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THE EFFECT OF HIGH-PRESSURE HOMOGENIZATION ON THE TEXTURE OF FERMENTED BEAN-BASED BEVERAGES®

Wpływ homogenizacji wysokociśnieniowej na teksturę fermentowanych napojów z fasoli®

Key words: plant-based beverages, bean-based beverages, milk substitutes, high-pressure homogenization, physical properties of food, texture.

Various types of technological treatments, including high-pressure homogenization (HPH), can be used to obtain plant-based yogurts. It is one of the technologies with a positive impact on food particles which leads to improvement of quality, shelf life, and popularity of the product. The aim of the study was to analyze the effect of HPH on the texture of fermented bean-based beverages. Selected texture discriminants (hardness, adhesiveness) for beverages prepared from germinated and non-germinated beans, subjected to and not subjected to HPH were analyzed. HPH bean-based beverages were lower pH values, before fermentation, after fermentation, and after 21 days of storage, which indicates that HPH results in a more efficient fermentation for bean-based beverages. In all the tested samples HPH significantly increased the hardness and adhesiveness of tested beverages. The values obtained for the tested texture determinants reached a similar level for the samples before fermentation, after fermentation, and after 21 days of storage, which indicates that the HPH was responsible for the texture shaping of the tested bean-based beverages. There is a need for further research into the physical properties of bean-based milk yogurt substitutes produced using HPH.

Słowa kluczowe: napoje roślinne, napój fasolowy, substytuty mleka, homogenizacja wysokociśnieniowa, właściwości fizyczne żywności, tekstura.

Różnego rodzaju zabiegi technologiczne, w tym homogenizacja wysokociśnieniowa (HPH), mogą być wykorzystane do produkcji jogurtów roślinnych. Jest to jedna z technologii pozytywnie wpływających na cząstki w żywności, która prowadzi do poprawy jakości, trwałości i popularności produktu. Celem pracy była analiza wpływu HPH na teksturę fermentowanych napojów na bazie fasoli. Analizie poddano wybrane wyróżniki tekstury (twardość, adhezyjność) napojów przygotowanych z fasoli skiełkowanej i nieskiełkowanej, poddanych i niepoddanych działaniu HPH. Napoje na bazie fasoli poddane HPH miały niższe wartości pH przed fermentacją, po fermentacji i po 21 dniach przechowywania, co wskazuje, że zastosowanie HPH skutkuje bardziej wydajnym procesem fermentacji napojów na bazie fasoli. We wszystkich badanych próbkach HPH istotnie zwiększyła twardość i adhezyjność napojów. Wartości uzyskane dla badanych wyznaczników tekstury osiągnęły podobny poziom dla próbek przed fermentacją, po fermentacji i po 21 dniach przechowywania, co wskazuje, że HPH odpowiadała za kształtowanie tekstury badanych napojów. Istnieje potrzeba dalszych badań nad właściwościami fizycznymi substytutów jogurtu mlecznego na bazie fasoli wytwarzanych z wykorzystaniem HPH.

INTRODUCTION

Sensory quality is one of the most important factors that consumers consider in their food choices. The sensory quality of food depends on many factors, including e.g. smell, taste, and texture [17]. Food texture is defined as all the rheological and structural attributes of the product perceptible using mechanical, tactile, and where appropriate, visual, and auditory receptors [7]. The texture of the product is of great importance in all food sectors, including the dairy industry. One of the most consumed dairy products worldwide is fermented milk and yogurts. Multiple researchers are concerned their texture and the possibility of its modification [8, 13].

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The texture of yogurt-type products is mainly influenced by the technological processes used during their production. The texture of milk yoghurt is mainly shaped by the fermentation process. During fermentation, lactic acid bacteria convert the lactose present in the milk into lactic acid, which results in the formation of a curd [2, 27]. The most frequently studied texture parameters of yogurts are hardness and adhesiveness. Hardness was defined as the necessary force to attain a given deformation. This factor is a critical texture property for yogurt-like products. Adhesiveness is the required work for prevailing attraction force between foodstuff surface and various substances coming into contact with them. It is the force required to separate the material that sticks to the teeth during eating [21].

Due to the growing consumer awareness related to intensified animal production, the popularity of plant-based diets is increasing. As a result, there is a growing demand for various types of alternatives to animal products, including dairy products [11, 15]. The size of the global dairy alternatives market has been estimated at $ 20.50 billion in 2020 and the compound annual growth rate (CAGR) is expected to be 12.5% in 2021–2028 [5]. The most popular alternative products to dairy products are plant-based beverages, which are mainly treated as milk substitutes [1]. They are produced from various raw materials, including e.g. cereals, legumes, nuts, and seeds [22]. With the growing popularity of dairy alternatives, the demand for fermented plant products containing probiotics is increasing [24].

The most popular plant-based beverages are soybean-based beverages, but also other legumes (e.g. beans, peas, chickpeas) can be a suitable matrix for their production [22]. Beans come in many varieties (e.g. white, red, adzuki, and mung beans), but all of them are characterized by a high protein content, which is two to three times higher than in cereal grains. In addition, beans contain large amounts of dietary fiber, starch, vitamins, and minerals, as well as a wide range of phytochemicals [3]. Bean-based beverages are not produced on a large scale but have been successfully produced under laboratory conditions [30].

Plant-based beverages can be used to make plant-based yoghurt substitutes [9]. Plant-based products should be similar to conventional yogurt in terms of textural and sensory properties and the ability to host viable lactic acid bacteria for long-time storage. Fermentation applied to plant-based matrices has been identified as a natural and effective biotechnological option to increase their technological, sensory, nutritional, and functional properties [20]. Various types of technological treatments, including high-pressure homogenization (HPH), can also be used to obtain plant-based yogurts. [14]. Homogenization is the ability to produce a homogeneous size distribution of particles suspended in a liquid, by forcing the liquid under the effect of pressure through a specifically designed homogenization valve. Homogenizers process fluid matrices at a pressure ranging between 20–100 MPa, which allows to reduction particle size and consequently increase the stability of emulsions [23]. It is one of the technologies with a positive impact on food particles that leads to improvement of quality, shelf life, and popularity of the product, which is considered a suitable alternative to thermal processes due to the lack of thermal damage [16].

The available literature lacks research on the texture of yogurt-type bean-based beverages and the factors influencing it. Therefore, the aim of the study was to analyze the effect of HPH on the texture of fermented bean-based beverages. Selected texture discriminants (hardness, adhesiveness) for beverages prepared from germinated and non-germinated beans, subjected to and not subjected to HPH were analyzed. The tests were performed for beverages before fermentation, after fermentation, and after 21 days of refrigerated storage (6°C).

MATERIALS AND METHODS

Preparation of bean-based beverages

The bean-based beverages were prepared from white kidney beans "Piękny Jaś Karłowy" (Lestello Sp. z o.o., Poland). The beverages were made in two variants - from germinated and non-germinated beans. The germination was carried out in a sprouter at 25°C for 72 hours (with changing the water every 24 hours). Germinated and non-germinated beans were sterilized at 121°C for 15 minutes, mixed with drinking water in a ratio of 1:9 (m/m), and blended until a homogeneous mass was obtained. The resulting mass was filtered through a sieve with a mesh size of 0.1 mm. The obtained beverages were sterilized at 121° C for 15 minutes. Before final sterilization, half of the obtained beverages were subjected to high-pressure homogenization with NS 1001 L2 PANDA, GEA Niro Soavi (GEA, Italy) at a pressure of 50 MPa.

Fermentation of bean-based beverages

Three industrial freeze-dried starter cultures were used in the study, including:

- Beaugel Soja 1 (Ets Coquard, France), containing *Lactobacillus casei* (currently classified as *Lacticaseibacillus casei*), *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*;
- YO-MIX 207 LYO 500 DCU (DuPont™ Danisco, Denmark), containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*;
- ABY-3 (Chr. Hansen, Denmark), containing *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* BB-12, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

The inoculums were prepared by dissolving the freeze-dried starter cultures in distilled water. The beverages samples were inoculated at 1.0% (m/m) and incubated at 45°C for 6 hours. After the fermentation was completed, the beverages were refrigerated at 6°C and stored for 21 days.

Active acidity and microflora analysis

The analysis of active acidity and microflora was performed before the fermentation, after the fermentation, and after 21 days of storage under refrigeration conditions (6°C). The active acidity was determined by measuring the pH using a CPO-505 pH meter (Elmetron, Poland). Measurements were made in triplicate for each sample.

Texture analysis

The texture analysis of bean-based beverages included the determination of hardness and adhesiveness. The test was carried out for beverages before fermentation, after fermentation, and after 21 days of storage at 6°C, for 100 ml samples. The tests were carried out using a Brookfield CT3 10K texturometer (AMETEK Brookfield, USA) with a TA4 / 1000 cylindrical probe with a diameter of 38.1 mm and a height of 20 mm. A pressure force of 0.04 N was applied during the experiment. The probe used was moved at a speed of 2 mm/s towards the inside of the test and 4.5 mm/s in the opposite direction during withdrawal from the test. Hardness was expressed in N units and cohesiveness in mJ. The tests were carried out in 3 replications, with single penetration. The results were analyzed using the TexturePro CT V1.4 Build 17 software included with the measurement kit.

Data analysis

The results of the study were subjected to one-way analysis of variance (ANOVA), using the software Statistica 13.1 (StatSoft, Poland). It allowed determining the effect of HPH on the studied texture properties of bean-based beverages. The significance of the differences was analyzed by Tukey's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Plant-based beverages are mainly treated as substitutes for dairy products. It is desirable to use technological processes that will allow to obtain plant-based products resembling their dairy analogs. Plant-based beverages usually differ in composition and sensory quality from milk; however, they can be fermented to produce dairy-free yogurt-type products while rendering the raw material into a more palatable form [15]. During the production of the tested bean-based beverages, fermentation and HPH were used to modify the quality of the final products.

The indicator of the progress of the fermentation is the pH of the product, which shows the level of active acidity. The acidity of fermented plant-based yogurts largely determines their taste, contributes to control microflora growth, and should be similar to the acidity of fermented commercial milk-based and plant-based yogurts [9, 28]. For most of the bean-based beverages samples tested, significant differences were observed in the pH value for homogenized and non-homogenized, germinated, and non-germinated beverages (Table 1). HPH bean-based beverages have lower pH values, both before and after the fermentation, and also after 21 days of storage. According to Mojka [19], the optimal pH for fermented milk beverages is in the range of 4.0 – 4.5. In the tested non-homogenized bean-based beverages, pH values close to 4.5 were achieved only in samples fermented with

Table 1. Changes in the active acidity in the high-pressure homogenized (HPH) and non-high-pressure homogenized (non-HPH) bean-based beverages. Values are presented as mean and standard deviation (± SD)

Tabela 1. Zmiany kwasowości czynnej w homogenizowanych (HPH) i niehomogenizowanych (non-HPH) wysokociśnieniowo napojach fasolowych. Wartości przedstawione są jako średnia i odchylenie standardowe (± SD)

Type of bean-based beverages	Time of the measurement	Starter cultures	pH	
			non-HPH beverages	HPH beverages
Non-germinated	before fermentation	-	6.25 ± 0.07 a	5.84 ± 0.14 b
	after fermentation	Beaugel Soja 1	4.95 ± 0.12 a	4.86 ± 0.12 a
		YO-MIX 207	4.84 ± 0.04 a	4.47 ± 0.11 b
		ABY-3	4.53 ± 0.11 a	4.15 ± 0.04 b
	after 21 days of storage	Beaugel Soja 1	4.94 ± 0.09 a	4.22 ± 0.05 b
		YO-MIX 207	4.77 ± 0.06 a	4.21 ± 0.12 b
		ABY-3	4.56 ± 0.05 a	4.14 ± 0.11 b
Germinated	before fermentation	-	6.37 ± 0.10 a	6.44 ± 0.12 a
	after fermentation	Beaugel Soja 1	4.56 ± 0.03 a	4.40 ± 0.10 a
		YO-MIX 207	4.86 ± 0.02 a	4.26 ± 0.06 b
		ABY-3	4.50 ± 0.09 a	4.12 ± 0.10 b
	after 21 days of storage	Beaugel Soja 1	4.76 ± 0.08 a	4.43 ± 0.11 b
		YO-MIX 207	4.73 ± 0.05 a	4.36 ± 0.04 b
		ABY-3	4.44 ± 0.04 a	4.24 ± 0.07 b

a, b – Within each row, the means values with the same letter do not differ significantly ($p \geq 0.05$).
a, b – W każdym wierszu średnie z taką samą literą nie różnią się istotnie ($p \geq 0,05$).

Source: The own study
Źródło: Badania własne

the ABY-3 starter culture, containing *L. acidophilus* La-5, *B. animalis* subsp. *lactis* BB-12, *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*. In homogenized beverages, pH values in the range of 4.0 – 4.5 were observed for all samples of germinated beverages and the majority of non-germinated samples after the fermentation and after 21 days of storage. The higher value (4.86) was shown only for the sample of non-germinated beverage, fermented with the starter culture Beaugel Soja 1, containing *L. casei*, *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus.* This indicates that HPH results in a more efficient fermentation for bean-based beverages. The reason may be the increased availability of sugars constituting a substrate for the production of lactic acid during fermentation in beverages subjected to HPH.

When analyzing selected texture discriminants of the tested bean-based beverages, it was found that in all the tested samples, the HPH process significantly increased hardness and adhesiveness, both in germinated and non-germinated beverages (Figure 1, Figure 2). Non-homogenized bean-based beverages were characterized by a low level of hardness (in the range of 0.2 – 0.3 N) and adhesiveness (in the range of 0.1 – 1.0 mJ). In all tested non-homogenized samples, these values did not change significantly after the fermentation process, and after the 21-days of storage. Homogenized bean-based beverages were characterized by a significantly higher level of hardness (in the range of 1.2 – 1.8 N) and adhesiveness (in the range of 11.8 – 15.4 mJ). The texture discriminants tested remained high in the samples, after fermentation, and after 21 days of storage. This may indicate that the HPH was mainly responsible for the texture shaping of the tested beverages. Fermentation did not affect the shaping of the texture but caused only a significant decrease in the pH value. Different results are obtained by the authors for milk yogurts in which the fermentation is sufficient to shape the appropriate texture of the products [6, 12, 29]. This may be due to the different structure of plant proteins and milk proteins, which affects the rate and mechanisms of the gelling process.

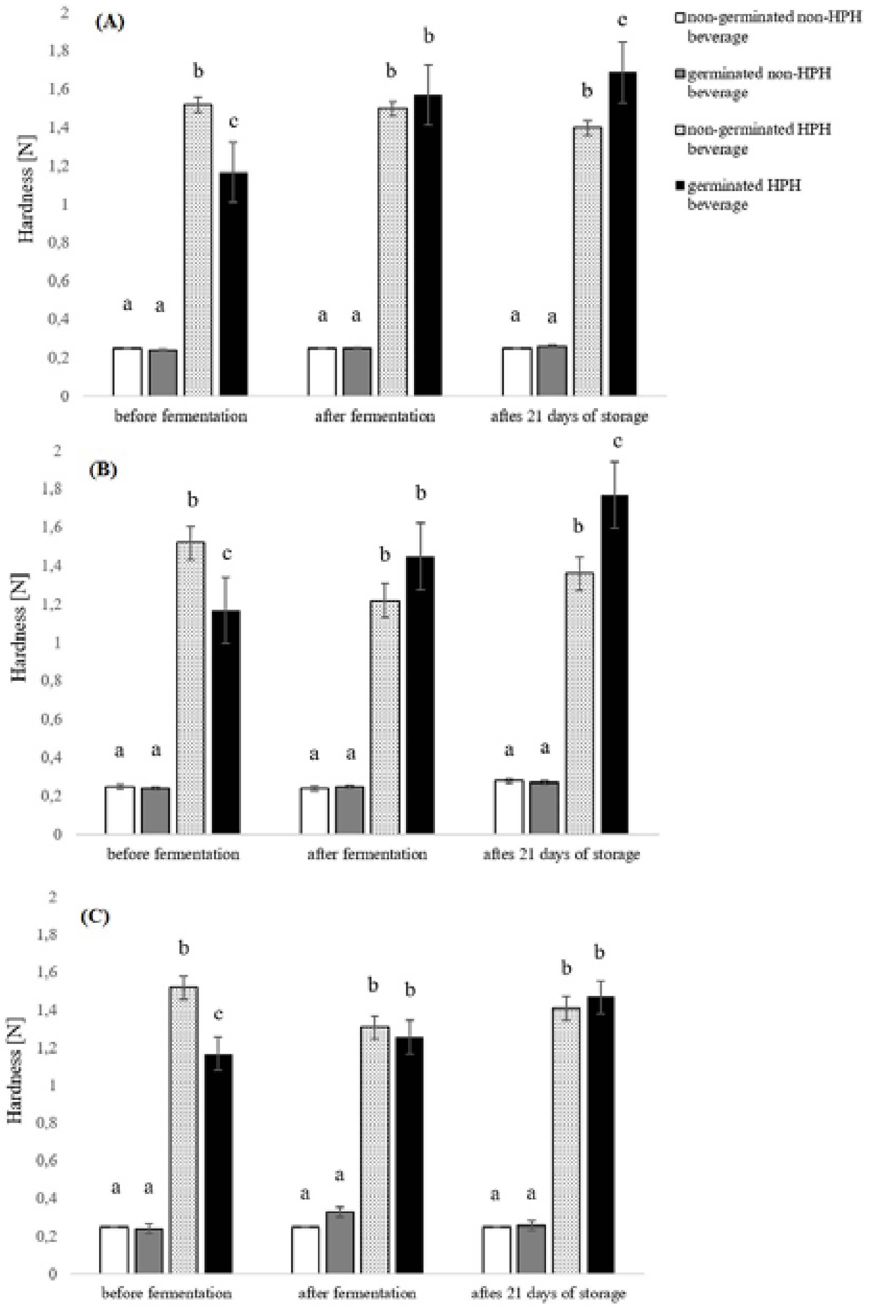

Fig. 1. The effect of HPH, germination and storage on hardness of bean-based beverages fermented using the Beaugel Soja 1 (A), YO-MIX 207 (B), ABY-3 (C) starter cultures.

Rys. 1. Wpływ HPH, kiełkowania i przechowywania na twardość napojów fasolowych fermentowanych z wykorzystaniem kultur starterowych Beaugel Soja 1 (A), YO-MIX 207 (B), ABY-3 (C).

a, b, c – Within each measuring period means with a common symbol are not significantly different ($p \geq 0.05$). Error bars represent standard error of the mean.

a, b, c – W ramach każdego okresu pomiarowego średnie z takim samym symbolem nie różnią się istotnie ($p \geq 0,05$). Słupki błędów reprezentują błąd standardowy średniej.

Source: The own study

Źródło: Badania własne

There was no unidirectional tendency for the influence of the germination process on the hardness and adhesiveness of the bean-based beverages tested. The changes in these parameters were mainly influenced by the HPH. Within the homogenized beverages, the changes in the values of the studied texture determinants were statistically insignificant or not greater than the decrease or increase in the range of 0.41 N for hardness and 3.4 mJ for adhesiveness.

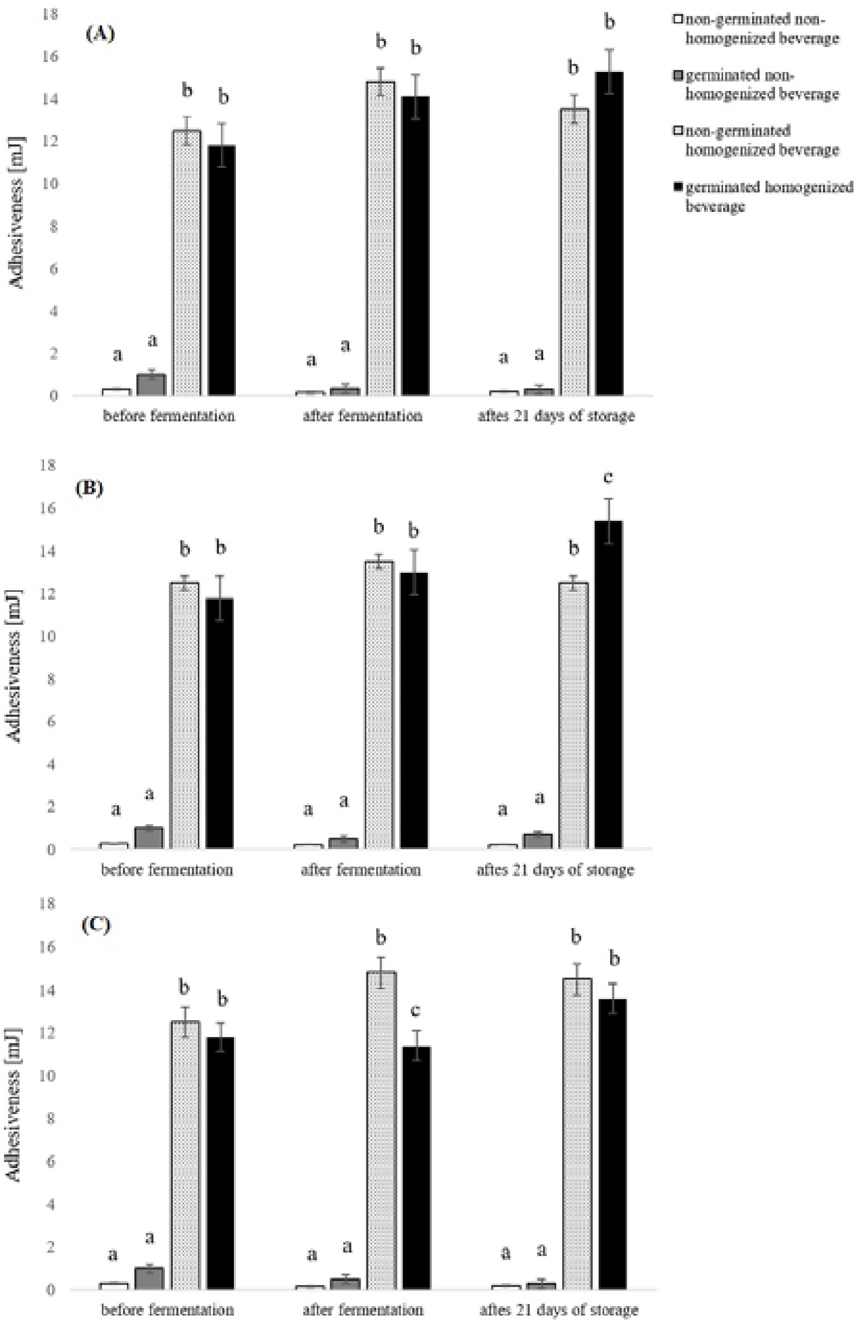

Fig. 2. The effect of HPH, germination and storage on adhesiveness of bean-based beverages fermented using the Beaugel Soja 1 (A), YO-MIX 207 (B), ABY-3 (C) starter cultures.

Rys. 2. Wpływ HPH, kiełkowania i przechowywania na adhezyjność napojów fasolowych fermentowanych z wykorzystaniem kultury starterowej Beaugel Soja 1 (A), YO-MIX 207 (B), ABY-3 (C).

a, b, c – Within each measuring period means with a common symbol are not significantly different ($p \geq 0.05$). Error bars represent standard error of the mean.

a, b, c – W ramach każdego okresu pomiarowego średnie z takim samym symbolem nie różnią się istotnie ($p \geq 0,05$). Słupki błędów reprezentują błąd standardowy średniej.

Source: The own study

Źródło: Badania własne

The available literature lacks research on the effect of HPH on the acidity and texture of fermented bean-based beverages. The use of this process in the production of other types of plant-based milk analogs has also not been extensively researched. Levy et al. [14] investigated the effect of HPH on the properties of a dairy-free yogurt-like fermented product made from potato protein isolate (PPI). PPI emulsions were homogenized at various pressures (0.1 MPa, 330–200 MPa), and inoculated (10 g/L) with LAB starter culture. In the HPH dairy-free yogurt samples tested, similar to the present test, a decrease in pH to about 4.5 was observed after 5-6 hours of fermentation at 37°C. The tested parameters of the texture (hardness, adhesiveness, and cohesiveness) showed that the 200 MPa yogurt alternative has the highest hardness (1.2 N). The mean adhesiveness in the samples tested was 2.5 N/mm and there was no difference between the samples treated with different pressure levels. In this study, a pressure of 50 MPa was used for the bean-based beverages samples and higher hardness values in the range of 1.2–1.8 N were achieved. The textural attributes may be related to the raw material used for the production of plant-based yogurts and gel microstructure differences.

Mei et al. [18] studied the effectiveness of different homogeneous methods on textural characteristics of soybean yogurt. One of the homogeneous methods used was HPH at a pressure of 100 MPa. Compared to the control samples, the yogurts subjected to HPH showed lower hardness and adhesiveness. Different results were obtained by Cruz et al. [4], who studied the effect of various parameters of ultra high-pressure homogenization (UHPH) (200–300 MPa, and 40–50°C) on the firmness (also defined as hardness) of a soy-yogurt product. There was a tendency to increase gel firmness as a combination of pressure and temperature increase, except in the more severe UHPH treatment (300 MPa, 50°C), in which this tendency was broken. In all samples subjected to homogenization, regardless of the parameters used, a significant increase in firmness was observed (values in the range of about 2.5 – 5.5 N were obtained) compared to the control sample. Differences between the results of other authors and the results obtained for bean-based beverages may result from differences in raw materials, the use of other technological processes, homogenization parameters, and the types of homogenizers.

The use of HPH in the production of milk yoghurts has been investigated to a much greater extent. It has been shown that this process can improve the quality, increase shelf life, and maintain the nutritional and sensory properties of milk and dairy products [16]. Serra et al. [25] investigated the effect of UHPH of 200 and 300 MPa on the firmness of set yogurt. The use of UHPH allowed to obtain yoghurts with high firmness (1.83 N for a pressure of 200 MPa, and 1.99 N for a pressure of 300 MPa), and during the 28-day storage period, these values did not change significantly or increased. In the tested fermented bean-based beverages, similar hardness values were obtained despite the use of lower pressure values (50 MPa) during homogenization.

In studies on milk yoghurts, HPH influences the reduction of milk particle size which leads to the formation of finer dispersions than those obtained by conventional homogenization combined with heat treatment. In addition, the density of the gel, aggregation rate, and water retention can be improved [6, 10, 26]. There is a need to expand research on bean-based yogurt-type milk substitutes to determine the precise effects of HPH on the physical properties of the system, including, for example, particle size, gelling, and water retention.

SUMMARY AND CONSLUSIONS

The high-pressure homogenization (HPH) can be successfully used to produce bean-based yogurt-type milk substitutes. In this study, the use of this technological process positively influenced the acidity and texture properties of the obtained products. The use of HPH significantly influenced the obtaining of the optimal pH for yoghurts (in the range of 4.0 – 4.5), both in beverages made from germinated and non-germinated beans. These values did not change significantly during 21 days of storage. These results indicate that HPH allowed for more efficient fermentation of the tested plant matrix. This may be the result of the increased availability of sugars, which are used during fermentation by lactic acid bacteria as a substrate for the production of lactic acid.

HPH also influenced the tested bean-based beverage texture discriminants. In all the tested samples, this process significantly increased the hardness and adhesiveness, both in beverages made from germinated and non-germinated beans. Moreover, the values obtained for the tested texture determinants reached a similar level for the samples before fermentation, after fermentation, and after 21 days of storage. This indicates that the HPH was responsible for the texture shaping of the tested bean-based beverages. The fermentation did not significantly affect the tested texture determinants, but only caused a significant decrease in pH in all fermented samples.

The available studies on the influence of HPH on the fermentation and physical properties of fermented milk substitutes are limited. This process can largely influence the unique features of this type of product and lead to the production of plant-based yoghurt substitutes with properties that meet the growing demands of consumers. Accordingly, there is a need for further research into the physical properties of bean-based yogurt substitutes produced by HPH.

PODSUMOWANIE I WNIOSKI

Proces homogenizacji wysokociśnieniowej (HPH – ang. high-pressure homogenization) może zostać z powodzeniem wykorzystany do wytwarzania roślinnych substytutów jogurtów na bazie fasoli. W niniejszym badaniu zastosowanie tego procesu technologicznego pozytywnie wpłynęło na kwasowość otrzymanych produktów oraz badane wyróżniki tekstury. Zastosowanie HPH wpłynęło istotnie na uzyskanie optymalnego pH (w zakresie 4.0–4.5) dla jogurtów, zarówno w napojach wytworzonych z fasoli skiełkowanej, jak i nieskiełkowanej. Wartości te nie zmieniły się istotnie w trakcie 21-dniowego okresu przechowywania. Otrzymane wyniki wskazują, że HPH umożliwiła bardziej efektywną fermentację badanej matrycy roślinnej. Może to być wynikiem zwiększonej dostępności cukrów, które w trakcie fermentacji wykorzystywane są przez bakterie mlekowe jako substrat do produkcji kwasu mlekowego.

Proces HPH wpłynął również na badane wyróżniki tekstury napojów fasolowych. We wszystkich badanych próbkach proces ten wpłynął na istotne zwiększenie twardości i adhezyjności, zarówno w napojach wytworzonych z fasoli skiełkowanej, jak i nieskiełkowanej. Ponadto wartości badanych wyróżników tekstury osiągnęły podobny poziom dla próbek przed fermentacją, po fermentacji i po 21 dniach okresu przechowywania. Wskazuje to, że proces HPH miał wpływ na kształtowanie tekstury badanych napojów fasolowych. Proces fermentacji nie wpłynął istotnie na badane wyróżniki tekstury, a jedynie spowodował istotne obniżenie pH we wszystkich fermentowanych próbkach.

Dostępne badania dotyczące wpływu HPH na przebieg procesu fermentacji i właściwości fizyczne fermentowanych substytutów mleka są dość ograniczone. Proces ten może w szerokim zakresie wpływać na kształtowanie się unikatowych cech tego typu produktów i prowadzić do wytworzenia substytutów jogurtów o właściwościach, które sprostają rosnącym wymaganiom konsumentów. W związku z tym istnieje potrzeba prowadzenia dalszych badań dotyczących właściwości fizycznych substytutów jogurtów na bazie fasoli, wytwarzanych z zastosowaniem HPH.

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