.14%)[14].

Histidine (26.27%) and glutamic acid (16.59%) make up the majority of the non-essential amino acids in BSG, which make up 70% of the total protein composition. Aspartic acid, valine, arginine, serine, alanine, tyrosine, glycine, asparagine, and glutamine are present in smaller levels. Compared with other

[12] Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications*, 2013.
[13] Ibidem.
[14] Waters DM, Jacob F, Titze J, Arendt EK and Zannini E, *Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment.* Eur Food Res Technol 235:767–778 (2012) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

agro-industrial by-products (such as rice straw, wheat straw, barley straw, oat straw, rice husks, barley husks) BSG presents lower cellulose content but similar lignin content, which allows its use as raw material for the production of valuable compounds such as activated charcoal, phenolic compounds, dispersants, emulsifier and chelate agents, pesticides, fertilizers, polymers, adhesives, components for resins, etc. The hemicellulose concentration of BSG, however, is high and greater than that of many other agricultural by-products, such as rice straw, wheat straw, rice husks, barley straw, and oat straw. Proteins are also present in BSG, but at higher concentrations than in other crop by-products. These benefits make BSG very interesting and promising material for food and biotechnological applications.

1.2.2 Nutritional aspects

The main BSG components of interest in terms of prospective health advantages are the phenolic (e.g., hydroxycinnamic acid) and fibre components. Given that BSG has a relatively high lysine content in contrast to other cereal products, the protein portion of the grain is also of importance.

AX is being viewed more and more as dietary fibre. The colonic microflora, which includes key components such as bifidobacteria and lactobacilli, can operate as a prebiotic by fermenting a major part of the water-extractable AX that enters the large intestine.

A healthy population of these bacteria is seen as important for the maintenance of gut health. For instance, bifidobacteria digest dietary fibres to produce short chain fatty acids (SCFA). The production of SCFA is typically seen as advantageous since it defends the host against infections, triggers immunological responses, lower cholesterol production, boosts colonic blood flow, improves muscle contractions and may guard against the development of colon cancer. The prebiotic activity of AX may be due to the breakdown of xylooligosaccharides (XOS), which are known prebiotics and have different degrees of polymerization[15]. It has also been noted that XOS produced by enzymatic treatment of wheat AX has prebiotic potential.[16]

Another important, although compositionally minor, fibre (~1% w/w), present in BSG is ß-glucan. Among cereals, oats and barley contain the highest levels of ß-glucan, with dry weight contents of 3-7 and 3-20 %, respectively[17]. It is a significant nutritional component of whole-grain diets since it has been linked to a lower risk of coronary heart disease. Consumption food that contain whole grains has been linked to a lower incidence of coronary heart disease, and β-glucan is thought to be a crucial nutritional ingredient[18]. Because β-glucan is soluble and can create a gel-like network, it is believed to have a physiological effect via increasing viscosity in the gastrointestinal tract. This viscosity is thought to have a net cholesterol-lowering impact by decreasing bile acid reabsorption and increasing bile acid production from cholesterol[19]. For barley β-glucan, people should consume at least 3 g of it daily to have this kind of cholesterol-lowering impact[20].

---

[15] Wang, J., Sun, B., Cao, Y., and Wang, C. (2010) *In vitro fermentation of xylooligosaccharides from wheat bran insoluble dietary fiber by Bifidobacteria*, Carbohydr. Polym. 82, 419–42 cit.in Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568.

[16] Grootaert, C., van den Abbeele, P., Marzorati, M., Broekaert, W. F., Courtin, C. M., Delcour, J. A., Verstraete, W., and Van de Wiele, T. (2009) *Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem*, FEMS Microbiol. Ecol. 69, 231–242 cit.in Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568.

[17] Steiner, J., Procopio, S., and Becker, T. (2015) *Brewer's spent grain: Source of value-added polysaccharides for the food industry in reference to the health claims*, Eur. Food Res. Technol. 241, 303–315. cit.in Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568.

[18] Truswell, A. (2002) *Cereal grains and coronary heart disease*, Eur. J. Clin. Nutr. 56, 1–14 cit.in Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568.

[19] Steiner, J., Procopio, S., and Becker, T. (2015) *Brewer's spent grain: Source of value-added polysaccharides for the food industry in reference to the health claims*, Eur. Food Res. Technol. 241, 303–315 cit.in Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568.

[20] EFSA Panel on Dietetic Products NDA (2011) Scientific opinion on the substantiation of a health claim related to barley beta-glucan and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006, EFSA J. 9, 2470 cit.in Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568.

Dietary fibre is thought to have positive effects on the host through a process known as immune system modulation[21].

A higher risk of colorectal cancer is linked to an inflammatory reaction. Increased dietary fibre consumption lowers proinflammatory effector levels.

To sum up, the ingestion of BSG provides several health benefits, such as accelerated transit time, increased faecal weight and fat excretion (alleviating both constipation and diarrhoea).

Furthermore, BSG is rich in antioxidant phenolic compounds that represent an alternative for application in food industry to avoid the use of synthetic antioxidants.

3.2 Food applications and future perspectives

BSG represents an inexpensive material for the natural fortification (through increasing fibre and protein content) of food products. Owing to all the above-mentioned benefits, the possibility of BSG application in food products has been extensively evaluated. There are many studies reporting, for example, the incorporation of BSG in the manufacture of bakery products such as bread, biscuits, cookies, muffins, cakes, waffles, pancakes, tortillas, snacks, doughnuts and brownies. To be incorporated in these products, BSG is firstly converted to flour, because its original form is too granular for direct application[22] [23] [24] [25].

Additionally, BSG has been used as a possible additive to increase the fibre content of non-bakery food items, such as frankfurters. BSG is able to increase total dietary

---

[21] Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications*, 2013.

[22] Hassona HZ, *High fibre bread containing brewer's spent grains and its effect on lipid metabolism in rats.* Nahrung 37:576–582 (1993) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[23] Miranda MZ, Grossmann MVE and Nabeshima EH, *Utilization of brewer's spent grain for the production of snacks with fiber. 1. Physicochemical characteristics*. BrazArchBiolTechnol 37:483–493 (1994) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[24] Miranda MZ, GrossmannMVE, Prudencio Ferreira SH and Nabeshima EH, *Utilization of brewer spent grain (BSG) for production of snacks with fiber. 2. Sensory analysis of snacks*. Braz Arch Biol Technol 37:9–21 (1994) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[25] Ozturk S,Ozboy O, Cavidoglu I and Ko¨ ksel H, *Effects of brewer's spent grain on the quality and dietary fibre content of cookies*. J Inst Brew 108:23–27 (2002) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

fibre and water holding capacity with its additions; however, appearance and texture were also changed, in part as a result of fat removal.

Bioactive substances, such as phenolic compounds, have received a lot of interest recently for their potential to improve human health through the reduction of risk factors for cardiovascular diseases, diabetes, and cancer as well as their antioxidant, antiallergenic, anti-inflammatory, and antimicrobial properties[26].

Bioactive phenolic compounds with antioxidant activity are abundant in BSG and can be extracted using a variety of techniques, such as alkaline reactions, enzyme reactions, solid-liquid extraction, and microwave-assisted extraction. Among these techniques, solid-liquid extraction—which involved extracting antioxidant phenolic chemicals from BSG for 30 minutes at 60 °C while using 60% (v/v) acetone as the solvent—proved to be the most effective[27]. The phenolic acids found in higher concentrations in BSG, ferulic and p-coumaric acids, may have a major impact on the material's antioxidant capacity. It has also been proposed that there is a considerable correlation between flavonoids and BSG's antioxidant ability.

In order to avoid using synthetic antioxidants, antioxidant phenolic compounds isolated from BSG offer a possible substitute for applications in the food business. Given that antioxidant phenolic compounds are economically expensive and have numerous industrial applications in the food and pharmaceutical industries, this would be a fascinating substitute for the use of BSG.

Reusing agro-industrial by-products is becoming more popular, both from an economic and environmental perspective. Breweries all over the world generate BSG in large quantities. Nevertheless, it is still primarily used as animal feed or is just dumped in landfills, despite the enormous range of potential applications, not the

[26] Martins S, Mussatto SI, Mart´ınez-Avila G, Montanez-Saenz J, Aguilar CN and Teixeira JA, *Bioactive phenolic compounds: production and extraction by solid-state fermentation*. A review. Biotechnol Adv 29:365–373 (2011) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[27] Meneses NGT, Martins S, Teixeira JA and Mussatto SI, *Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains*. Separ Purif Technol 108:152–158 (2013) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

least of which is as a useful ingredient for the food sector or a valuable source of bioactive compounds. As a result, there is a lot of interest in novel applications and methods for using this brewing by-product, especially since BSG is an inexpensive substance that is consistently available in huge quantities all year long.

The primary market for cereal goods that can earn money through the use of BSG as an ingredient is anticipated to be cereal products and more research into the technological elements associated with such applications is encouraged.

1.2.4 Other industrial applications of BSG

BSG is a raw material of interest for application in different areas (not only in food) because of its low price, large availability throughout the year and valuable chemical composition such as in feed, energy production, and in chemical and biotechnological processes.

The main current application of BSG is as cattle feed, where it can be utilized directly in wet form (as separated from the mash tun) or as dry material. BSG is a substrate of great interest for use as animal feed, indeed it can provide all the essential amino acids required for animal nutrition when paired with less expensive nitrogen sources, like urea, for example. In addition, adding BSG to a cow's diet causes an increase in milk production and total solids content while a decrease in fat content[28] [29]. The significant benefits that came from using BSG in cattle feed include the use of this substance in the feeding of other animals like pigs, poultry, and fish[30]. Once more, the addition of BSG to these animals' diets has good benefits including body weight gain. Today, BSG is considered a cheap substitute for the feed components present in most poultry diets. However, because these birds lack the enzymes required for hydrolyzing the polymer chains, the majority of the cell wall

[28] Belibasakis NG and Tsirgogianni D, *Effects of wet brewers grains on milk yield, milk composition and blood components of dairy cows in hot weather*. Anim Feed Sci Technol 57:175–181 (1996) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[29] Sawadogo L, Sepehri H and Houdebine LM, *Presence of a factor stimulating prolactin and growth hormone secretion in brewers' spent grains.* Reprod Nutr Dev 29:139–146 (1989) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[30] Kaur VI and Saxena PK, *Incorporation of brewery waste in supplementary feed and its impact on growth in some carps*. Bioresour Technol 91:101–104 (2004) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

polysaccharides included in BSG, such as arabinoxylan and β-glucan, are not digested in their gastrointestinal tracts[31]. Therefore xylanase and β-glucanase enzymes have been commonly added to BSG to overcome this problem.

Thermochemical conversion (pyrolysis, combustion), the creation of biogas, and the production of ethanol are a few methods that have been suggested for the use of BSG in energy production. Additionally, BSG is a raw material of great interest for application in this field due to its wide availability, chemical make-up, and inexpensive cost. Utilising thermochemical conversion techniques like pyrolysis and combustion is one of the potential solutions for producing energy from biomass materials like BSG. There has been research[32] on the potential to create biogas from BSG. This application is particularly well suited for generating thermal energy in breweries with little environmental impact.

Anaerobic fermentation processes for producing biogas can be broken down into two stages: a first hydrolytic stage to encourage complete material degradation; and a second methanogenic stage in which acidogenic microorganisms transform the macromolecules released during the previous hydrolytic stage into volatile fatty acids, such as acetate, butyrate, and propionate, and methanogenic bacteria then convert these volatile acids to methane. Different techniques, such as chemical/thermal treatment, crushing (by wet centrifugal grinding or ball milling), or enzymatic treatment, can be used to complete the hydrolysis process.

Corn is the primary raw material utilised to produce ethanol at the current high quantities seen worldwide. This situation is going to change, though, because of the incentive provided for the production of second-generation ethanol, or ethanol made from lignocellulosic waste materials, since this type of feedstock is essentially food. Because of its high hemicellulose and cellulose fraction composition, BSG has also been taken into consideration while studying other lignocellulosic raw materials for this purpose. Research in this area has increased over the past ten years due to

---

[31] Denstadli V, Westereng B, Biniyam HG, Ballance S, Knutsen SH and Svihus B, *Effects of structure and xylanase treatment of brewer's spent grain on performance and nutrient availability in broiler chickens*. Br Poultry Sci 51:419–426 (2010) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[32] Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

interest in developing a competitive method for producing second-generation ethanol. BSG was identified as a promising raw material for this application and tested in various processes with various microbial strains. BSG's cellulose and hemicellulose fractions can both be utilized to make ethanol.

For chemical procedures to either extract (by extraction) or generate (by chemical reaction) molecules of industrial interest, BSG is a raw material of interest. BSG has a number of potential uses that have been made up in this field, but one of its most promising uses is as an adsorbent material for wastewater treatment. As was already mentioned, BSG is abundant in cellulose and hemicellulose polysaccharides, added-value substances with a wide range of industrial uses.

BSG is considered as an appropriate raw material for use in the production of papers[33] because of its fibrous nature. Paper towels, business cards, and coasters have previously been prepared with BSG, giving these products a premium sensation of touch.

Due to its abundance in polysaccharides, proteins, and minerals, BSG is a substrate with a significant potential for use in biotechnological processes. The use of BSG as a raw material to extract or synthesize useful chemicals through fermentation or enzymatic processes has thus been the subject of numerous studies. The fact that BSG is a very unstable substance that can degrade quickly due to microbial activity when it is separated from the lauter tun is the fundamental proof that it is an effective substrate for the culture of microorganisms. Due to its high water content (800 g/kg) after being removed from the lauter tun as well as the presence of proteins and carbohydrates in its composition, it degrades so quickly.

However, some biological techniques can also be employed for this, and they have the added benefit of not producing toxic effluents, making them more environmentally friendly. In this way, many enzymes have been applied to the extraction of beneficial chemicals from BSG. Polysaccharide hydrolase mixtures, such as those from *Aspergillus japonicus, Aspergillus versicolor,* and *T. reesei*, may

[33] Ishiwaki N, Murayama H, Awayama H, Kanauchi O and Sato T, *Development of high value uses of spent grain by fractionation technology*. Tech QMaster Brew Assoc Am 37:261–265 (2000) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

hydrolyze more than 42% of the total polysaccharides in BSG to sugars in just one day[34].

In addition to extracting sugars, enzymes can also be employed to separate hydroxycinnamic acids (ferulic and p-coumaric acids) from the BSG structure. Thirty percent of the total amount of ferulic acid in BSG could be extracted using a combination of xylanase from *Trichoderma viride* and esterase from *Aspergillus niger*. Almost all of the ferulic acid and 9% of the p-coumaric acid found in BSG were liberated by an enzyme preparation from the thermophilic fungus *Humicola insolens.*

In general, BSG is a significant agro-industrial by-product in terms of production volume and valuable chemical composition. The reuse of this material has been the subject of studies for many years but in the last 10 years, the research has increased, maybe due to environmental and financial concerns. Mentioned uses of BSG are mostly in culinary, energy, chemical, and biotechnological processes. These applications have been shown to have significant advantages. More intensive use of BSG can be expected in the future.

1.2 Purpose of the research

Since BSG is a valuable plant-based material, its utilization in plant-based products may offer the production of products with an additional nutritional value. In this perspective, the main focus of this study has been the use of BSG for the fortification of soy yogurt.

Due to its nutritional profile, including high-quality proteins, polyunsaturated fatty acids, and dietary fibres, as well as its significant contribution to the reduction of the risk of cardiovascular diseases, type 2 diabetes, cancer, and osteoporosis[35], soybean is becoming a more important component of the human diet.

---

[34] Khan AW, Lamb KA and Schneider H, *Recovery of fermentable sugars from the brewers' spent grains by the use of fungal enzymes*. Process Biochem 23:172–175 (1988) cit.in Solange I Mussatto, *Brewer's spent grain: a valuable feedstock for industrial applications,* 2013.

[35] Chen, Li-Jun, Jian- Jun, Bo, & Rui, 2010; Rinaldoni, Campderrós, & Padilla, 2012; Xiao, 2008 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

Indeed, a plant-based lifestyle is becoming increasingly popular, and people are shifting from an animal-based diet to a plant-based diet[36]. Plant-based alternatives to dairy products are referred to as drinks, beverages, dairy alternatives, or some other names other than "milk" or "cheese" or "yogurt"[37]. This fast growth is driven by increasing prevalence toward allergenicity of cow's milk, lactose intolerance, changing consumer lifestyles, and interest in alternative diets (i.e., vegan and flexitarian)[38] [39]. In 2020, the COVID-19 pandemic accelerated this process since it made consumers rethink their lifestyle and deviate to a more plant-based diet as a healthier option[40]. Therefore, the demand for a variety of vegan alternatives to cheese and yogurt, among others, is gradually gaining importance in the market[41].

[36] Fehér, A.; Gazdecki, M.; Véha, M.; Szakály, M.; Szakály, Z. A Comprehensive Review of the Benefits of and the Barriers to the Switch to a Plant-Based Diet. Sustainability 2020, 12, 4136 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[37] McClements, D.J.; Newman, E.; McClements, I.F. Plant-based Milks: A Review of the Science Underpinning Their Design, Fabrication, and Performance. Compr. Rev. Food Sci. Food Saf. 2019, 18, 2047–2067 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[38] Munekata, P.E.S.; Domínguez, R.; Budaraju, S.; Roselló-Soto, E.; Barba, F.J.; Mallikarjunan, K.; Roohinejad, S.; Lorenzo, J.M. *Effect of innovative food processing technologies on the physicochemical and nutritional properties and quality of non-dairy plant-based beverages*. Foods 2020, 9, 288 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[39] Boukid, F.; Rosell, C.M.; Rosene, S.; Bover-Cid, S.; Castellari, M. *Non-animal proteins as cutting-edge ingredients to reformulate animal-free foodstuffs: Present status and future perspectives.*

[40] Research and Markets Global Dairy Alternatives Market Report 2021: *Opportunities in Growing Demand from Emerging Economies* cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[41] Markets and Markets Dairy Alternatives Market Size|Trends–Forecasts to 2026|*COVID-19 Impact on Dairy Alternatives Market* cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

It has also been seen that[42] okara, a soybean waste generated from soymilk production, could be employed in the food industry to improve nutritional and functional properties of final products.

Soymilk fermentation, particularly with lactic acid bacteria, is a key step that may enhance the product's flavour and texture as well as its health aspects.

According to several studies, soy products, especially soy yoghurt, may be effective vehicles for probiotic microorganisms[43]. Prebiotic substances like inulin, when combined with probiotic microorganisms, may have a protective effect that enhances the survival and activity of probiotic bacteria both during the transit through the gastrointestinal tract and during the preservation of probiotic food products[44].

The strains of probiotic bacteria most frequently used are from the genera *Lactobacillus* and *Bifidobacterium*.

The first step for making soy yogurt is to prepare soymilk. Soymilk is a nutritious plant protein beverage and it's traditionally manufactured in several steps, namely, soybean soaking, grinding, filtering of the slurry and heating above 95°C for 5 – 10 min. Soymilk can be used to produce an innovative yogurt-like gel food called soy yogurt through lactic fermentation[45]which involves the use of very specific cultures such as *Lactobacillus Bulgaricus* and *Streptococcus thermophilus.* Soybean oligosaccharides can be partially degraded through fermentation to improve the

---

[42] Jiménez-Escrig, Tenorio, Espinosa-Martos, & Rupérez, 2008 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[43] Bedani, Rossi, & Saad, 2013; Champagne, Green-Johnson, Raymond, Barrette, & Buckley, 2009; Donkor, Henriksson, Vasiljevic, & Shah, 2007; Farnworth et al., 2007; Wang et al., 2009 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[44] Akin, Akin, & Kirmaci, 2007; Buriti, Castro, & Saad, 2010; Hernandez-Hernandez et al., 2012 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

[45] Buono, Setser, Erikson, & Fung, 1990; Pinthong, Macrae, & Rothwell, 1980 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

nutritional value of soymilk. Moreover, fermentation flavours that are produced from lactic fermentation can increase the consumer preference of soymilk products[46].

After the addition of the cultures, the mixture has to be incubated at 40 – 45°C for 4-8 hours until its pH increases from 6.5 (pH of soymilk) to 4.5 (typical pH of yogurt) and then cooled and stored at refrigeration temperature.

In the next chapter the manufacturing of two types of soy yogurt will be described: a common one (BSG-free) and a fortified one, i.e. with the addition of BSG.

[46] Blagden & Gilliland, 2005; Donkor, Henriksson, Vasiljevic, & Shan, 2007 cit.in Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443.

CHAPTER 2

2. Materials and methods

2.1 Classic soy yogurt preparation

Soybeans were bought in a local supermarket. 200 g of beans were weighed and soaked overnight in tap water. The next day, after 3 washes in cold water, 1400 mL of water was added to the soaked beans. Then this mixture was blended using a kitchen mixer (speed 2) for 45 seconds and filtered through filter papers to separate the solid part of the beans and obtain soy milk.

The milk was poured into 500 mL bottles and each bottle contained 300 mL of milk. After putting 300 mL in the bottles, gelatin powder and sugar were added into cold milk and the solution was blended.

Milk was heated to 85°C for 15 minutes and cooled down to 35 – 40°C and then two different lyophilized yogurt cultures (*YoFlex* and *Biomix*) were added; the mixtures were mixed for about 10 minutes.

YoFlex contained *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus.*

Biomix, contained the following microorganisms: *Streptococcus thermophilus, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium adolescentis* (which are probiotic starter cultures) and other strains of *Lactobacillus* such as *Lactobacillus gasseri, Lactobacilus rhamnosus, Lactobacillus acidophilus.*

Four different treatments were performed on the basis of the cultures chosen and the use or not of gelatin as shown in **Table 1**:

Table 1: Samples of yogurts prepared with gelatin and two different cultures

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Soymilk	300 mL	300 mL	300 mL	300 mL
Gelatin (0.3%)	-	0.9 g	-	0.9 g
Sugar (5%)	15 g	15 g	15 g	15 g
YoFlex(0.02%)	0.06 g	0.06 g	-	-
Biomix(0.04%)	-	-	0.12 g	0.12 g

For each treatment, soymilk was transferred into: two plastic containers (80 g each), two 15 ml centrifuge tubes (10 mL each) for syneresis, one 50 mL centrifuge tube (30 mL) for pH measurement.

All these containers were incubated for 4 h at 42 °C to reach the required acidity (pH<4.6), then cooled and stored at 4°C.

After one day, samples in all plastic containers were used to measure the viscosity, hardness, and particle size (**Fig.2**).

The test was repeated in triplicate.

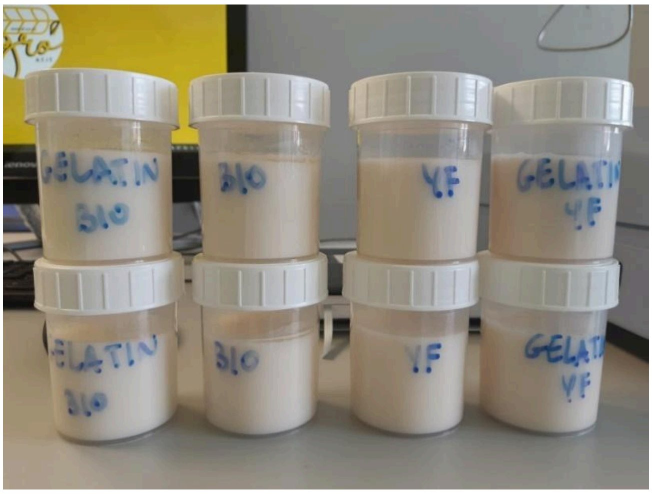


Figure 2: Yogurt sasmples object of the research: Gelatin Bio = Gelatin with Biomix cultures; Bio= Biomix cultures; YF= YoFlex cultures; Gelatin YF= Gelatin with YoFlex cultures.

2.2 Preparation of fortified yogurts

The preparation of the fortified yogurts was almost identical to the preparation of the classic yogurts: 510 g of beans were used and 1190 mL of water was added to the soaked beans the next day.

BSG was provided by the small university brewery of the Czech University of Life Sciences. BSG was ground into smaller particles and sieved. Only the powder was used to make yogurts (Fig.3), the remaining part that did not pass through the sieve was thrown away (Fig.4).

Figure 3: BSG powder after sieving

Figure 4: BSG residue not passing the sieve

The milk was poured into two 500 mL bottles until filled. After putting 500 mL in the bottles, gelatin powder, sugar and BSG powder were added into cold milk and the solution was blended. A treatment without BSG powder served as a control (**Tab.2**).

Milk was heated to 85°C for 15 minutes and cooled down to 35 – 40°C. *YoFlex* was then added to the soymilk and mixed for about 10 minutes.

Table 2: Three different treatments object of the research

	Treatment 1	Treatment 2	Treatment 3
Soymilk	500 mL x 2 bottles	500 mL x 2 bottles	500 mL x 2 bottles
Gelatin (0.3%)	1.5 g x 2 bottles	1.5 g x 2 bottles	1.5 g x 2 bottles
Sugar (5%)	25 g x 2 bottles	25 g x 2 bottles	25 g x 2 bottles
BSG (2 + 100)	-	10 g x 2 bottles	-
BSG (4 + 100)	-	-	20 g x 2 bottles
YoFlex (0.02%)	0.1 g x 2 bottles	0.1 g x 2 bottles	0.1 g x 2 bottles

Each treatment of soymilk was transferred to: ten plastic containers (80 g each), ten 15 mL centrifuge tubes (10 mL each) for syneresis, five 50 mL centrifuge tubes (20 mL each) for pH measurement.

All the containers were incubated for 4 h at 42 °C to reach the required acidity (pH<4.6), then cooled and stored at 4°C (**Fig.5**).

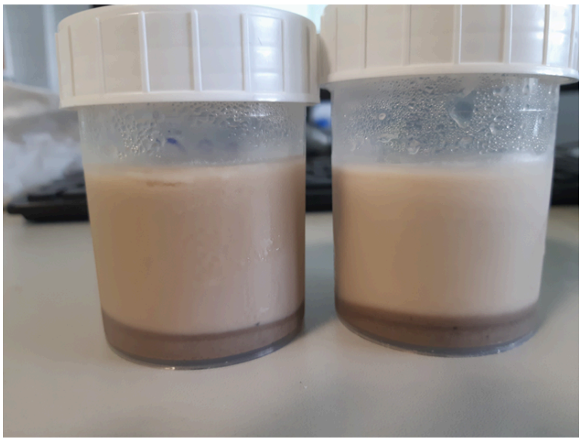


Figure 5: two examples of yogurts with the addition of BSG

This experiment was repeated in triplicate. Each batch was investigated on days 1, 7, 14, 21, 28 and hardness, particle size, viscosity, syneresis and pH were evaluated.

2.3 Analysis of hardness

Hardness was measured by a Texture Analyzer Shimadzu EZ-X and with a cylindrical probe (diameter 35 mm) penetrating into the yogurt sample at constant speed of 1 mm/s (**Fig.6**).

Typical parameters quantified were 'cohesiveness' (the extent to which a material can be deformed before it ruptures), 'hardness' (the force necessary to attain a given deformation), 'springiness' (or 'elasticity' which is the rate at which the deformed material goes back to its undeformed condition after the deforming force has been removed) and 'adhesiveness' (work necessary to overcome the attractive forces between the surface of the yogurt and the surface of other materials with which it comes in contact.

The mean and standard deviation of the values for each textural parameter were determined and one-way analysis of variance was used to determine significance.

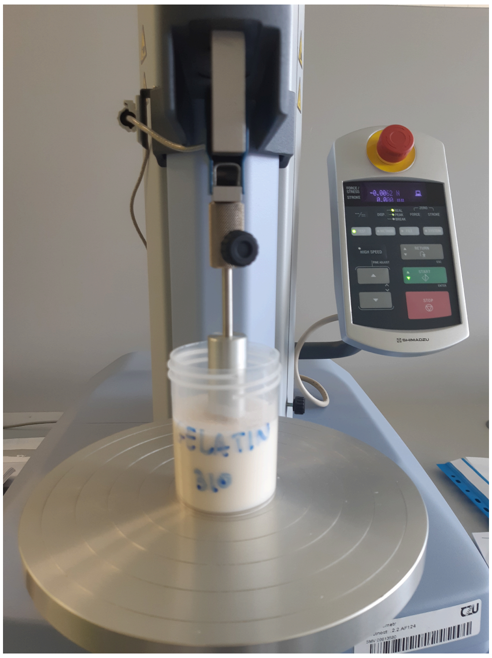


Figure 6: Texture Analyzer for the analysis of hardness of yogurt

2.4 Analysis of rheological behaviour

Rheological behaviour measurements of yogurt were performed by using a Modular Compact Rheometer (MCR) series – MCR 72 (Anton Paar) in a geometry system plate-plate (gap of 1 mm). Before the analysis, yogurt samples were kept for 30 min at room temperature. Then, 5 mL were applied to the plate for measurement. The viscosity was expressed in mPa · s , the shear rate was determined in s^{-1}.

2.5 Analysis of particle size

BSG powder was analyzed using a Malvern Mastersizer 3000 equipped with an Aero unit (Malvern Instruments Ltd., Worcestershire, UK, 2013). Samples were added to the disperser, and compressed air at 0 bar was used to transport powders to minimize damage to the powder during measurement.

A Hydro LV dispersion unit was also included with the Malvern Mastersizer 3000 to measure the particle sizes of the yogurts under investigation.

2.6 Syneresis

The syneresis was measured on 50 mL centrifuge tubes. Four different samples were centrifuged for 10 min at 1500 rpm (**Fig.7**).

The supernatant was separated and the syneresis was calculated according to the equation:

$$syneresis\ (\%) = \frac{weight\ of\ supernatant\ (g)}{weight\ of\ yogurt\ (g)}\ x\ 100$$

Figure 7: centrifuge containing yogurt tubes for syneresis analysis.

2.7 Analysis of pH

The pH was measured by a laboratory pH-metre. The pH-metre was calibrated with pH 4.0, 7.0 and 9.0 buffer solution.

2.8 Statistical analysis

Statistical analysis was performed by using IBM SPSS Statistics (version 29.0.0.0). A significance level of $P < 0.05$ was applied.

CHAPTER 3

3. Results

3.1 Analysis of pH

The pH decrease of soy yogurts associated with the two cultures used is shown in Fig.8. Compared to Biomix cultures, YoFlex seems to have a faster pH decrease drop in 4 h of fermentation.

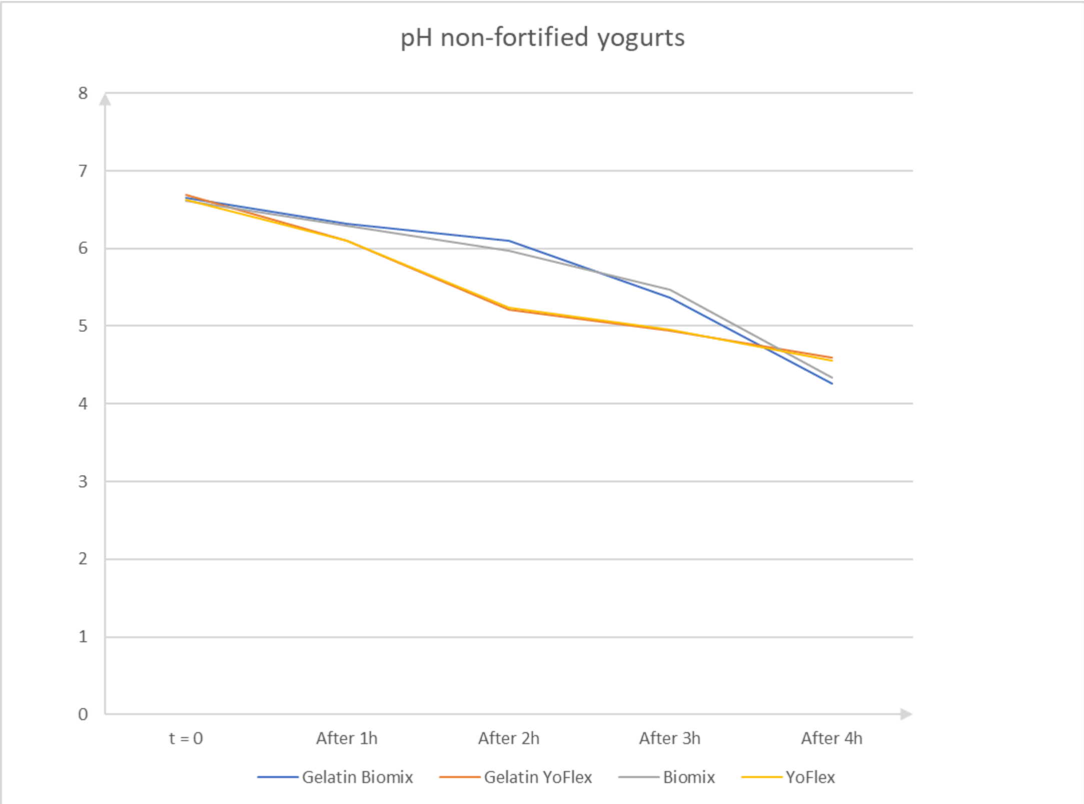


Fig.8: Fermentation curves of non-fortified yogurts during fermentation process.

Table 3: pH (mean, n=3) during fermentation process of fortified yogurts.Values are mean ± standard deviation.

pH					
	t=0	After 1h	After 2h	After 3h	After 4h
CONTROL	6,34±0,24	5,77±0,41	5,02±0,22	4,69±0,15	4,49±0,08
BSG 10	6,29±0,29	5,87±0,27	5,19±0,24	4,69±0,09	4,52±0,06
BSG 20	6,29±0,28	5,8±0,31	5,13±0,26	4,67±0,07	4,47±0,06

The desired range of pH (4.4-4.5) was obtained after 3-4 h of fermentation (Table 3). In general, the addition of BSG significantly increased the fermentation rate (Supplement 1). During the observation period, the highest derivation of pH occurred between the second and third hour of fermentation. This impact of BSG on pH change during the fermentation could be associated with an improved rate growth of LAB promoted by the BSG addition. By this, BSG might contribute to the availability of amino acids for LAB growth. This effect might be due to the potential of BSG as a prebiotic for its high amount of dietary fibre and protein[47]. BSG consists indeed of arabino-xylooligosaccharides which are considered as prebiotic nutraceuticals[48] and thus might promote the fermentation rate.

Moreover, Table 3 shows that the addition of BSG to soy yogurts before the start of fermentation had no effect on the pH (in fact all yogurts initially have a similar pH

[47] Amorim et al., 2019; Lao et al., 2020; Wen et al., 2019 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage.*

[48] Amorim et al., 2019; Xiros & Christakopoulos, 2012 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage.*

i.e. around 6.3). However, the derivation of pH during fermentation might influence the release of certain compounds from BSG such as protein and phenolic compounds. It is indeed reported that pH significantly impacts protein solubility[49]. During the pasteurization at 85°C, the disruption of BSG cell walls might occur thus allowing the protein release from the matrix.

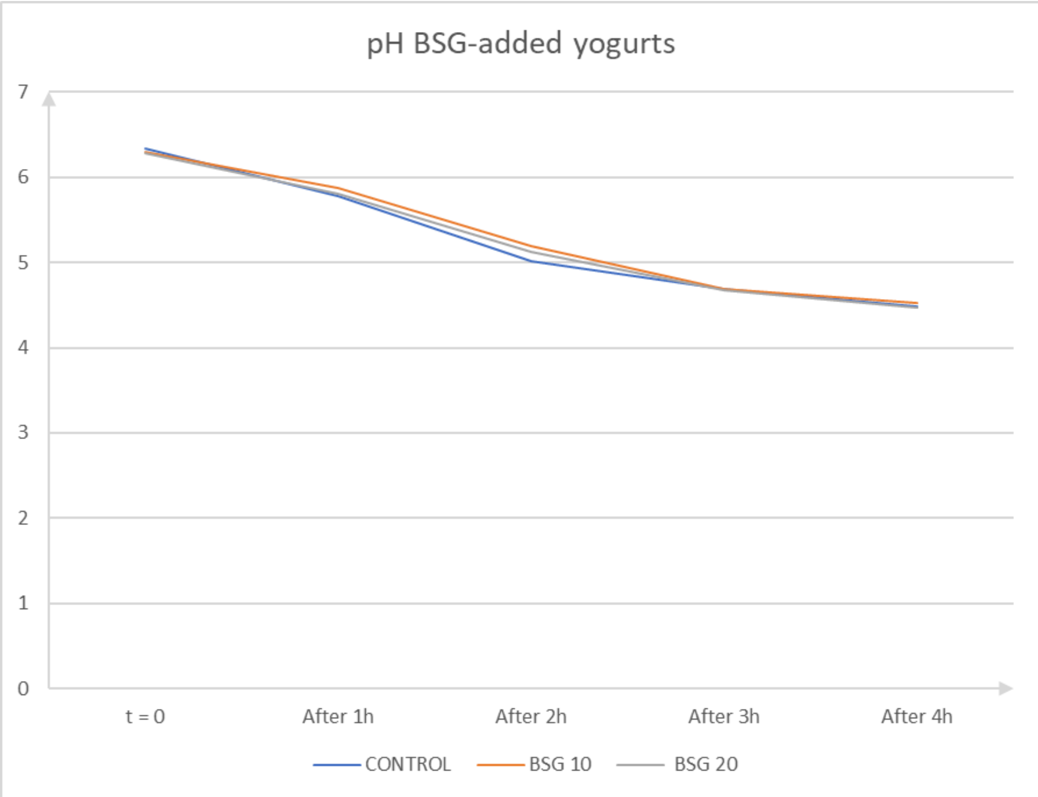


Fig.9: pH in BSG-added yogurts during fermentation process.

[49] He et al., 2019; Vieira et al., 2014, Vieira, Teixeira, & Ferreira, 2016 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage.*

Table 4: pH decreasing after 7,14,21,28 days of storage in BSG-added yogurts. Values are mean ± standard deviation.

pH storage				
	Day 7	Day 14	Day 21	Day 28
Control	4,42 ± 0,11	4,38 ± 0,09	4,35 ± 0,09	4,32 ± 0,07
BSG 10	4,47 ± 0,09	4,42 ± 0,08	4,37 ± 0,07	4,34 ± 0,05
BSG 20	4,42 ± 0,07	4,41 ± 0,12	4,34 ± 0,04	4,3 ± 0,02

After measuring the pH value at the end of fermentation, the pH was also measured every 7 days. The pH of BSG-added yogurts during the storage period is shown in Table 4. In general, the pH decreased during the storage in all observed groups. Moreover, the pH of BSG-added yogurt is higher than control (0% BSG), except in the 4% of BSG substitution after 7 days of storage, thus showing the impact of BSG on pH of BSG-added yogurt.

3.2 Analysis of hardness

The texture is considered a crucial indicator to measure the quality of fortified yogurt. Hardness, adhesiveness, and cohesiveness of fortified (**Tab.5b**) and non-fortified yogurts (**Tab.5a**) were measured with a texture analyzer .

Cold storage increased the hardness of yogurt, (Table 5b), while the addition of BSG, determined a little reduction of the hardness of yogurt. However, the hardness was not a significant parameter which has influenced fortified-soy yogurt (Supplement 2).

Besides, the addition of gelatin and the use of different yogurt cultures did not significantly influence the hardness of the samples.

Tab 5: (a) hardness of non-fortified yogurts. (b) hardness of fortified yogurts during 28 days of cold storage. Values are mean ± standard deviation. All values are expressed in Newton (N).

a

Biomix	0,38 ± 0,16
YoFlex	0,52 ± 0,15
Gelatin Biomix	0,39 ± 0,14
Gelatin YoFlex	0,42 ± 0,11

b

	Day 1	Day 7	Day 14	Day 21	Day 28
Control	0,29 ± 0,04	0,44 ± 0,13	0,42 ± 0,13	0,36 ± 0,08	0,41 ± 0,1
BSG 10	0,26 ± 0,07	0,39 ± 0,12	0,28 ± 0,1	0,35 ± 0,1	0,4 ± 0,09
BSG 20	0,22 ± 0,02	0,34 ± 0,12	0,32 ± 0,09	0,34 ± 0,06	0,36 ± 0,12

3.3 Analysis of rheological behaviour

Rheological behaviour is one of the key properties for the quality of fermented products. The apparent viscosity is a significant factor in determining the general properties of the gel and stability of dairy products. **Fig. 10** illustrates the changes in the viscosity of yogurt samples - viscosity curves: YoFlex cultures showed a lower viscosity than the Biomix cultures. This means that yogurts prepared with YoFlex cultures were less viscous than yogurts prepared with Biomix cultures.

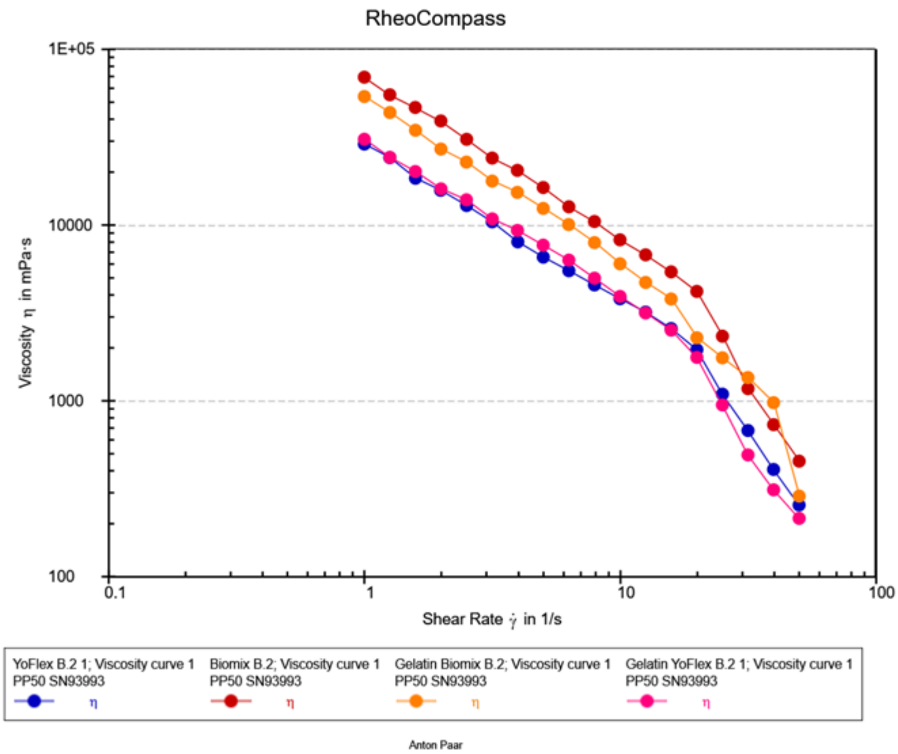


Fig.10: viscosity curves by using different yogurt cultures without BSG .

The flow behaviour of BSG-added yogurts is shown as viscosity curves in Fig.11a, Fig.11b and Fig.11c. They show the viscosity of the prepared yogurts are dependent on the shear rate - yogurts exhibited pseudoplastic behaviour. In general, the addition of BSG increased the viscosity of yogurt. Evaluation of the flow behaviour in general, the addition up to 2% of BSG seems to have the same flow behaviour as control (BSG 0%). An excessive amount of BSG (4%) changes the flow behaviour of the yogurt which might negatively affect the acceptability of the yogurt. During the storage, the flow behaviour fluctuated depending on the amount of BSG substituted. Shear stress describes the required energy to destroy the structure of the yogurt matrix[50]. The decrease in the viscosity as the shear rate increases, shows a shear-thinning behaviour. Both viscosity and shear stress fluctuated during the storage: interestingly, 14 and 21 days storage had a different pattern of flow behaviour (shear rate vs shear stress) compared to 1 and 7 days of storage (Fig.11c). In comparison to other BSG level addition, 4% BSG had an increase in shear stress in the increase of shear rate. This shows that 4% BSG influenced a stronger structural interaction between protein-fat and polysaccharide that might occur due to the LAB growth which generated metabolites and peptides in different levels[51]. Moreover, an increase in the formed network could occur due to the hydration of macromolecules and stabilization properties of certain ingredients during the refrigerated storage [52]. The unstable flow behaviour of BSG-added yogurt might be related to the firm stability of the yogurt which is affected by the fermentation process. A lower fermentation time could diminish the protein network[53] and generate a lower amount

[50] V´enica, Spotti, Pav´on, Molli, & Perotti, 2020 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage.*

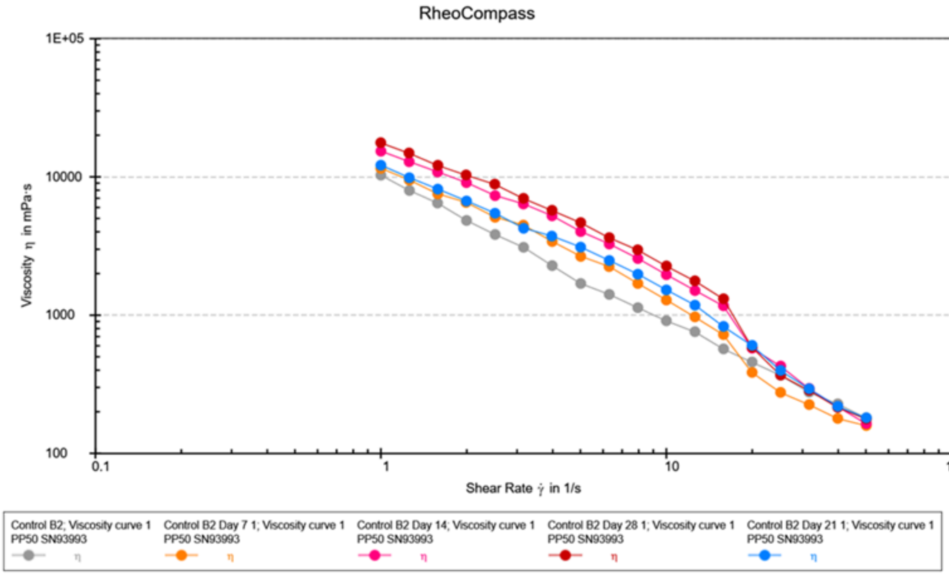
[51] Chandan & O'Rell, 2013 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage*

[52] Ramírez- Sucre & V´elez-Ruiz, 2013 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage*

[53] Pachekrepapol et al., 2021 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage*

of free fat and protein[54]. This could lead to the formation of a weaker gel and irregular protein network [55].

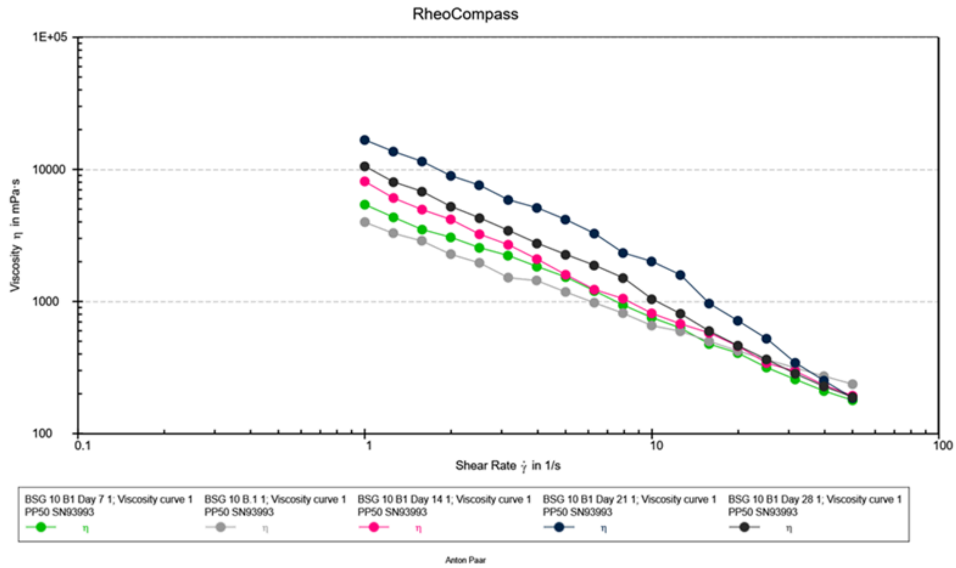
a



[54] Sinamo, Hasan, & Hasanah, 2020b cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage*

[55] Pachekrepapol et al., 2021 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage*

b



c

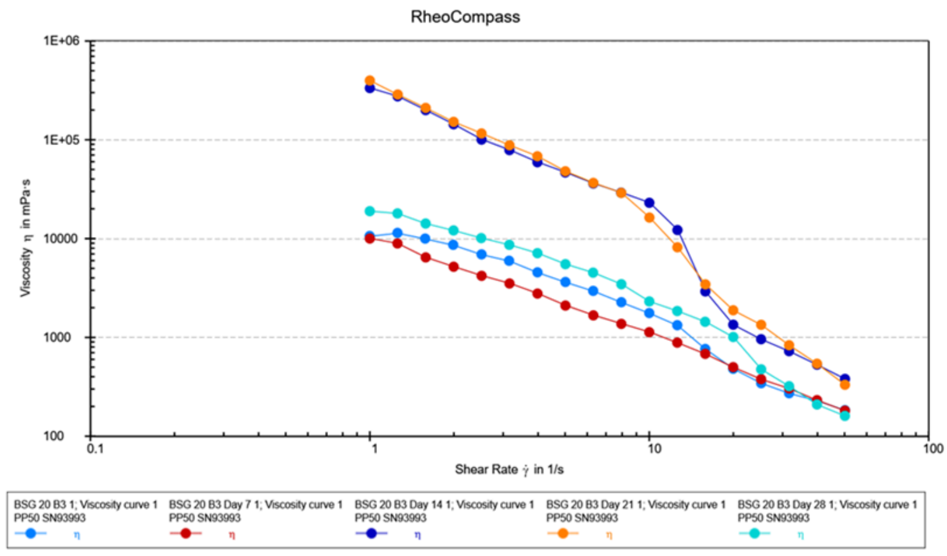


Fig. 11: (a) viscosity curves - 0% BSG. (b) viscosity curves - 2% BSG. (c) viscosity curves - 4% BSG.

3.4 Analysis of particle size

The stability of soy yogurt depends mainly on the particle size. Results related to the particle size parameters of soy yogurt with different yogurt cultures are presented in Fig. 12 and Tab. 6.

The particle size exhibited a monomodal distribution in all soy yogurt samples. Overall, YoFlex cultures resulted in larger particles.

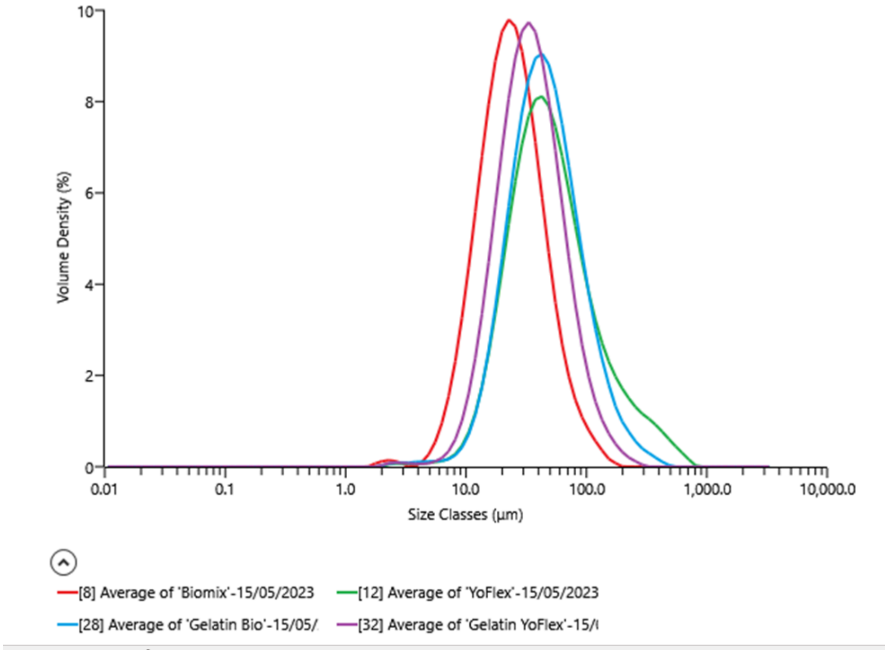


Fig. 12: volume distribution of particle size by using different yogurt cultures without BSG.

Table 6: mastersize parameters of non-fortified yogurts.

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	8	Average of 'Biomix'	10.6	23.3	53.2
	12	Average of 'YoFlex'	19.2	47.1	163
	28	Average of 'Gelatin Bio'	19.0	43.8	111
	32	Average of 'Gelatin YoFlex'	15.3	33.7	77.3
Mean			**16.0**	**37.0**	**101**
1xStd Dev			**4.03**	**10.8**	**47.6**
1xRSD (%)			**25.1**	**29.1**	**47.1**

From the results obtained it can be deduced that, when using YoFlex cultures in yogurt, the particles tend to be larger than those prepared with Biomix. However, this cannot be confirmed if gelatin is used.

As for BSG, its particles in the form of powder were measured (**Fig.13**). From the results obtained it can be understood that the particles were very large and on average 90% they measured 422 μm (Tab.7).

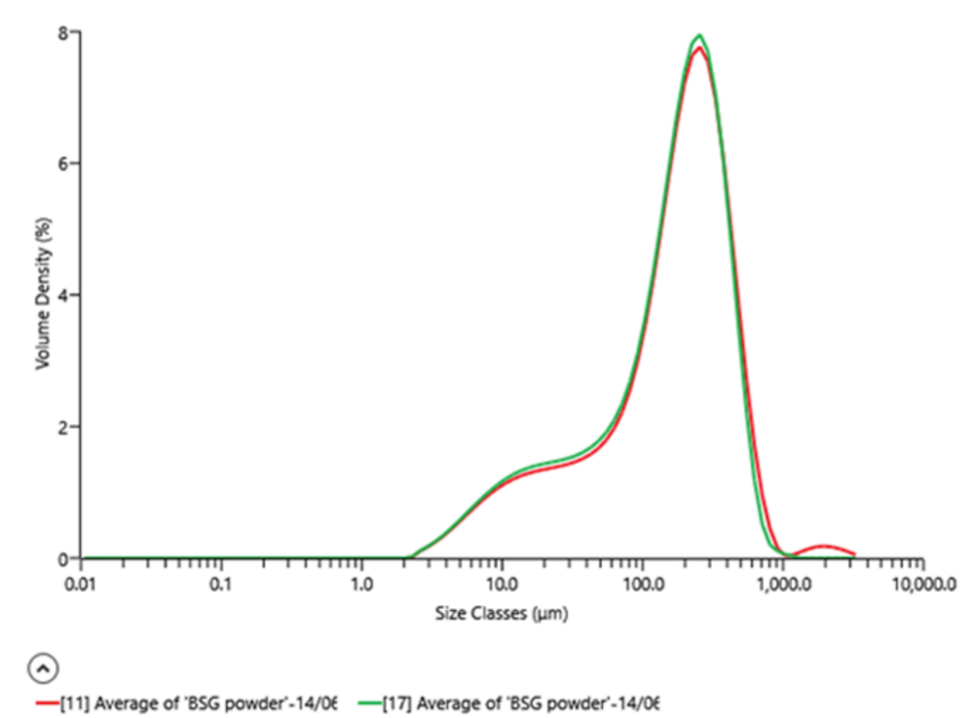


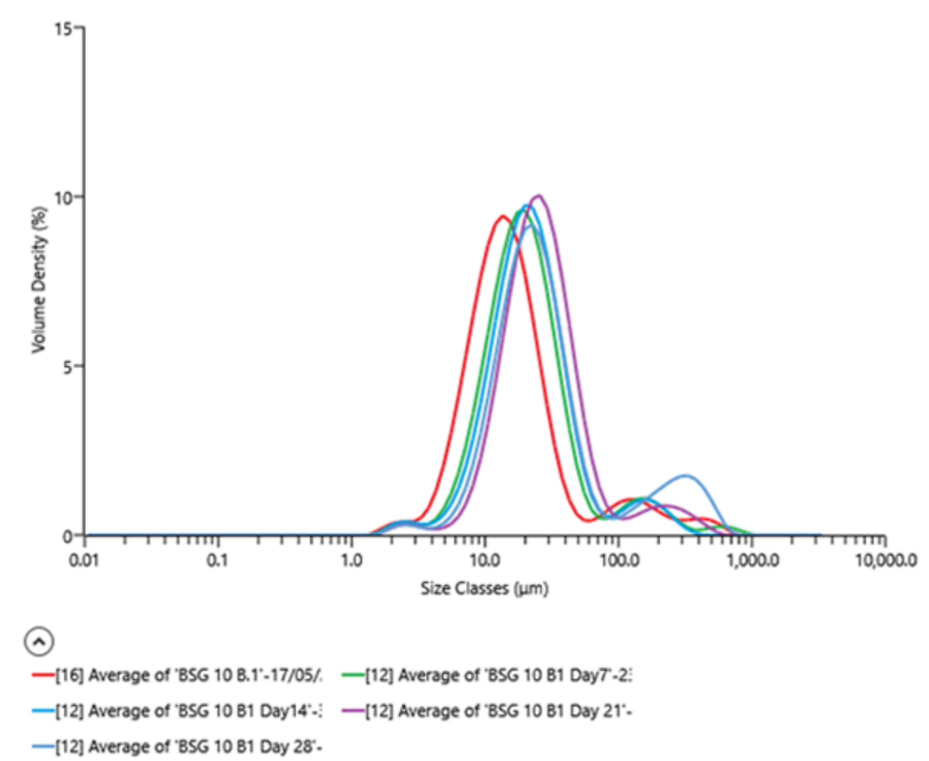
Fig. 13: volume distribution of particle size of BSG powder.

Table 7: mastersize parameters of BSG powder.

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	11	Average of 'BSG powder'	18.0	183	441
	17	Average of 'BSG powder'	17.0	174	404
Mean			**17.5**	**179**	**422**
1xStd Dev			**0.719**	**6.34**	**25.8**
1xRSD (%)			**4.11**	**3.55**	**6.10**

Then, the addition of BSG in two different amounts produced the following results: the graph (**Fig.14a**) represents yogurts containing 2% BSG powder. It can be noted that the sizes of most particles tended to increase, in fact after 28 days 90% of the particles were around 211 µm in diameter (Fig. 14b). This can be justified by the fact that BSG is able to bind more water and reduce the syneresis in yogurt (*paragraph 3.5*). However, the binding of higher amounts of water by BSG can cause a higher number of large particles in the sample.

a



b

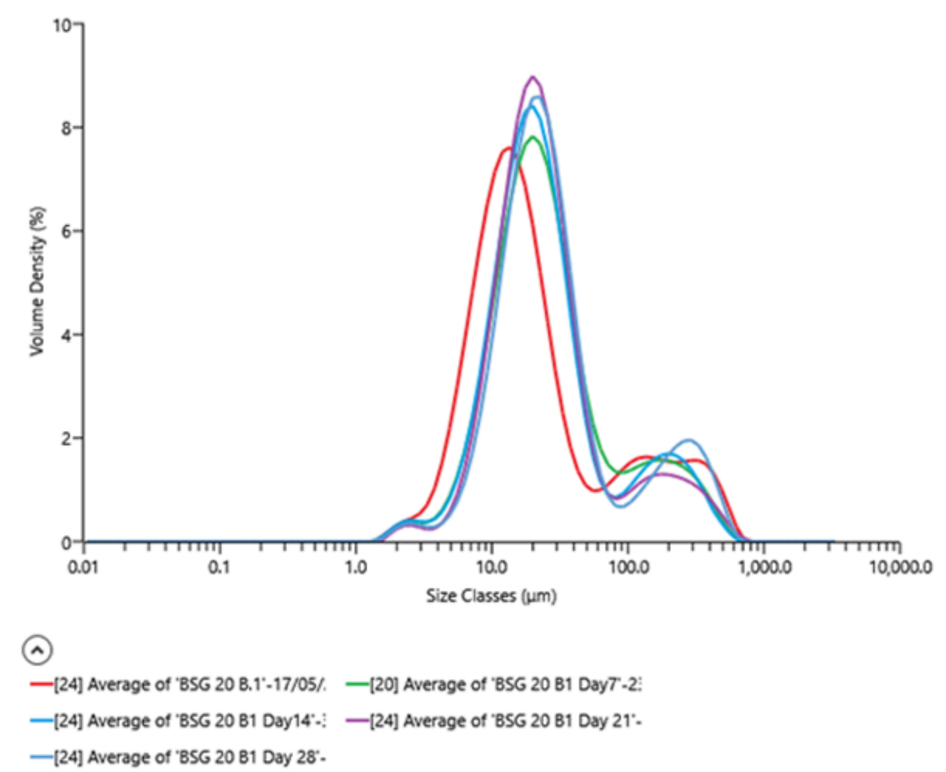


Fig.14:(a) volume distribution of particle size during 7,14,21,28 days - 2% BSG. (b)volume distribution of particle size during 7,14,21,28 days - 4% BSG.

Table 8: (a) mastersize parameters of fortified yogurt with 2% BSG. (b) mastersize parameters of fortified yogurts with 4% BSG.

a

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	16	Average of 'BSG 10 B.1'	6.39	14.4	61.8
	12	Average of 'BSG 10 B1 Day7'	8.48	19.3	55.4
	12	Average of 'BSG 10 B1 Day14'	9.15	20.9	52.5
	12	Average of 'BSG 10 B1 Day 21'	11.6	25.3	63.5
	12	Average of 'BSG 10 B1 Day 28'	10.5	24.0	211
Mean			**9.21**	**20.8**	**88.8**
1xStd Dev			**1.98**	**4.30**	**68.3**
1xRSD (%)			**21.5**	**20.7**	**76.9**

b

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	24	Average of 'BSG 20 B.1'	6.24	16.1	195
	20	Average of 'BSG 20 B1 Day7'	8.56	22.6	151
	24	Average of 'BSG 20 B1 Day14'	8.48	21.2	154
	24	Average of 'BSG 20 B1 Day 21'	9.27	21.7	133
	24	Average of 'BSG 20 B1 Day 28'	9.79	23.7	210
Mean			**8.47**	**21.1**	**169**
1xStd Dev			**1.36**	**2.92**	**32.5**
1xRSD (%)			**16.0**	**13.9**	**19.2**

3.5 Syneresis

Syneresis is a marker of the ability of the matrix to bind water. It is expressed as the amount of water phase on the top after the centrifugation of yogurt samples. As shown in Table 9, by using different yogurt cultures, there's no significant difference (p>0.05). Syneresis of non-fortified samples was around 1.5-2%.

Tab. 9: parameters to measure level of syneresis in non-fortified yogurt by using different yogurt cultures.Values are mean ± standard deviation.

weight before centrifuge (g)	weight after centrifuge (g)	weight of water (mL)	weight of container (g)	weight of yogurt (g)	Cultures	Syneresis (%)
18,23	18,05	0,18	6,62	11,61	Biomix	1,55±0,06
17,18	17,01	0,17	6,62	10,56	Biomix	1,61±0,07
18,46	18,27	0,19	6,62	11,84	YoFlex	1,60±0,11
16,63	16,38	0,25	6,62	10,01	YoFlex	2,49±0,26
16,08	15,94	0,14	6,62	9,46	Gelatin Biomix	1,48±0,3
17,55	17,35	0,2	6,62	10,93	Gelatin Biomix	1,82±0,14
18,12	17,92	0,2	6,62	11,5	Gelatin YoFlex	1,74±0,16
17,46	17,25	0,21	6,62	10,84	Gelatin YoFlex	1,94±0,25

As is shown in Table 10a, the incorporation of BSG in yogurt significantly ($p<0.05$) decreased the syneresis level. In general, the higher the amount of BSG the lower the amount of syneresis. The results show the ability of BSG in binding water thus preserving the consistency of the yogurt and its flow behaviour. However, the syneresis increased during the storage period (Tab.10b) (Supplement 3). This phenomenon shows that the ability of BSG in binding water decreased due to the refrigerated storage. Therefore, water was released from the matrix. Interestingly, the ability of BSG in preserving the syneresis of the yogurt is higher than that of non-fortified yogurts. Moreover, results show that BSG had a higher ability to maintain the stability of the yogurt. It has been indeed reported that the addition of dietary fibre in yogurt enhanced the stability of the yogurt during the storage from the perspective of syneresis[56]. By this, BSG could have acted as a stabilizer agent in yogurt products.

Tab. 10:(b) parameters to measure level of syneresis in BSG-added yogurts during storage time. (a) parameters to measure level (%) of syneresis with different amounts of BSG.Values are mean ± standard deviation.

b

weight before centrifuge (g)	weight after centrifuge (g)	weight of water (ml)	weight of container (g)	weight of yogurt (g)	BSG level (%)	Syneresis (%)	Storage time (Day)
16,09	16	0,09	6,62	9,47	0%	0,95±0,12	7
16,06	16,01	0,05	6,62	9,44	0%	0,53±0,08	
16,48	16,34	0,14	6,62	9,86	2%	1,42±0,04	
16,25	16,15	0,1	6,62	9,63	2%	1,04±0,03	

[56] Bouaziz et al., 2021; Choobari et al., 2021; Khubber et al., 2021 cit in Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., Yang, B. (2022). *Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage*

weight before centrifuge (g)	weight after centrifuge (g)	weight of water (ml)	weight of container (g)	weight of yogurt (g)	BSG level (%)	Syneresis (%)	
16,16	16,1	0,06	6,62	9,54	4%	0,63±0,2	
16,39	16,3	0,09	6,62	9,77	4%	0,92±0,11	
16,26	16,15	0,11	6,62	9,64	0%	1,14±0,02	14
16,66	16,45	0,21	6,62	10,04	0%	2,1±0,12	
16,31	16,22	0,09	6,62	9,69	2%	0,93±0,03	
16,5	16,4	0,1	6,62	9,88	2%	1,01±0,01	
16,64	16,56	0,08	6,62	10,02	4%	0,79±0,05	
16,34	16,27	0,07	6,62	9,72	4%	0,72±0,03	
16,25	16,09	0,16	6,62	9,63	0%	1,66±0,22	21
16,6	16,32	0,28	6,62	9,98	0%	2,8±0,27	
16,26	16,12	0,14	6,62	9,64	2%	1,45±0,06	
16,13	15,96	0,17	6,62	9,51	2%	1,78±0,07	
16,55	16,39	0,16	6,62	9,93	4%	1,61±0,03	
16,67	16,53	0,14	6,62	10,05	4%	1,39±0,02	
16,35	16,22	0,13	6,62	9,73	0%	1,33±0,11	28
16,27	16,15	0,12	6,62	9,65	0%	1,24±0,13	
16,39	16,29	0,1	6,62	9,77	2%	1,02±0,05	
16,48	16,3	0,18	6,62	9,86	2%	1,82±0,02	
16,34	16,12	0,22	6,62	9,72	4%	2,26±0,06	
16,39	16,18	0,21	6,62	9,77	4%	2,15±0,07	

a

weight before centrifuge (g)	weight after centrifuge (g)	weight of water (ml)	weight of container (g)	weight of yogurt (g)	BSG level (%)	Syneresis (%)
16,36	16,19	0,17	6,62	9,74	0%	1,74±0,04
16,23	16,1	0,13	6,62	9,61	0%	1,35±0,04
16,6	16,51	0,09	6,62	9,98	2%	0,90±0,02
16,69	16,6	0,09	6,62	10,07	2%	0,89±0,03
16,53	16,45	0,08	6,62	9,91	4%	0,81±0,04
16,25	16,19	0,06	6,62	9,63	4%	0,62±0,03

CHAPTER 4

4. Conclusions

BSG is a prevalent by-product generated by breweries all over the world. Considering all potential uses of BSG, its application in the food production chain is very promising. It is however still mostly limited to application as animal feed or just wasted in landfills. It can be used in food industry, or it could be a great source of bioactive compounds. This is why there is an interest in developing new products based on this brewing by-product, especially since BSG is an inexpensive substance that is consistently available in large quantities all year long.

BSG is a very promising material for the incorporation in the human diet due to its high protein and fibre content.

This by-product is a valuable source of chemicals that, when extracted, may be used as bioactive and functional components in human nutrition. It is also a valuable raw material for utilization in food products. Therefore, consuming products made from BSG can be beneficial for human health in a number of ways.

This study demonstrates the high potential of BSG as a food ingredient in soy yogurts. In fact, the incorporation of BSG in soy yogurt benefited due to its impact in increasing the fermentation rate and supported the LAB growth. BSG-added yogurt allowed the LAB growth and increased the pH which can be a benefit for industrial production because of the lower need of a sweetener to cover the acidity. Yogurt became firmer when kept in cold during the storage period. On the contrary, the hardness of the yogurt was significantly decreased by the addition of BSG. It seems that adding up to 2% of BSG will produce a result that is identical to the control (0% BSG), based on an analysis of the flow behaviour in general. An excessive amount of BSG (4%) may negatively change the yogurt's flow characteristics affecting the acceptability of yogurts. BSG increases the viscosity of the yogurt depending on the particle size of BSG added. Therefore, the presence of high dietary fibre in BSG contributed to a lower syneresis, a higher shear stress and maintenance of viscosity at certain levels of addition during the storage period. Furthermore, the addition of BSG at certain levels strengthened the survival level of LAB during the storage. A maximum substitution of 2% BSG is suggested to be more suitable in yogurt products due to its ability to preserve the flow behaviour and syneresis in addition to other physico-chemical properties. In general, syneresis decreases with increasing BSG levels. The results demonstrate BSG's capacity to bind water, maintaining yogurt's flow characteristics and consistency. Nevertheless during the storage time, the syneresis increased.

To conclude, from the results obtained, it can be noted that a soy yogurt enriched with BSG could offer better nutritional aspects than non-fortified soy yogurts also maintaining its physical characteristics.

Further study on chemical and sensory analysis, together with the nutritional and nutraceutical characterization of BSG-added yogurt are needed.

Appendix

Supplement 1: Two way ANOVA analysis (main parameters BSG_level and Storage time) of pH results.

Test di effetti tra soggetti

Variabile dipendente: pH

Origine	Somma dei quadrati di tipo III	df	Media quadratica	F	Sig.
Modello corretto	,369[a]	14	,026	5,588	<,001
Intercetta	1743,104	1	1743,104	369162,666	<,001
BSG_level	,019	2	,009	1,995	,143
Storage_time	,343	4	,086	18,151	<,001
BSG_level * Storage_time	,008	8	,001	,205	,989
Errore	,354	75	,005		
Totale	1743,828	90			
Totale corretto	,724	89			

a. R-quadrato = ,511 (R-quadrato adattato = ,419)

Supplement 2: Two way ANOVA analysis (main parameters BSG_level and Storage time) of hardness results.

Test di effetti tra soggetti

Variabile dipendente: Hardness

Origine	Somma dei quadrati di tipo III	df	Media quadratica	F	Sig.
Modello corretto	,182[a]	14	,013	1,155	,327
Intercetta	11,557	1	11,557	1026,756	<,001
BSG_level	,068	2	,034	2,999	,056
Storage_time	,068	4	,017	1,505	,209
BSG_level * Storage_time	,047	8	,006	,519	,839
Errore	,844	75	,011		
Totale	12,583	90			
Totale corretto	1,026	89			

a. R-quadrato = ,177 (R-quadrato adattato = ,024)

Supplement 3: Two way ANOVA analysis (main parameters BSG_level and Storage time) of syneresis results.

Test di effetti tra soggetti

Variabile dipendente: Syneresis

Origine	Somma dei quadrati di tipo III	df	Media quadratica	F	Sig.
Modello corretto	7,059[a]	14	,504	2,490	,006
Intercetta	172,345	1	172,345	851,176	<,001
BSG_level	1,664	2	,832	4,108	,020
Storage_time	3,005	4	,751	3,710	,008
BSG_level * Storage_time	2,391	8	,299	1,476	,180
Errore	15,186	75	,202		
Totale	194,590	90			
Totale corretto	22,245	89			

a. R-quadrato = ,317 (R-quadrato adattato = ,190)

References

1. Bedani, R., Vieira, A. D. S., Rossi, E. A., & Saad, S. M. I. (2014). Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in synbiotic soy yoghurt with okara during storage. *LWT - Food Science and Technology*, *55*(2), 436–443. https://doi.org/10.1016/j.lwt.2013.10.015
2. Boukid, F., Lamri, M., Dar, B. N., Garron, M., & Castellari, M. (2021). Vegan Alternatives to Processed Cheese and Yogurt Launched in the European Market during 2020: A Nutritional Challenge? *Foods*, *10*(11), 2782. https://doi.org/10.3390/foods10112782
3. CHENG, Y. J., THOMPSON, L. D., & BRITTIN, H. C. (1990). Sogurt, a Yogurt‐like Soybean Product: Development and Properties. *Journal of Food Science*, *55*(4), 1178–1179. https://doi.org/10.1111/j.1365-2621.1990.tb01631.x
4. Gantumur, M.-A., Sukhbaatar, N., Jiang, Q., Enkhtuya, E., Hu, J., Gao, C., … Li, A. (2024). Effect of modified fermented whey protein fortification on the functional, physical, microstructural, and sensory properties of low-fat yogurt. *Food Control*, *155*, 110032. https://doi.org/10.1016/j.foodcont.2023.110032
5. Han, J., Fitzpatrick, J., Cronin, K., Maidannyk, V., & Miao, S. (2021). Particle size, powder properties and the breakage behaviour of infant milk formula. *Journal of Food Engineering*, *292*, 110367. https://doi.org/10.1016/j.jfoodeng.2020.110367
6. Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568. https://doi.org/10.1002/jib.363

7. McCann, T. H., Fabre, F., & Day, L. (2011). Microstructure, rheology and storage stability of low-fat yoghurt structured by carrot cell wall particles. *Food Research International*, *44*(4), 884–892. https://doi.org/10.1016/j.foodres.2011.01.045
8. Mussatto, S. I. (2014). Brewer's spent grain: a valuable feedstock for industrial applications. *Journal of the Science of Food and Agriculture*, *94*(7), 1264–1275. https://doi.org/10.1002/jsfa.6486
9. Naibaho, J., Butula, N., Jonuzi, E., Korzeniowska, M., Laaksonen, O., Föste, M., … Yang, B. (2022). Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage. *Food Hydrocolloids*, *125*, 107412. https://doi.org/10.1016/j.foodhyd.2021.107412
10. Naibaho, J., Jonuzi, E., Butula, N., Figiel, A., Yang, B., Föste, M., … Korzeniowska, M. (2021). Valorisation of brewers' spent grain in different particle size in yogurt production. *E3S Web of Conferences*, *332*, 01008. https://doi.org/10.1051/e3sconf/202133201008
11. Olivares-Galván, S., Marina, M. L., & García, M. C. (2022). Extraction of valuable compounds from brewing residues: Malt rootlets, spent hops, and spent yeast. *Trends in Food Science & Technology*, *127*, 181–197. https://doi.org/10.1016/j.tifs.2022.06.002
12. Osundahuns, O. F., Amosu, D., & Ifesan, B. O. T. (2007). Quality Evaluation and Acceptability of Soy-yoghurt with Different Colours and Fruit Flavours. *American Journal of Food Technology*, *2*(4), 273–280. https://doi.org/10.3923/ajft.2007.273.280

13. Pabbathi, N. P. P., Velidandi, A., Pogula, S., Gandam, P. K., Baadhe, R. R., Sharma, M., … Gupta, V. K. (2022). Brewer's spent grains-based biorefineries: A critical review. *Fuel*, *317*, 123435. https://doi.org/10.1016/j.fuel.2022.123435

14. Peng, X., & Guo, S. (2015). Texture characteristics of soymilk gels formed by lactic fermentation: A comparison of soymilk prepared by blanching soybeans under different temperatures. *Food Hydrocolloids*, *43*, 58–65. https://doi.org/10.1016/j.foodhyd.2014.04.034

15. Saavedra Isusi, G. I., Marburger, J., Lohner, N., & van der Schaaf, U. S. (2023). Texturing of Soy Yoghurt Alternatives: Pectin Microgel Particles Serve as Inactive Fillers and Weaken the Soy Protein Gel Structure. *Gels*, *9*(6), 473. https://doi.org/10.3390/gels9060473

16. Wu, J., Dai, T., Lin, R., Niu, J., Li, Z., Chang, Z., … Gao, H. (2023). Effect of protein-glutaminase on the texture, rheology, microstructure and sensory properties of skimmed set-type yoghurt. *Food Chemistry*, *429*, 136831. https://doi.org/10.1016/j.foodchem.2023.136831

17. Zhu, H., Chen, Z., Li, G., Yao, X., Hu, Y., & Zhao, W. (2023). Physicochemical, sensory, and antioxidant characteristics of stirred‐type yogurt enriched with *Lentinula edodes* stipe powder. *Food Science & Nutrition*, *11*(10), 6231–6240. https://doi.org/10.1002/fsn3.3563