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# Functional Fermented Beverage Prepared from Germinated White Kidney Beans (*Phaseolus vulgaris* L.)

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## Abstract

The current demand for plant-based food indicates that the food market is providing alternatives for products that are currently commercially available. This chapter discusses the possible use of germinated bean seeds as a raw material in the production of substitutes for dairy products, including fermented ones. Beans are a valuable source of easily digestible protein, carbohydrates, minerals, and various vitamins (e.g., B vitamin group). They also contain significant amounts of fiber which affects the proper functioning of the digestive system and antioxidant compounds. The fat content is low and is estimated to be around only 1–2%. However, it is mainly (about 70%) constituted by unsaturated fatty acids, including the polyunsaturated ones such as linoleic acid or linolenic acid, which are desirable in the human diet for the prevention of cardiovascular diseases or cancer. Biological processes such as germination or fermentation may improve the nutritional value of bean seeds (by increasing the content, digestibility, and bioavailability of some nutrients and by eliminating undesirable components) and deliver live cells of prohealth bacteria (lactic acid bacteria, propionic acid bacteria, or bifidobacteria).

**Keywords:** bean-based beverages, white kidney beans, germination, fermentation, lactic acid bacteria, bifidobacteria

## 1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an annual angiosperm belonging to the Fabaceae family. It originates from Central and South America, where it was cultivated as early as 7000 years ago. Due to their ability to self-pollinate, beans can produce seeds after pollination with pollen from their own flower [1]. There are about 200 species identified so far, of which the first known were green pods with dark seeds. Based on the evolutionary rate, organisms of the genus *Phaseolus* are estimated to be about six million years old, which suggests that it is a relatively young group of plants [2, 3]. Currently, most varieties of beans are cultivated for food, on all continents and in various climatic zones [1].

Bean varieties are classified into two main groups: dwarf and tic. In the case of dwarf varieties, a poorly developed stem reaches 60 cm, whereas in climbing varieties, the stem can grow up to 200–300 cm. Features such as shape, color, and size of the pods are related to the variety. There is a relationship between the shape of the seeds and the shape of the pod. Elongated and cylindrical seeds are found in

round and long pods, while flattened seeds are found in flat pods. The bean fruit is an elongated pod, which varies in color, shape, and fiber content, depending on the variety.

Beans are one of the most important plants that are directly consumed in the world. Due to their nutritional and health benefits, they are used in many dishes and are also consumed by people following vegan and vegetarian diets as a valuable source of vegetable protein. In some regions, such as South and Central American and African countries, beans are a staple in the daily diet and usually consumed after soaking and cooking.

Consuming the seeds of legumes, which include beans, can result in many physiological and health benefits, including the prevention of cardiovascular disease, diabetes, and cancer. Beans are high-fiber, high-protein vegetables that contain a less amount of fats. They are valuable sources of not only easily digestible protein but also minerals and various vitamins (e.g., B vitamins). Furthermore, they contain a wide range of phytochemical and antioxidant compounds, including flavonoids, such as anthocyanins, flavonols, phenolic acids, and isoflavones, which are compounds regulating the expression of genes responsible for the processes of  $\beta$ -fat oxidation, lithogenesis, and hepatic gluconeogenesis. Beans are also rich in oligosaccharides, lectins, saponins, phytates, and polyphenolic compounds, the main classes of which are tannins, phenolic acids, and flavonoids. Phenolic extracts from various types of beans exhibit antioxidant properties [4]. The polyphenolic components present in beans are mainly concentrated in the seed coat and give the seeds their color. Legume seeds also have a lower glycemic index compared to other starchy foods such as cereals and potatoes. When added in the daily diet, legumes can exert many beneficial physiological effects and prevent common metabolic diseases such as diabetes, coronary artery disease, and cancer [5–7]. They are effective in lowering blood pressure, normalizing body weight and lipid metabolism, reducing insulin resistance, and thus regulating the components of the metabolic syndrome and are therefore recommended for its prevention and treatment [8].

Use of the germination of bean seeds and then the fermentation process of beverages obtained from the germinated bean seeds allow to increase the health and nutritional values of these beverages. This chapter presents various studies about technology, quality, and nutritional value of fermented bean-based beverages prepared from germinated white kidney beans (*Phaseolus vulgaris* L.).

## 2. Preparation of beverages from beans

Plant milk substitutes are obtained mainly by water extraction of selected plant material. The production process is of different types, depending on the raw material used (legumes, cereals, vegetables, nuts, seeds), but all the variants have a common outline. Generally, the preparation process involves the following stages: selection of the raw material, soaking and wet or dry grinding of the raw material, water extraction of the raw material, heating, separation of the solid fraction, cooling, standardization, homogenization, thermal fixation, aseptic packaging, and storage [9–12].

In some cases, additional blanching or roasting of the raw material is carried out prior to soaking and grinding. Blanching is usually done in boiling water for 1–5 minutes, for example, to inactivate trypsin and lipoxygenase inhibitors in the case of soybean beverages. Moreover, it prevents the formation of foam during the technological process. Roasting is carried out at a temperature above 100°C, in hot air, and its duration is determined by the type of the raw material and the temperature of the process. This process is used to improve the taste and aroma of the final

product; however, it may reduce the protein solubility and extraction efficiency [9]. In the case of bean-based beverages, before soaking and grinding the seeds, it is advisable to carry out germination [10, 12].

Soaking and grinding (or only grinding) of the raw material are employed for further processing steps and to facilitate the release of nutrients contained in it. Water inactivates some of the inhibitors and reduces the amount of phytic acid, which increases the absorption of nutrients and their bioavailability [9–14]. In the case of beans, wet grinding is performed after thermal treatment of the seeds to induce starch thermohydrolysis. For some plant materials, enzymes are also added at this stage to induce enzymatic hydrolysis of starch or the polysaccharides present. An example of an enzyme used is alpha-amylase, which catalyzes the hydrolysis of the  $\alpha$ -1,4-glycosidic linkage of amylose and amylopectin present in starch and results in the formation of shorter-chain compounds, mainly in the form of dextrans. Proteolytic enzymes are also used for increasing the protein digestibility and extraction efficiency, as well as for improving the suspension stability [9, 15]. Beans can also be subjected to such a process. Alternatively, initial dry grinding of the raw material can be employed, followed by its aqueous extraction at an elevated temperature [9].

The next step in the production of plant-based beverages is the separation of the solid from the liquid fraction, by filtration or centrifugation of the obtained suspension. The resulting plant-based beverage may undergo the standardization process in order to obtain a product with a previously assumed composition, enriched with vitamins or minerals. In the case of bean seeds, subsequent additional heating is carried out to a particular temperature depending on the specificity of the raw material [9, 10, 12].

Usually, ultrahigh temperature (UHT) treatment is applied, where the product is heated in flow to 135–150°C for a few seconds to obtain a commercially sterile one. This process degrades and converts the vegetative forms into microorganisms, while maintaining the taste and aroma of the product. The obtained, microbiologically safe product is poured into unit packages, stored, and distributed [9, 10, 12].

Plant-based beverages exhibit low suspension stability due to the presence of solid particles (e.g., protein, starch, fiber, and other solid residues of plant material). To increase the stability of cow milk substitutes, hydrocolloids of plant origin are added before the final thermal run. Alternatively, the resulting suspension is subjected to homogenization and micronization, which leads to an increase in the stability of the system without the addition of hydrocolloids. This process involves simultaneous grinding and mixing of the particles of the dispersed phase, while forcing the liquid heterogeneous system under high pressure (15–25 MPa) through the homogenizing gap. After micronization, the particle size usually ranges from 0.5  $\mu\text{m}$  to 10  $\mu\text{m}$ . Consequently, the obtained product will have greater creaminess and homogeneity compared to the beverage subjected only to the homogenization process [9, 12].

### **3. Fermentation of bean-based beverages using lactic acid bacteria and propionic acid bacteria**

For many years, attempts have been made to obtain fermented plant-based beverages with the same amount of lactic acid bacteria as in the fermented dairy beverages [16–18]. Several biotechnology companies offer starter cultures for the fermentation of plant products. The addition of these cultures is aimed at obtaining vegan fermented beverages, which are substitutes for milk yogurts. These products most often contain microorganisms that are used for the fermentation of milk,

including lactic acid bacteria and bifidobacteria such as *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Bifidobacterium animalis*, and *Bifidobacterium lactis* [19]. Furthermore, Wajcht's research [14] proves the possibility of using *Propionibacterium freudenreichii* subsp. *shermanii* for the production of fermented bean-based beverages (obtained from germinated adzuki bean and mung bean seeds). After 24-hour fermentation of bean beverages at 37°C, the pH was in the range of 4.3–4.7, and the population of propionibacteria was not lower than 7 log<sub>10</sub> CFU/mL [14]. Which proves that *Propionibacterium freudenreichii* subsp. *shermanii* show the ability to grow and ferment sugars contained in bean-based beverages to an extent no worse than the lactic acid bacteria do.

The population size of these microorganisms throughout the shelf life of such beverages is an important factor. It is one of the indicators of the quality and health-promoting properties of these products. The minimum number of cells in such products should be at least 7 log<sub>10</sub> CFU/mL or g for starter bacteria and at least 6 log<sub>10</sub> CFU/mL or g for additional microorganisms (e.g., probiotics) [20]. It has already been established that the selection of bacteria used for fermentation and the composition and acidity of the product have a significant impact on the viability of the starter and prebiotic bacteria, as well as on the maintenance of the required bacterial population [18, 21]. Ziarno et al. [10] used two commercially available industrial yogurt starter cultures, namely Yo-Mix 205 LYO (DuPont Danisco; consisting of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, and *B. lactis* with sacharose and maltodextrins as carriers) and ABY-3 Probio-Tec (Chr. Hansen; consisting of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and documented probiotic strains *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12), for the fermentation of bean-based beverages obtained from germinated bean seeds of the "Piękny Jaś Karłowcy" variety. In the fermented beverages thus obtained, the level of bacteria of the starter culture and additional microorganisms was, respectively, at least 7 log<sub>10</sub> CFU/mL and at least 6 log<sub>10</sub> CFU/mL. During 28 days of storage at 6°C, there was a significant reduction in the population of *S. thermophilus*, *L. acidophilus*, and *Bifidobacterium* in the beverages; however, the levels of microorganisms were not below 7 log<sub>10</sub> CFU/mL and 6 log<sub>10</sub> CFU/mL, respectively. Maciejak [12] noted that the population of starter microorganisms was at the level of 8.1 log<sub>10</sub> CFU/mL in the bean beverage fermented with the industrial starter culture ABY-3. Zaręba and Ziarno [18] showed that the fermentation of plant-based beverage matrices (soy, rice, and coconut beverages) is more conducive to the development and survival of streptococci compared to lactobacilli. However, the survival rate of both lactobacilli and lactobacilli depends on the type of plant-based beverages and the starter culture used, as well as the degree of fermentation of the beverage (final pH). Therefore, the starter culture should be carefully selected for the specific type of plant beverage, and the storage temperature of the final product should also be adjusted.

#### 4. Nutritional and health value of bean seeds and bean-based beverages

Depending on the variety, white bean seeds contain approximately 21–23 g of total proteins, approximately 0.8–1.6 g of fat, approximately 60–63 g of total carbohydrates, including approximately 40 g of starch and approximately 15–24 g of dietary fiber per 100 g [22–26].

The nutritional and health value of bean beverages depends on the recipe composition and the amount of bean seeds in the product [10, 12, 14, 19, 27]. Ziarno et al. [10] obtained a bean-based beverage by blending 100 g of presoaked white

bean seeds of the “Piękny Jaś Karłowcy” variety with 900 g of drinking water. Then, the homogenate was boiled to gelatinize the starch contained in the beans. The obtained mixture was filtered through a sieve and sterilized at 121°C for 20 minutes. Among other components, the protein content of the beverage obtained was estimated at 2.3 g/100 g. The study by Jeske et al. [28] reported that commercial plant-based beverages have a comparable protein content.

The main types of proteins found in legume seeds, including bean seeds, are globulins and albumin, which account for 50–70% and 20% of all proteins, respectively [25]. Globulins, which are the dominant fraction of proteins, are referred to as storage proteins. These are stored in membrane-bound organelles, vacuole stores, or protein bodies. In parenchymal cells, globulins survive drying out during seed maturation and undergo proteolysis during germination, providing free amino acids [7, 24, 29, 30].

The storage proteins of bean seeds, like other legumes, have a relatively low content of methionine (approximately 0.1–0.37 g/100 g), cysteine and cystine (0.23–0.25 g/100 g), and also tryptophan (0.25–0.27 g/100 g); however, they are rich in lysine (1.4–1.6 g/100 g), which is the limiting amino acid [26]. Consuming legumes and cereals together in a ratio of 35:65 significantly improves the quality of the supplied protein, making the meal wholesome with a favorable composition of amino acids [7, 24, 29–31].

Most legume seeds, including the white bean ones, contain a maximum of about 5% fat in dry matter (DM), except chickpeas and soybeans, which contain approximately 15% and 47% of fat, respectively [32]. However, in the fermented bean-based beverages prepared by Maciejak [12], the total fat content was 0.16 g/100 g. The fat level in commercial plant beverages is usually regulated (and increased), for example, by the addition of vegetable oils [19]. According to the research of Jeske et al. [28], commercial plant-based beverages have a comparable fat content as the bean-based beverages prepared by Maciejak [12].

The main lipid components of bean seeds are triacylglycerols (TAGs) and phospholipids [33]. The lipid fraction is rich in mono- and polyunsaturated fatty acids (PUFA) (approximately 0.07–0.10 g/100 g and 0.36–0.51 g/100 g, respectively), which constitute approximately 60% of all fatty acids [26, 34]. In some legumes, PUFA are present in the form of linoleic acid (C18:2, included in the omega-6 acid fraction) and  $\alpha$ -linolenic acid (C18:3, included in the omega-3 acid fraction). These acids cannot be synthesized by the human body, so they must be constantly supplied in the diet [31]. Among the unsaturated fatty acids, linoleic acid dominates (0.19–0.27 g/100 g), accounting for 21–53% of all fatty acids in beans [24, 29]. The content of linolenic acid is in the range of 0.26–0.23 g/100 g or accounts for 4–22% of the total pool of fatty acids. The seeds of all types of legumes are characterized by a very low content of trans fatty acids, constituting less than 1% [26, 35]. The fat present in legumes does not contain cholesterol [34]. While examining the lipid profile of white bean seeds of the “Piękny Jaś Karłowcy” variety, Ziarno et al. [36] showed that the dominant fatty acid was linolenic acid (C18:2 n-6c), amounting to 39.23% of the total pool. Other unsaturated acids present in significant amounts were  $\alpha$ -linolenic acid (23.23% of all fatty acids) and oleic acid (17.58% of all fatty acids). The most abundant saturated acids were palmitic acid (12.78% of all fatty acids) and stearic acid (3.68% of all fatty acids). Overall, all unsaturated and saturated acids, respectively, amounted to 81.87% and 18.13% of the total pool of fatty acids. The remaining acids present were about 0.5% or less of all fatty acids. For comparison, Ryan et al. [37] estimated the share of  $\alpha$ -linolenic, linoleic, and oleic acid in kidney beans, which amounted to 45.69%, 26.04%, and 11.97% of all fatty acids, respectively. Palmitic acid and stearic acid, respectively, amounted to 14.2% and 1.3% of the total pool. The proportion of unsaturated fatty

acids was 83.8% and that of saturated fatty acids was 16.5%. In another work [38], the fatty acid profile was determined for beans (*P. vulgaris*) and other legumes. For common beans, the content of linoleic acid,  $\alpha$ -linolenic acid, and oleic acid (C18:1 n-9) was, respectively, 43.1%, 12.4%, and 13.9% of all fatty acids. The following saturated fatty acids were found in the highest content: palmitic C16:0 (16.8% of all fatty acids) and stearic acids C18:0 (3.5% of all fatty acids). In Adzuki bean seeds, the dominant fatty acids were palmitic, stearic, oleic, linoleic, and  $\alpha$ -linolenic acids. Of these, unsaturated fatty acids, mainly linoleic,  $\alpha$ -linolenic, and oleic acids, were found in large proportions, which constituted from 70.6% to 73.8% of the total content of fatty acids depending on the variety [34]. Another type of legume seeds studied were mung bean seeds, in which the total amount of lipids was 1.2–1.56% of dry weight depending on the cultivar. As in the previous case, the dominant fatty acid was linoleic acid, which was in the range of 340.5–465.7 mg/100 g of dry weight, while there were significant amounts of palmitic, oleic,  $\alpha$ -linolenic, and stearic acids as well [39].

In addition, the positional distribution of fatty acids in the TAG molecules has found interest in increasing research works. Significant differences have been observed in the distribution of fatty acids in TAGs depending on the variety of beans and other legume seeds [33, 37, 38, 40, 41]. In his diploma thesis, Ziarno et al. [36] described the positional distribution of fatty acids in the TAG molecules in the lipids of bean seeds of the “Piękny Jaś Karłowcy” variety (**Table 1**). Ryan et al. [37] determined the content of  $\alpha$ -linolenic, linoleic, and oleic acids in kidney beans to be, respectively, 45.69%, 26.04%, and 11.97% of all fatty acids. Palmitic acid and stearic acid, respectively, amounted to 14.2% and 1.3% of the total pool of fatty acids. Unsaturated fatty acids amounted to 83.8% of all fatty acids, and saturated acids to 16.5%. In another study, Grela and Gunter [38] determined the fatty acid profile for beans (*P. vulgaris*) and other legumes. In common beans, linoleic acid,  $\alpha$ -linolenic acid, and oleic acid amounted to 43.1%, 12.4%, and 13.9% of all fatty acids, respectively. Among the saturated fatty acids, palmitic (16.8% of all fatty acids) and stearic (3.5% of all fatty acids) acids were found in the highest content.

For comparison, in Adzuki bean seeds, most of the unsaturated fatty acids (>96% of fatty acids) were accumulated in the sn-2 position of the TAG molecules. Saturated acids were accumulated in the sn-1 and sn-3 positions, except oleic acid, which was accumulated evenly in all three positions [34]. As for the positional distribution of the TAG molecules in broad bean seeds, similarly to Adzuki beans, a significant amount of unsaturated fatty acids (>95% of fatty acids) were accumulated in the sn-2 position. Only oleic acid was found to be almost evenly occupying the sn-1, sn-2, and sn-3 positions. Saturated acids, such as palmitic and stearic

Type of fatty acid	TAG content of fatty acid [% of fatty acids]	Content of fatty acid [% of fatty acids] in position		Content of fatty acid in the sn-2 position [% of fatty acids]
		sn-2	sn-1,3	
Palmitic (C16:0)	12.78	8.68	14.83	22.63
Stearic (C18:0)	3.68	2.16	4.44	19.55
Oleic (C18:1 n-9c)	17.58	16.79	17.97	31.83
Linoleic (C18:2 n-6c)	39.23	46.52	35.59	39.53
$\alpha$ -Linolenic (C18:3 n-3c)	23.23	22.67	23.51	32.53

**Table 1.**

Positional distribution of fatty acids in triacylglycerol (TAG) molecules in the lipids of the white bean seeds of the “Piękny Jaś Karłowcy” variety (based on [36]).

acids, were accumulated in the sn-1 and sn-3 positions. An almost identical distribution was noted in peas, where more than 90% of unsaturated fatty acids were accumulated in the sn-2 position, while saturated acids were mainly accumulated in the sn-1 and sn-3 positions [33, 40, 41].

The carbohydrates contained in legume seeds, including bean seeds, are mainly composed of starch, fiber, nonstarch polysaccharides, and nondigestible oligosaccharides, which together constitute 30–40% of the dry weight of seeds in the case of species with a high protein content (e.g., lupines and soybeans) or 50–65% of DM in those containing less protein in seeds [25, 42]. However, in the obtained unfermented bean beverages, Cichońska [19] determined a total carbohydrate content of 1.8–3.7 g/100 g depending on the production recipe used.

Bean seeds contain more than 40% of starch and 10–25% of dietary fiber [26]. They are also rich in the resistant fraction of starch, which is not hydrolyzed in the small intestine but is fermented by colonic microorganisms at different rates [7]. The ratio of soluble fiber to insoluble fiber is similar to that found in cereals, which is approximately 1:3 [35]. Consumption of bean fiber is associated with many health and physiological benefits, including improvement in the metabolism of glucose and lipids [24, 43]. When resistant starch in beans is fermented, short-chain fatty acids (such as acetic, butyric, and propionic acid) are produced, the concentration and distribution of which depend on the microorganisms and the carbohydrate content in the gut. Therefore, resistant starch is often considered a prebiotic component as it can regulate the amount and activity of the intestinal flora. It is a precursor of butyrates, which are known for their anti-inflammatory and anti-cancer properties [44]. In addition, resistant starch binds with bile acids, lowers the absorption of cholesterol and fat, and reduces the absorption of glucose. It also influences the acceleration and regulation of intestinal peristalsis, prevents constipation, and supports the development of beneficial microflora in the large intestine. Furthermore, it reduces hunger and increases satiety after a meal, as it swells in the stomach [24, 43].

Consuming legume seeds, including bean seeds, can cause gas production due to bacterial fermentation, including undigested leftovers. The main oligosaccharide found in legumes is raffinose, which is attributed to the properties of a prebiotic (fermented by probiotic bacteria to short-chain fatty acids); thus, legumes can improve colon health and reduce the risk of colon cancer [31].

Proper preparation of bean beverages can reduce the content of nonenzymatically decomposed ingredients in the human digestive tract [10, 13, 14]. Studies have shown that when lactic acid bacteria are used for the fermentation of legume seeds, the level of stachyose is reduced, which in turn reduces digestive discomfort and flatulence [45–49]. Maciejak [12] obtained fermented and unfermented bean beverages from the germinated white bean seeds of the “Piękny Jaś Karłowcy” variety (Table 2). Their data showed a significant reduction in the content of all analyzed saccharides. For instance, the content of sucrose was reduced by 82.46%, and raffinose content reached 82.84%. The content of stachyose after the fermentation process was reduced by 68.64%, while the smallest change, amounting to 60.61%, was recorded for verbascose.

Of all the seeds of legumes, beans have the highest content of minerals. They can act as an important source of iron (7–11 mg/100 g), zinc (3–4 mg/100 g), copper (0.6–1.0 mg/100 g), phosphorus (300–450 mg/100 g), and potassium (1500–1800 mg/100 g) in the daily diet [23, 24, 26, 50, 51]. Although the content of minerals varies depending on the variety of beans [23, 24], the seeds of white beans are identified as a source of calcium (170–240 mg/100 g) and magnesium (180–190 mg/100 g) [26, 44]. In bean-based beverages, the content of minerals is

Bean beverages	Sacharose [mg/g]	Raffinose [mg/g]	Stachyose [mg/g]	Verbascose [mg/g]
Nonfermented	1.725 ± 0.601	0.425 ± 0.177	2.725 ± 0.015	0.15 ± 0.071
Fermented with lactic acid bacteria	0.303 ± 0.086	0.073 ± 0.019	0.855 ± 0.219	0.059 ± 0.058

**Table 2.**

Content of selected carbohydrates in the bean-based beverage obtained from the germinated white bean seeds of the “Piękny Jaś Karłowcy” variety (based on [12]).

determined by the recipe composition, especially the amount of bean seeds in the products.

Legume seeds, including beans, are also a good source of water-soluble vitamins, such as B vitamins [10, 26, 52]. White bean seeds contain thiamine (0.4–0.7 mg/100 g), riboflavin (0.1–0.2 mg/100 g), niacin (0.5–1.4 mg/100 g), and acid folic (370–390 µg/100 g) [26, 52]. Similar to minerals, the content of vitamins varies depending on the variety of beans [23, 24]. Legume seeds, including bean seeds, are deficient in fat-soluble vitamins and vitamin C [26]. The vitamin content of bean-based beverages is also determined by the recipe composition, in particular the amount of bean seeds in such products, as well as by the thermal (pasteurization, sterilization) and enzymatic (germination, fermentation) treatments used. Although, Zhang et al. [53] showed that in fortified oat beverages subjected to UHT treatment by direct steam injection, the content of vitamins A, D3, K, B6, B12, thiamine, riboflavin, niacin, and folic acid was not influenced by the thermal process. In addition, the UHT sterilization of the bean-based beverage had a similar effect on the vitamins mentioned. Ziarno et al. [10] showed that the unfermented bean-based beverages obtained from bean seeds of the “Piękny Jaś Karłowcy” variety contained 0.69 mg/kg thiamine, 0.20 mg/kg riboflavin, 2.34 mg/kg niacin, and 0.55 mg/kg pyridoxine.

An interesting group of compounds present in legume seeds, including bean seeds, are active biological substances, such as phytochemicals and antioxidants. These include polyphenols, lignans, isoflavonoids, protease inhibitors, trypsin and chymotrypsin inhibitors, saponins, alkaloids, phytoestrogens, and phytates [31, 53–55]. Most of them are referred to as antinutritional ingredients. Although they are nontoxic substances, they can cause physiological side effects affecting the digestion of proteins or the bioavailability of certain minerals. However, many of them show a different, positive biological activity [31, 53]. The polyphenol content in beans depends, among others, on the species, cultivar, and agrotechnical and climatic conditions of cultivation [30, 56]. Due to the high content of polyphenolic compounds, such as tannins, flavonoids, isoflavonoids, phenolic acids, phytates, or lignans, legume seeds, including bean seeds, are known for their anticancer properties [24, 29, 30, 50, 57]. These properties of beans are especially related to the presence of protease inhibitors, which help to naturally regulate the levels of inhibitors found in the human body. Antioxidant components also influence the anticarcinogenic properties of beans. A proper diet, including bean seeds, helps to prevent cancer, while in people with advanced-stage cancer, it can support oncological therapy. Furthermore, beans have a high content of hemagglutinins and lectins that direct atypical cells to the apoptotic pathway [30, 57, 58]. The content of these bioactive substances in bean seeds also depends, among others, on species, cultivar, and agrotechnical and climatic conditions of cultivation [56]. In the case of bean-based beverages, the polyphenol content is influenced by many factors, mainly the recipe composition and technological activities used in the production process [19, 59]. In addition, the content of these bioactive substances in beverages is

influenced by the various technological processes used during the initial processing of bean seeds and the processing of the prepared product (germination, blending, mixing, homogenization, pasteurization or sterilization, oxygenation) [59–61].

Legume seeds, including bean seeds, are characterized by a low glycemic index (<55), and can therefore limit hyperglycemia [52]. They can contribute to reducing the risk of type 2 diabetes and control the levels of sugars and lipids in the human body [24, 29, 35, 50]. In people with type 2 diabetes, increased consumption of beans can reduce tissue insulin similarity [30, 62]. Consuming legumes four times a week or more may also reduce the risk of coronary heart disease by 22%. Increased consumption of legume seeds contributes to lowering the levels of total cholesterol and low-density lipoprotein cholesterol [42]. Moreover, bioactive compounds, such as isoflavonoids or lignans, present in legumes may play a role in the prevention of osteoporosis [44].

## **5. Influence of germination on the quality of bean-based beverages**

Legumes contain many antinutritional ingredients, such as trypsin inhibitors, phytic acid, or  $\alpha$ -galactosides, as well as indigestible carbohydrates; therefore, they must be subjected to appropriate treatments before consumption [11, 63]. Methods such as dehulling, heating, germination, fermentation, soaking, or partial hydrolysis using proteolytic enzymes can be applied. These improve the quality of seeds and increase their nutritional quality [64–66]. The optimal time of these processes is determined as 3–5 days, and they should be performed at room temperature [10, 12, 67].

Germination is one of the most important and effective processes that can improve the nutritional value of seeds, by increasing the content of certain nutrients (e.g., proteins or polyphenols) or eliminating undesirable components (e.g., trypsin inhibitors, stachyose, raffinose) [10, 12, 36, 68]. During this process, all lipolytic, amylolytic, and proteolytic enzymes are activated, which catalyze the breakdown of storage substances, respiratory processes, and the synthesis of macromolecular compounds needed for the growing parts of the embryo [69]. Germinated bean seeds are characterized by a lower level of stachyose and raffinose but a higher content of polyphenols and protein [64, 65, 70]. Proteolytic enzymes help in the breakdown of endosperm proteins, while dipeptides, tripeptides, and new embryonic proteins are synthesized simultaneously [69].

The lipid content and fatty acid profile of legume seeds, including bean seeds, also change during the germination process [36, 71, 72]. Frias et al. [68] found that the germination process carried out for 9 days led to an increase in the inhibition of lipid oxidation. On the other hand, Ziarno et al. [36] showed that in the bean seeds of the “Piękny Jaś Karłow” variety, the germination process significantly reduced the oxidative stability of the fat isolated from the seeds. Compared to raw beans, the researcher recorded an almost fourfold reduction in the time of oxidation. The maximum oxidation time of raw beans was 44.93 minutes, while for a beverage made from the germinated bean seeds, the oxidation time was only 11.82 minutes.

Moreover, during the germination process of legume seeds, including bean seeds, the content of phenolic compounds [69] has been found to be significantly increased. In the research on lupine seeds, the germination process was found to increase the content of  $\alpha$ -tocopherol (from 0.19 mg/100 g DM in the control to 3.91 mg/100 g DM after 9 days of germination) and decrease the content of  $\gamma$ -tocopherol (from 20.1 mg/100 g DM in the control to 13.4 mg/100 g DM after 9 days of germination), but it did not significantly affect the content of  $\delta$ -tocopherol (0.22 mg/100 g DM in the control compared to 0.25 mg/100 g DM

after 9 days of germination). Additionally, a significant increase in the content of vitamin C was found (from 6.48 mg/100 g DM in the control to 56.1 mg/100 g DM after 9 days of germination). Germinated lentil or chickpea seeds were also characterized by an increase in the bioavailability of calcium (respectively 29.3% and 19.3% in the control and 46.5% and 32.9% in germinated seeds) and iron (respectively 10.2% and 11.3% in the control and 18.5% and 18.6% in germinated seeds) [73].

The use of various technological and biological procedures (e.g., soaking, cooking, germination, fermentation) also greatly influences the fatty acid profile of legume seeds, including bean seeds [36, 74, 75]. Germination increases the availability of free amino acids and vitamins. It also improves the content and digestibility of proteins as well as the content of crude fiber [76]. Furthermore, germination of legume seeds, including bean seeds, partially minimizes the activity of trypsin inhibitors, and eliminates flatulence caused by oligosaccharides from the raffinose family [11]. This contributes not only to significant biochemical and nutritional changes but also to sensory changes in legumes [10, 12, 14, 59, 63, 77].

The influence of germination on other substances such as bioactive compounds and antinutritional ingredients has also been investigated [10, 67, 69, 78]. The antinutritional ingredients present in materials of plant origin include e.g., protease inhibitors, phytates, glucosinolates, saponins tannins, lectins, oligosaccharides and non-starch polysaccharides, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, cyclopropenoid fatty acids, and antivitamin. Meanwhile, the identified bioactive compounds can be divided into six categories, namely flavonoids, phenolic acids, saponins, alkaloids, polysaccharides and others (i.e., (e.g., terpenoids, stilbene glycosides, coumarins). Valdes et al. [56] showed that bean germination may affect the accumulation of polyphenols in black bean sprouts. The researchers observed a 1.54-fold increase in polyphenol content in bean sprouts after 6 days of bean fermentation. Other studies [64, 65] indicated that after 5 days of bean seed germination, the content of polyphenols increased from 2.28 mg/g DM to 2.95 mg/g DM, whereas there was a reduction in the content of raffinose (from 5.90 mg/g DM to 1.98 mg/g DM) and stachyose (from 60.28 mg/g DM to 5.87 mg/g DM).

Some researchers reported that the germination process influenced the level of B vitamins in legume seeds [53]. The direction of these changes was dependent on the cultures and processing parameters [10, 79–81]. El-Adawy [82] showed a significant increase in riboflavin content (from 1.733 g/kg DM to 2.013 g/kg DM) and a slightly lower increase in pyridoxine content (from 4.663 g/kg DM to 4.830 g/kg DM) after 3 days of germination of chickpea seeds. In addition, there was a significant reduction in the content of thiamine (from 4.533 g/kg DM to 2.833 g/kg DM) and niacin (from 16.027 g/kg DM to 15.186 g/kg DM). Ziarno et al. [10] observed a significant reduction in the content of riboflavin, niacin, and pyridoxine in bean-based beverages fermented with yogurt bacteria.

## 6. Effect of fermentation on the quality of bean-based beverages

Another process that can improve the nutritional value of legumes, including bean seeds, is fermentation. A wide range of microorganisms can be involved in the fermentation of legume seeds as follows: lactic acid bacteria of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Enterococcus*; molds of the genera *Rhizopus*, *Aspergillus*, and *Mucor*; yeasts of the genera *Saccharomyces* and *Zygosaccharomyces*; or bacteria of the genus *Bacillus* [12, 14, 18, 83, 84]. Fermentation reduces the level of antinutritional substances, increases digestibility, and enhances

the level of valuable nutrients [10, 12, 14, 59, 77, 85–92]. Spontaneous fermentation is quite widespread, especially in developing countries. However, this technology has disadvantages such as low efficiency, variable product quality, and safety drawbacks [89]. One of the alternatives is controlled fermentation with the use of lactic acid bacteria, which can be used for different raw materials, not only legumes but also cereals and root crops [18, 93–95].

Lactic acid fermentation has been used for years for obtaining fermented beverages. For the production of fermented foods, starter cultures of known composition are used, which allows reproducibility of the process [83, 91, 96]. Moreover, such products become a source of bioactive substances and prebiotic substances, which are extremely important for health ( $\beta$ -glucan, oligosaccharides, and resistant starch), as well as live cells of lactic acid bacteria and probiotic strains. This also applies to nondairy fermented beverages [12, 14, 18, 90–92]. The most popular types of bacteria used for fermentation purposes, which also exhibit probiotic properties, are lactic acid bacteria *Lactobacillus*, *Bifidobacterium* and *Propionibacterium* [12, 14, 97, 98].

Fermentation does not significantly affect the protein content of bean seeds, although an increase in the content of exogenous amino acids has been noted after the process, especially in leucine (from 7.98 mg/g to 16.68 mg/g), threonine (from 4.16 mg/g to 7.31 mg/g), and isoleucine (from 4.26 mg/g to 6.39 mg/g) [64, 65, 99]. Similar observations were made with regard to other legume seeds. In broad bean seeds, fermentation with the use of a *Lactobacillus plantarum* strain caused an increase in the content of free amino acids (from 7.10 g/kg to 17.66 g/kg), mainly essential amino acids and  $\gamma$ -aminobutyric acid. The protein digestibility improved to a small but statistically significant extent (from 75.1% to 76.6%) [100]. Furthermore, protein digestibility improved *in vivo* (3.5%) as well as *in vitro* (12.55%) [48]. Similarly, Czarnecka et al. [101] used *L. plantarum* for the fermentation of bean seeds and found a significant improvement in the *in vitro* digestibility of protein (from 59.1% to 72.2%).

A slight reduction in fat content was also observed in fermented bean seeds, probably due to the hydrolysis of fatty acids [64–66]. This is evidenced by the effect of fermentation using different bacterial cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* on the fatty acid profile [36, 72, 102]. Ziarno et al. [36] showed in the white bean seeds of the “Piękny Jaś Karłowcy” variety that the combined processes of germination and fermentation with lactic acid bacteria of the genus *Lactobacillus* contributed to an increase in the amounts of saturated acids (palmitic and stearic) and oleic acid in fatty acid profile compared to raw beans. However, the direction of changes depended on the selection of the *Lactobacillus* strain for fermentation of the bean-based beverage. The dominant fatty acid in the fatty acid profile of raw bean seeds was linolenic acid, which constituted 39.23% of the total fatty acid pool. Other unsaturated acids that were present in a significant amount were  $\alpha$ -linolenic (23.23% in fatty acid profile) and oleic (17.58% in fatty acid profile) acids. The most abundant saturated acids were palmitic acid (12.78% in fatty acid profile) and stearic acid (3.68% in fatty acid profile). Overall, the total amount of all unsaturated acids was 81.87% in the fatty acid profile of raw bean seeds, and that of saturated acids was 18.13%. The remaining acids were present at about 0.5% or less in the total pool. On the other hand, in bean-based beverages fermented by variants of the *Lactobacillus* genus, an increase in the content of palmitic acid (except for the beverage fermented by a strain of *L. plantarum*) and stearic acid, compared to raw beans, was noted. Moreover, in fermented bean-based beverages, an increase in the content of oleic acid and a decrease in the content of PUFA (i.e., linoleic acid and  $\alpha$ -linolenic acid) were observed. However, Barampama and Simard [103] obtained contrasting results in their work. They used *L. plantarum* to ferment beans. After 16 hours of fermentation at 37°C, they observed a reduction

in the content of stearic acid (from 12.20 mg/100 g to 120.6 mg/100 g), palmitic acid (from 124.22 mg/100 g to 118.57 mg/100 g), oleic acid (from 56.39 mg/100 g to 52.39 mg/100 g), linoleic acid (from 130.97 mg/100 g to 113.26 mg/100 g), and linolenic acid (from 137.69 mg/100 g to 110.34 mg/100 g).

The combination of the germination and fermentation processes also affects the positional distribution of fatty acids in the middle (sn-2) and external (sn-1,3) positions in bean seeds. The differences in the amount of individual fatty acids in the middle position (sn-2) were found to be statistically insignificant [36]. In the current literature, there is only limited information on the impact of various biological and technological processes on the positional distribution of fatty acids in the TAG molecules present in legume seeds, including bean seeds. One study assessed the effect of microwave heating on the distribution of fatty acids in the hypocotyl TAGs of two soybean seeds. It was found that after 12 minutes or more of heating, there were minor but statistically significant changes in the distribution of fatty acids. These changes were manifested, inter alia, as an increase in the percentage of palmitic acid in the sn-1 and sn-3 positions and a reduction in the percentage of linoleic acid in the sn-2 position [104].

In the fermented bean seeds, changes in the content of complex carbohydrates, such as stachyose, raffinose, and verbascose, were found, but the degree of reduction in their concentration depended on the type of microorganisms used in the process [48, 64, 65, 101]. Germination and fermentation by lactic acid bacteria (LAB) or bifidobacteria increased the amount of simple sugars in the beans, while they induced the breakdown of raffinose and stachyose into galactose, glucose, sucrose, and fructose [13]. Ziarno et al. [10] showed that the fermented beverages produced from germinated white bean seeds of the “Piękny Jaś Karłowcy” variety using two yogurt starter cultures contained about 31% and 17% of stachyose and raffinose, respectively, compared to those before the fermentation (2.73 mg/kg and 0.43 mg/kg, respectively), but the reduction in verbascose was not significant. As a result of germination, maltose was released from complex carbohydrates contained in beans [69, 105]. Granito and Alvarez [48] showed an increase in the content of resistant starch by about 13% and in the content of available starch by about 35%, as well as a decrease in the content of soluble (by over 63%) and insoluble fiber (by 39%). Czarnecka et al. [101] showed an improvement in *in vitro* starch digestibility in beans by 55–58% after germination.

Fermentation with LAB and bifidobacteria may also change the content of B vitamin group, but the direction of these changes is dependent on the cultures and processing parameters that influence the biosynthesis of these vitamins [10, 79–81, 106, 107]. In addition, fermentation contributes to the reduction of antinutritional components. The content of polyphenols was also shown to be increased by 43.4%, whereas the content of non-nutrients such as tannins was reduced (by approximately 84%) [64, 65]. Fermentation with the fungus *Rhizopus oligosporus* was found to cause an almost threefold increase in the content of polyphenols and a twofold increase in their antioxidant activity [99]. In the bean seeds fermented with the fungus of the genus *Rhizopus*, an increase in the content of polyphenols by 43.4% and a reduction in the content of non-nutrients such as tannins (by approximately 84%) were found [64, 65]. In broad beans fermented with *L. plantarum* bacteria, a reduction in the activity of trypsin inhibitors by approximately 56% and a reduction in the content of condensed tannins by approximately 50% were found [100].

## 7. Conclusions

Use of the germination and fermentation processes in combination in the production of fermented beverages from the seeds of ordinary beans has not been

discussed in the scientific literature so far. This chapter presents various studies proving that it is possible to obtain fermented bean-based beverages using these processes together, and the health and nutritional values of these beverages are higher than that of raw bean seeds. The most promising results were reported for the lactic acid fermentation of bean-based beverages, which yields wholesome nondairy products with similar quality as their dairy counterparts. The use of lactic acid bacteria has a positive effect on the digestibility of fermented beverages, owing to the reduction of oligosaccharides that cause digestive discomfort. Most importantly, the obtained fermented bean-based beverages are ideal for the survival of the starter bacterial cells during both fermentation and refrigerated storage, and therefore, they can be considered as functional products acting as a carrier for probiotics.

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## Conflict of interest

Authors have declared that they do not have any conflict of interest for publishing this research.

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