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Sprouting of soybean: a natural process to produce unique quality food products and additives

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REVIEW ARTICLE

Abstract

Seed germination (sprouting) offers a broad spectrum of quality changes to the design of novel food components/additives/products with improved composition, enhanced nutritional value and/or with dedicated functional properties compared to ungerminated seed. This review summarises the general physiological and compositional changes that occur during the germination process in soybean seed. The changing pattern of macro- and micro components, different bioactive compounds and anti-nutritive factors are highlighted and their nutritional and functional characteristics summarised. Furthermore, the benefits of a strictly controlled short-term germination process (germination time <48 h) are summarised and the potential of an innovative sprouting procedure is discussed. Short-term germination technology and the processed bean materials provide a special quality seed product as raw material or additive, which can be used in a wide variety of sectors, such as the food industry, households, and public and hospital catering. It can also be applied in the healthy and functional food production segment.

Keywords: germination/sprouting, soybean, raw material, food additive, bioactive components, short-term germination

1. Introduction

There is an ongoing need to ensure sufficient quantities of good quality food for a growing global population living fast-pace lives, and to develop new, economically produced, and preferably versatile food products. A growing percentage of the population is eating an unbalanced diet, primarily due to lack of sufficient income, a busier lives and various popular dietary trends. In a large part of the world millions of people are starving and do not have access to a fraction of the daily required nutrients. Meanwhile, the number of people suffering from diseases requiring special diets continues to grow.

It is a major challenge to develop new food raw materials/products to solve the above-mentioned problems. The ideal scenario would be to select raw materials that can be inexpensively produced and have an outstanding biological value. The widely grown soybean has these properties and its original functional properties are also excellent. It is suitable for human and animal consumption, as well as industrial processing. Soybean has high protein and low carbohydrate levels compared to other legumes. The mature soybean contains approximately 38% protein, 30% carbohydrate and 18% high quality oil. Soybean is a complete protein source, with unique properties compared to other plant-based protein sources. Other beneficial properties include a rich source of vitamins, minerals and bioactive components and high levels of polyunsaturated fatty acids (Erdman and Fordyce, 1989).

The nutritional value of plant seeds can be effectively increased by germination. The valuable role of sprouts in nutrition has been known for thousands of years, and the consumption of sprouts or sprouted seeds and the practice of germination date back to well before Christ. Germination is a natural, holistic biological process in which the seed, containing all the ingredients necessary for its life, starts life. Sprouts have a higher nutritional value than mature seeds and contain nutrients in a form that is easily accessed and easily processed by the human body. Sprouted seed materials increase the versatility of food

consumed in different protein-rich and special diets. This paper presents the major changes that occur during soybean germination and highlights the unique, high quality raw material/food product/additive produced in the process.

2. Germination in general

Germination can be regarded as an unfolding of new plant life. It consists of numerous cellular and molecular events which transform the embryo into a young plant. This biological process occurs in the vegetative part of the plant life cycle. The development of the seed begins with water uptake and is directly followed by the release and mobilisation of reserve nutrients. Before germination starts, a dormancy phase is necessary for each seed, in order for it to survive adverse environmental conditions and complete the endogenous processes that are essential for germination. Dormancy is the result of, for example, the presence of compounds which inhibit germination (e.g. salicylic acid, coumarin, abscisic acid) or various physical factors. Germination only starts when there are sufficient amounts of water, light, oxygen and in some cases only when the temperature is optimal. The optimum values of these environmental factors are different for each species. For example, the minimum germination temperature for soy is 15 °C (Graeber *et al.,* 2012).

Germination is a heterotrophic process in which the reserved nutrients are mobilised, so that anabolic processes and growth can begin. During imbibition the seed rapidly swells and changes in size and shape. The metabolic processes speed up, enzyme activity increases and the operation of the plant hormones is becomes more active during germination. Until the appearance of the green leaves, the reserved nutrients (carbohydrates, lipids, proteins) provide sufficient organic matter and energy for the plant. In a later phase, autotrophic nutrition will be typical for a plant that has green parts and is able to photosynthesise (Izsáki and Lázár, 2004).

The low moisture content (5-15%) and the inactivity of the metabolic processes are typical for ungerminated seed, however the structure, macromolecular content and genetic information carried by ungerminated seed are at a stage where metabolism can be resumed (Obroucheva and Antipova, 1997). Hydration and oxygen uptake is necessary for the continuation of the metabolic processes. Therefore, water intake is considered the first step of germination, while the beginning of the embryonic axis elongation is the last step. As the embryonic axis elongates in the core, the radicle breaks through the seed coat and the first visible sign of germination (the radicals) appears (Bewley and Black, 1994).

Germination can be divided into two main phases. In the first phase, swelling of the seed and reactivation of the germ can be observed. The second phase is the mobilisation of the reserve nutrients. The major cellular and metabolic processes of germination are shown in Figure 1. The sequence of events is derived from studies of various species. The remarkable intake of water, i.e. the rapid swelling, starts the basic metabolic processes. The macromolecules, cell compartments, hormones and enzymes are reactivated in the seed (Bove *et al.,* 2002). The reserved mRNAs and proteins are sufficient for germination, so the reactivation of the metabolic processes depends greatly on the stored proteins and metabolites (Sano *et al.,* 2012). The newly synthesised macromolecules, including enzymes, contribute to the later phase of germination. *De novo* enzyme and protein synthesis from the reserved mRNAs occurs in the early phase of germination (Rajjou *et al.,* 2006). In this initial phase the water intake occurs via a physical path and the metabolic pathways are activated step by step. Respiration intensifies causing a rapid increase in oxygen intake and it reaches the multiple value of what is typical for the dormancy stage. The glycolysis, citric acid cycle and pentose-phosphate cycle are activated. The free amino acids and saccharides are easy to access so they are digested initially, and the poorly degradable starches and proteins are digested and used only much later. It is typical for the new macromolecule synthesis that the mRNA synthesis is initiated first, followed by the protein synthesis – also the enzyme protein synthesis – and relatively later by the DNA synthesis. The second phase of germination begins when the level of hydration exceeds 60%. At this point water intake slows down and the cells begin to grow towards the embryonic axes. Osmotically active substances accumulate, e.g. saccharides, amino acids and potassium ions. The germ produces gibberellins, which induce the *de novo* synthesis of the hydrolytic enzymes (α-amylases, proteases, ribonucleases, etc.); these enzymes penetrate the endosperm and start to digest the reserves. The acidification of the cell wall causes the bonds between the polymers of the cell wall to loosen and disintegrate. Simultaneously the H^+ ATPase is activated in the plasma membrane, causing a further increase in the water intake. Finally, the surrounding tissues weaken decreasing the mechanical constraints imposed by the surrounding layers, the embryo passes through the seed coat and the endosperm, and germination finishes (Nonogaki, 2006). The radicle appears on the surface of the seed only due to cell elongation, without any cell division (Sliwinska *et al.,* 2009). The previously mobilised reserve nutrients are used as long as the plant is not able to photosynthesise. If the cell cycle continues during germination then the first cell division will occur after the germination phase (Bove *et al.,* 2002; Obroucheva and Antipova, 1997; Salgó, 1986).

Germination can be delayed temporarily by maintaining the dormant state. The plant development process is determined by two endogenous plant growth factors: abscisic acid and the gibberellins. They operate in

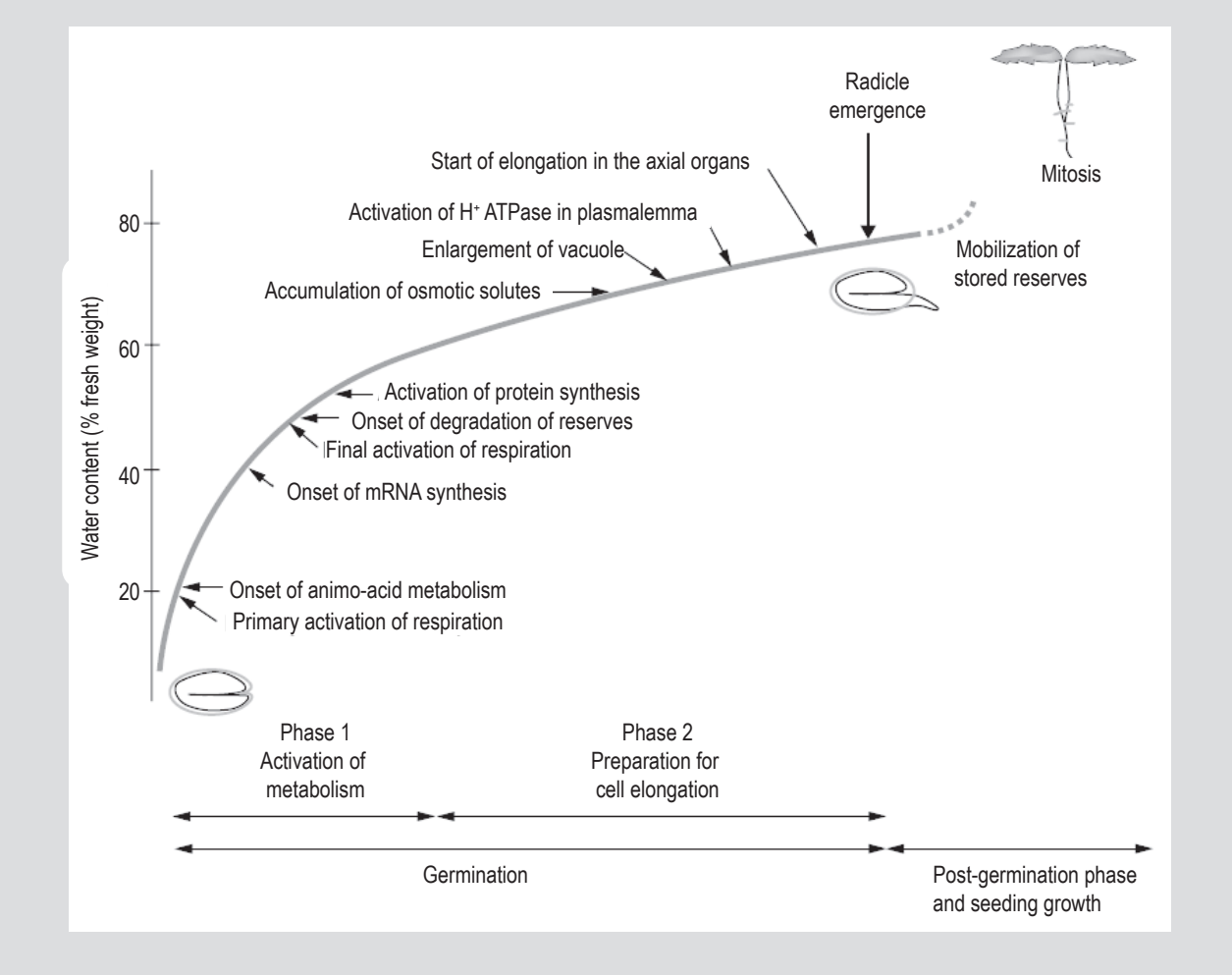

Figure 1. The main events occurring during germination (Obroucheva and Antipova, 1997).

opposition to each other (Grappin *et al.,* 2000). During dormancy and at the beginning of germination the hormone abscisic acid plays a major role. Later gibberellins become the most important regulators. To maintain dormancy the *de novo* synthesis of abscisic acid is required; however, desiccation also maintains dormancy even when abscisic acid levels are extremely low (Penfield and King, 2009).

3. Changes in macro components of soybean

For the growth of the new specimen a sufficient supply and quality of storage nutrients are available in the mature seed. These nutrients provide the raw material and part of the energy necessary for the anabolic processes of the germinated seed. The rapid imbibition of the initially sound seeds (i.e. the first step of germination) leads to the progressive resumption of metabolic activity, gene expression (transcription), protein synthesis and processing, and DNA repair (Weitbrecht *et al.,* 2011). 8 h of initial soaking in water results in an increase from 6 to 57% of the moisture content of the soybean. Within the seven-day germination period the moisture content continues to increase gradually from 57% to 82% (Shi *et al.,* 2010). The changes in the individual macro components during seed germination depend on the soybean cultivars and on the germination conditions. However, it can be said that there is an increase in relative protein content, and a decrease in sugar and lipid content during germination (Bau *et al.*, 1997). Shi *et al.* (2010) germinated soybeans for seven days at 25 °C. They observed an increase of approximately 4% in the protein level and a 5-6% reduction in the carbohydrate and lipid content after the 7-day germination. They examined the effect of light but the presence of light during germination had only a little, if any, effect on the levels of the micro- and macronutrients in soybeans. Wilson and Kwanguen (1986) reported that the mature soybean seed contained approximately 20 w/w% oil, 90% of which was triacylglycerols. For the growing of the seed (during the germination process), one half of the triacylglycerols was used as the major carbon source (Wilson and Kwanguen, 1986). Although there are differences in the data provided by different authors concerning the amino acid profile, it is assumed that it does not change greatly during the germination of the soybean. Only a noticeable increase in

aspartic acid was observed, whereas there was a gradual decrease in the available lysine (Bau *et al.,* 1997).

Enzymes necessary for the synthesis of various macromolecules are gradually activated after the rehydration of the core. Poly-ribosomes appear quickly, link with the mRNAs and so the synthesis of parallel multiple copies of the same protein progresses. Simultaneously, as the protein synthesising complex aggregates, the numbers of free ribosomes decreases. The ribosomes, tRNAs and amino acids required for protein synthesis are already present in the dry seed; moreover, all of them are available in sufficient quantities for the synthesis which will take place in the dry embryo. Therefore, when the legume seed is hydrated, the synthesis can begin immediately. However, the mRNAs required for the translation dissolve slowly, so the synthesis of the mRNA starts very quickly, earlier than the protein synthesis. In this way these newly synthesised mRNAs are incorporated into the protein synthesising complex. In addition, it is likely that the mRNAs found in the dry seed are also conserved in a form that allows early protein synthesis. It has been observed in many plant species that their mRNA pattern changes in a few hours after water uptake, because of the complicated utilisation and degradation of the newly synthesised and already available mRNAs (Bewley and Black, 1994).

Changes in the protein composition during sprouting in soybean

A significant percentage of the stored nutrients are proteins. Degradation of these proteins provides the amino acids and part of the energy for seed growth. The reserved proteins are found mainly in the aleurone layer and in the endosperm of the legume seed. The biosynthesis of proteins starts shortly after water absorption, even in the first stage of germination. Among these newly synthesised proteins there are also the enzyme proteins which are important regulators of the germination process and are essential for elongation.

Soybean has an extremely high protein content; its crude protein content varies between 36 and 41% (Andriska and Ponyi, 1989). It also has a balanced amino acid composition; however, it contains relatively little cystine, cysteine and methionine, but is rich in lysine (Sági, 1997).

The proteins of legumes such as soybean are usually classified on the basis of Osborne fractionation. Soybean has only those kinds of proteins that belong to the albumin and globulin fractions. Among them the globulin type proteins account for 90% of the bean's proteins. Globulins can be separated into two further fractions by ultracentrifugation or by chromatographic methods: to 7S fraction (vicilins) and to 11S fraction (legumins). In soybeans these proteins are called glycinin and β-conglycinin, respectively (Wolf, 1969). The amino acid composition of the 7S- and 11S-globulins is very similar except with regard to their methionine content. Greater differences can be observed in their carbohydrate content because 7S-globulins contain significantly higher amounts of carbohydrates than the members of the legumins fractions. The 11S-globulins consist of six subunits while the 7S consist of three. The thermal stability of the two groups (which is significantly influenced by the association or the dissociation of the molecule) differs. The dissociated proteins precipitate easily when the temperature increases (Belitz and Grosch, 1985; Hajós, 2008).

The conformational stages of the proteins are different according to whether the protein is present in the dormancy phase or in the germination phase. Bogomolov *et al.* (1997) examined the conformational changes of the reserved proteins during soybean germination. In the dormancy phase the reserved proteins have a particular secondary structure, which is probably resistant to proteases. Thanks to water penetration, at the start of swelling another kind of secondary structure is formed. Finally, in the last phase of swelling, the protein molecule is defenceless against proteases. The conformation of globulins alters significantly, i.e. the secondary structure changes, the former H-bridges split up and new H-bridges are formed. These changes have a noticeable effect on the denaturation temperature of the proteins. The molecular weight of the proteins present in the dormant soybean seeds varies widely; it contains relatively high molecular weight globulins as well as relatively low molecular weight albumins. The temperature required for the denaturation of the proteins increases gradually as the size of the molecules grows. At approx. 70 °C only the albumin-like proteins are denatured, while a higher temperature is required for globulins. In addition to the alteration in temperature required for protein denaturation, the denaturation enthalpy also changes during germination. As germination progresses the change in denaturation enthalpy is different in the 7S and 11S proteins, while the change in temperature required for denaturation is similar (it decreases gradually) in the two proteins (Bogomolov *et al.,* 1997).

Reserved proteins accumulate in the middle and late stages of seed maturation and are placed in so-called protein bodies, structures that are surrounded by a membrane (Müntz, 1998). They can be found in the storage tissues, in the embryonic axes and also in the cotyledons. Since proteins are protected against premature proteolytic digestion, their degradation during accumulation is negligible (Madison *et al.,* 1981). So far no study has observed any inactive enzyme stores that would have been activated during germination. However, many research results confirm that the protein bodies of dry seed contain mature, i.e. active, enzymes. Endopeptidases and carboxypeptidases are also present in the protein bodies of the dormant seed. These enzymes

play an important role in the initiation of storage protein mobilisation. Mobilisation continues during germination and during seedling growth but is only possible when protection against proteolytic digestion has ceased. Either the enzyme turns into its active form or the protein conformation is changed (so protection ceases). In the case of soybean the protection of the proteins ceases because of the degradation of protease inhibitors (Wilson *et al.,* 1988). The *de novo* synthesised proteases (peptidases) play a significant role in degradation only in the later phase of germination and after germination. Researchers have reported stored proteases only in the cotyledons of soybean. The stored proteases identified so far belong to the metalloproteases, which can degrade the globulins of soybean in *in vitro* conditions (Müntz *et al.,* 2001).

Catsimpoolas *et al.* (1968) detected six immunochemically different protein components in the protein bodies. One of these is the 11S component, which is the same as glycinin. The 7S protein component was also identified. The N terminal amino acid composition of the two abovementioned proteins is different. The protein bodies begin to swell in the presence of water and they often exceed more than twice their original diameter. Finally, they disintegrate into particles with a diameter of less than 0.5 μm. This can be prevented if the pH value is held at 5 because the solubility of the glycinin is the lowest at this pH value. Glycinin is the most abundant protein in the soybean, often referred to as soy-protein. Protein bodies contain carbohydrates and lipids in only a small amount: in general 5% is lipid, of which 0.75% is triglyceride, 1.5% is free fatty acid and the rest is phospholipid. Phytic acid binds strongly to glycinin so the protein can be found in a bound form in the protein bodies (Tombs, 1967).

Changes in the protein composition of the soybean due to various abiotic stresses have been examined by several scientists. Ferritin plays an important role in protection against stress caused during germination or seedling growth. Ferritin is a protein that stores iron. Its level decreases gradually during normal germination conditions (Masuda *et al.,* 2001), but increases during drought or if the seed is planted in saline soil. Although the effects caused by the stress are different, all of them presumably induce oxidative stress. Ferritin is an important member of the iron-mediated defence mechanism against oxidative stresses (Alam *et al.,* 2010; Briat *et al.,* 1999). The so-called PM36 protein, a seed maturation promoting protein, was observed when examining the effect of high salinity soils. The protein is synthesised during late embryogenesis and degrades rapidly in the early stages of germination, however, its level increases due to saline stress. The PM36 is presumably a storage form of biotin, which promotes growth during germination (Hsing *et al.,* 1998; Xu *et al.,* 2011).

Changes in carbohydrate composition during sprouting in soybean

Mature, dry soybean contains approximately 30% carbohydrates, half of which is dietary fibre. The majority of the soluble carbohydrates are oligosaccharides, containing sucrose, stachyose, raffinose and trace amounts of verbascose. The consumption of soy and soy products is greatly affected by indigestible, flatulence-causing oligosaccharides (Kuo *et al.,* 1997).

The quantity of soluble saccharides and the degradative process of saccharides have been examined in various plant seeds during germination by Kuo *et al.* (1988). Among the soluble saccharides of soybean, stachyose is the most abundant, followed by sucrose. The germination process was carried out at 27 °C for five days, taking samples on each day. As a result of soaking, the raffinose family oligosaccharide content of soybean increased slightly compared to dormant seed. However, after a short period of germination the soluble saccharide content began to decrease, and reached less than half the quantity of dormant seed after two days of germination. During five days of germination the flatulence-causing saccharides disappeared. While the amount of sucrose decreased, fructose and smaller amounts of glucose accumulated in the embryonic axis. The cotyledons' monosaccharide content was undetectable. These results suggest that the breakdown of sucrose takes place in the embryonic axes. Researchers assume that raffinose family oligosaccharides present in the cotyledons are hydrolysed directly during germination, that they produce sucrose and that their further metabolism – after transporting – takes place in the embryonic axes. This degradation process offers energy to the developing seed in the initial stages of germination.

Martín-Cabrejas *et al.* (2008) germinated soybean and non-conventional legumes at 25 °C for 96 h under different light conditions. They observed that the biological process resulted in a 20% decrease in the insoluble dietary fibre fraction of soybean in all conditions used. Moreover, the total soluble sugar content of soybean showed a 16% decrease and the raffinose family oligosaccharides a more than 80% decrease. The level of glucose, fructose, galactose, ribose, sucrose, maltose and mannotriose increased slightly. The increasing levels of reducing sugar are the result of the gradual consumption of the carbohydrate store, including residual starch and oligosaccharides of the hydrolysed raffinose family.

Donangelo *et al.* (1995) observed a small decrease in the total amount of carbohydrates in the soybean after 48 h germination at 28 °C in the dark. Starch, non-starch polysaccharides, low-molecular-weight sugars and dietary fibre content were examined. The major reductions were 79.6, 66.7 and 64.7% in the content of sucrose, raffinose

and stachyose, respectively. Dietary fibre and non-starch polysaccharides showed a slight increase after the two-day germination process.

Changes in the lipid composition during sprouting in soybean

The protein and lipid content of soybean is extremely high. Its fat content is approximately 20%, depending on the type and growing conditions (Dornbos and Mullen, 1992). Almost 87% of this is unsaturated fatty acid, of which linoleic acid is present in the largest amount, oleic acid in a smaller amount and linolenic acid in an even smaller amount. However, the linolenic acid content of soybean is still remarkable at 6.4 g/100 g fatty acid (Grela and Günter, 1995).

Dhakal *et al.* (2009) examined the fatty acid content of six soybean varieties. They investigated separately the properties of raw, 5, 6 and 7 day germinated beans and in addition to whole seeds and sprouts they also analysed individual parts (cotyledon, seed coat, etc.). The fatty acid composition changed, both among species and among different parts of the seed or germ. Among the investigated fatty acid components of the seeds, when the germination time was different only the amount of palmitic acid, linoleic acid and linolenic acid changed significantly. The amount of linolenic acid increased significantly until the sixth day of germination in each tested soybean varieties except one. However, in all cases there was less linolenic acid in the seven day than in the six day germinated seeds.

Mostafa *et al.* (1987) germinated soybean for six days and examined the fatty acid composition of the plant during that time. The amount of total unsaturated fatty acids decreased gradually as germination progressed; as a result the total unsaturated fatty acids/total saturated fatty acid ratio also decreased. The reason for this is presumably the desaturation of fatty acids during β-oxidation (Dutton and Mounts, 1966).

Kim *et al.* (2013) germinated soybean germ at room temperature for 24 h. (The actual meaning of soy germ according to Kim *et al.* was described above.) Among others, changes in the fatty acid content was also investigated during the biological process. Of the unsaturated fatty acids linoleic acid (53.59%) and linolenic acid (21.06%) can be found in the largest quantity in the soybean sprouts. Only minor changes were observed in the fatty acid composition during the one-day germination process; there were a slight decrease in saturated fatty acids, as well as a slight increase in unsaturated fatty acids (oleic, linoleic, linolenic).

4. Changes in micro components of soybean during germination

Sprouting is the fastest growing period of plant life and is known to increase numerous essential nutrient levels and bioavailability in soybeans. For example, the levels of various vitamins increase significantly during germination. Furthermore, the anti-nutritive factors, harmful for the human body, are partly or fully degraded.

Wai *et al.* (1946) examined the carotene, thiamine, riboflavin, niacin and ascorbic acid content of germinated soybean. They measured the amount of the abovementioned molecules in the original dry seed, in soaked seed soaked for 10 h and after 24, 48, 54 and 72 h of germination. Only for thiamine an increase in the amount could not be observed during the biological process. Although thiamine levels first increased to approximately 1.4 times of the soaked value until the 24-h germination time, thereafter they decreased to levels of the 54-h germination time. In the case of carotene, riboflavin, niacin and ascorbic acid, increases were observed 48 h after soaking. Comparing the 72-h germination values to the values of the soaked seeds, carotene and riboflavin increased approximately 2.5 times, niacin approximately 2 times and ascorbic acid approximately 6.8 times. Of all the vitamins, ascorbic acid content showed the most marked increases during germination (Wai *et al.,* 1946). Soybean contains no or undetectable amounts of ascorbic acid in the mature (non-germinated) seed, which increases greatly during germination. Huang *et al.* (2014) measured 5.27 μg/g ascorbic acid content after two days' germination of soybean. During the 5-day germination it reached the highest value.

The tocopherol and phytosterol content of soybean also varied during the germination process. The total phytosterol level (in which β-sitosterol is the predominant form) increased by almost 98% until the third day of germination. Tocopherols have antioxidant properties and soybean is a rich source of them. The amount of γ- and δ-tocopherols increased from the first day of germination till the third day and then decreased gradually. In addition to this the α-tocopherol concentrations were low and remained constant during the biological process (Shi *et al.,* 2010).

The total phenolic content of soybean increases as germination progresses. Scanning the germination process for 5 days, the sample germinated for 4 days showed the highest total phenolic content. This value was approximately 330% higher than the value of the non-germinated soybean. The beans germinated for two days showed a slight decrease in total phenolic content compared to the beans germinated for one day, but the two-day value was still 208% higher than the value of the non-germinated soybean. Isoflavones represent the majority of total phenolic compounds. They help protect the plant from environmental factors (Huang *et al.,* 2014).

Mostafa *et al.* (1987) reported that trypsin inhibitor activity decreased gradually as germination progressed. They measured the fastest decrease from the second to the fourth day of germination. According to their data the reduction in trypsin inhibitor activity was more than 10% after two days of germination and almost 30% after four days of germination. Changes in the amount of the Bowman-Birk soybean trypsin inhibitor and Kunitz trypsin inhibitor were examined separately during germination. Before the extensive degradation of the two inhibitors, they underwent an initial limited proteolysis. The degradation of the Bowman-Birk inhibitor is started by the protease B1 enzyme. This enzyme appears in the first day of germination and peaks on the fourth day, so the level of the inhibitor declines from the second day. Levels of the Kunitz trypsin inhibitor begin to fall on the third day of germination (McGrain *et al.,* 1989). Protease K1 is the enzyme cleaved at the carboxy-terminal and removes five amino acid residues from the native Kunitz inhibitor. It is a cysteine protease that peaks in activity on the fourth day of germination. As germination continues, more enzymes are involved in the degradation of both inhibitors. Presumably these inhibitors are localised in the protein bodies (Wilson *et al.,* 1988). So it can be stated that the changes in the profile of the Bowman-Birk trypsin inhibitor occur earlier and are more pronounced than the changes in the Kunitz trypsin inhibitor profile. As the proteinase inhibitors function as a storage place for amino acids or amino groups, it makes sense to degrade the inhibitors during germination.

It is known that soybean contains relatively high levels of Ca, Zn and Fe. The sodium, potassium, magnesium, calcium, iron, zinc, phosphorus and chlorine content of raw soybean is 33 mg/kg, 14 g/kg, 2.1 g/kg, 2.1 g/kg, 71 mg/kg, 8.3 mg/kg, 4.9 g/kg, 58 mg/kg, respectively (Belitz and Grosch, 1985). The amount of Ca, Mg, Mn, K and Cu differs slightly in the germinated and ungerminated soybean seeds. Zn concentration increased slightly in the late stage of germination, while the Fe concentration fluctuated during germination (Shi *et al.,* 2010). In contrast to these results, Bau *et al.* (1997) reported an 11.0% increase in Ca and a 21.5% increase in Mg in the 5-day germinated soybean, but this increase may have resulted from the tap water used for steeping and rinsing seeds.

Changes in the allergens during sprouting

Unfortunately, the number of people suffering from food allergies is still increasing, and soybean is responsible for a remarkable percentage of them. For example, in the USA soybean is the most common source of food allergy caused by plants aside from peanut (Astwood *et al.,* 1996). Among children soy allergy is even more frequent. Unfortunately, the proteins causing allergic reactions are mostly resistant to food processing, i.e. they retain their allergenicity after cooking, baking or other similar processes. The only way to successfully treat this disease is to completely avoid the allergen in the diet.

Three main soy allergens are distinguished with the help of IgE antibodies obtained from the serum of soybean-sensitive people: they are the Gly m BD 68K, Gly m BD 30K and Gly m BD 28K molecules. These proteins belong to the 7S globulin fraction of soybean. The Gly m BD 68K is actually the α-subunit of the β-conglycinin (Tsuji *et al.,* 2001). About 25% of those allergic to soybean respond to it (Ogawa *et al.,* 1991). β-conglycinin is a salt-soluble globulin consisting of three subunits (α', α and β) and has a 180 kDa molecular weight (Breiteneder and Ebner, 2000; Burks *et al.,* 1988). More than 65% of people allergic to soybean respond only to the Gly m BD 30K protein. Although it is present only at low levels in the soybean seed, it should be considered as a major allergen (Helm *et al.,* 1998). This protein belongs to the cysteine proteases in the papain superfamily. It has not been reported to have any enzymatic activity and its function is unknown (Herman *et al.,* 2003). The Gly m BD 28K is very similar to the MP27/MP33 protein of the pumpkin seed, and to the globulin-like protein of the carrot. So Gly m BD 28K is a vicilin-like storage protein (Tsuji *et al.,* 2001). The allergen triggers a response from around 25% of the sensitive individuals (Ogawa *et al.,* 1991). Luckily, soybean varieties have been found in nature that do not contain Gly m BD 28K. Almost 80% of the Japanese varieties do not contain this protein (Yamanishi *et al.,* 1996). Soybean also contains profilin which shows cross-reactivity with IgE antibodies of the patient's serum. Profilin has a size of 12-15 kDa; it can be bound to actin monomers and regulate the polymerisation of actin filaments (Carlsson *et al.,* 1977; Tsuji *et al.*, 2001). Soybean also contains minor allergens, such as lecithin or Kunitz-trypsin inhibitor (Astwood *et al.,* 1996). The Kunitz-trypsin inhibitor can trigger anaphylaxis as well (Moroz and Yang, 1980). Most people with food allergies only respond to one protein in the food, but unfortunately some people show over-reactivity to several soy proteins.

The spatial and temporal expression of the individual allergen proteins gradually change during the development of the seed and during germination. In the case of soybean the allergenic proteins accumulate along the embryonic axis as well as in the cotyledons; however, they accumulate faster and in larger amounts in the cotyledons. This is also due to the fact that their degradation starts later in the cotyledons. The degradation of the proteins starts in order to ensure the nutrients required for the development of the seedling. The level of Gly m BD 28K already begins to increase 14 days after flowering, which is earlier than any other known allergens. Its degradation is also relatively fast and itis more effective in the embryonic axes. However, the level of protein starts to decrease already 24 h after water intake in the cotyledons, then increases significantly between 36 and 48 h, only to decrease rapidly again after 72 h. Finally, after

108 h it is fully degraded. On the other hand, the amount of Gly m BD 28K gradually decreases in the embryonic axes after water intake from 24 h after water intake up to 72 h. The Gly m BD 30K protein level starts to degrade after 36 h of germination in the cotyledons; however, its degradation starts 12 h earlier in the embryonic axes. The kinetics of the degradation of the Gly m BD 68K molecule is also different in the two places. Its level starts to decrease 36 h after water intake in the cotyledons and 12 h earlier in the embryonic axes. The protein becomes undetectable in the embryonic axes after 96 h of germination. Thus the regulation of the synthesis and the degradation of the three main soybean allergens are dissimilar during the development of the seed. The temporal distribution of their quantity is therefore different. Proteases involved in the degradation activity are regulated by several mechanisms. Different proteases are probably involved in the degradation of the three proteins whose activities are different during the germination period. Eventually 108 h after the beginning of germination all three abovementioned allergens are fully degraded (Wu *et al.,* 2012).

Sprouting and the subsequent heat treatment, such as an allergenic capability lowering procedure, have several advantages. They are simple, economical and easy to achieve on an industrial scale compared to those technological operations that aim to eliminate the allergen proteins or decrease their numbers. The food prepared this way is safe because no microbiological enzyme or detergent is used during this process. A further advantage is that the amount of various functional components increases during germination, with the result that, after germination and subsequent heat treatment, functional food can be obtained.

Changes in bioactive components during sprouting

Common features of bioactive components are their health benefits for the human body. They contain thousands of compounds. Bioactive components are usually present in small quantities in food. They have antimicrobial, antioxidant, antithrombotic, anti-tumour, immunomodulatory and anti-inflammatory effects, among others. They may have a positive effect on blood pressure, cholesterol and blood sugar levels. Their chemical structures are very different and their biological functions can be diverse. They can be found mostly in plants or in specific parts of them (Kris-Etherton *et al.,* 2002). Luckily, the levels of many bioactive compounds increase during the biological process of germination. The following sections will review the most important ones.

Phytoestrogen content

Phytoestrogens are compounds of the original plant that have antioxidant properties and a steroid skeleton. One group of phytoestrogens, the isoflavones, can be found in abundance in soybean. They play an important role in many biological processes. For example, they competitively inhibit the thyroperoxidase, which assists in the conversion of thyroxine to triiodothyronine (Divi *et al.,* 1997). Therefore it can be used in areas where iodine intake is very low. It has been observed in some research studies that isoflavones reduce the probability of osteoporosis in postmenopausal state and slightly reduce the level of LDL cholesterol.

Twelve different kinds of isoflavones have been identified in soybean; these are arranged into four subgroups according to their functional group (Table 1). According to several researchers these compounds, and especially the aglycones, are beneficial against cardiovascular disease and cancer. Furthermore, they act as anti-oestrogens, and can help to preserve the flexibility of the blood vessel walls (Wang and Murphy, 1994).

The levels of isoflavones and their ratio show a significant change during the plant life cycle. Lin and Lai (2006) examined the isoflavone content of the immature, one-day and four-day germinated seeds (Figure 2). According to their measurements the total isoflavone content of soybean increases at the beginning of germination, but the total isoflavone content of the four-day germinated seed is lower than the total isoflavone content of the immature soybean. Glycitin, acetyl-daidzin and acetyl-genistin are present only in a barely detectable amount in the three examined stages. Both researchers also recorded the chromatogram of the soybean's isoflavones in the three abovementioned stages. The levels of malonyl-glucoside were highest in all stages. The levels of aglycon and all the isoflavones increased significantly after the first day of germination. At the beginning of germination the levels of isoflavone rise in soybean and in most parts of the legumes for two different reasons. The first is that aglycons are released

Table 1. The classification of soybean isoflavones (Wang and Murphy, 1994).

aglycon	glucoside	malonyl-glucoside	acetyl-glucoside
daidzein	daidzin	malonyl-daidzin	acetyl-daidzin
genistein	genistin	malonyl-genistin	acetyl-genistin
glycitein	glycitin	malonylglycitin	acetyl-glycitin

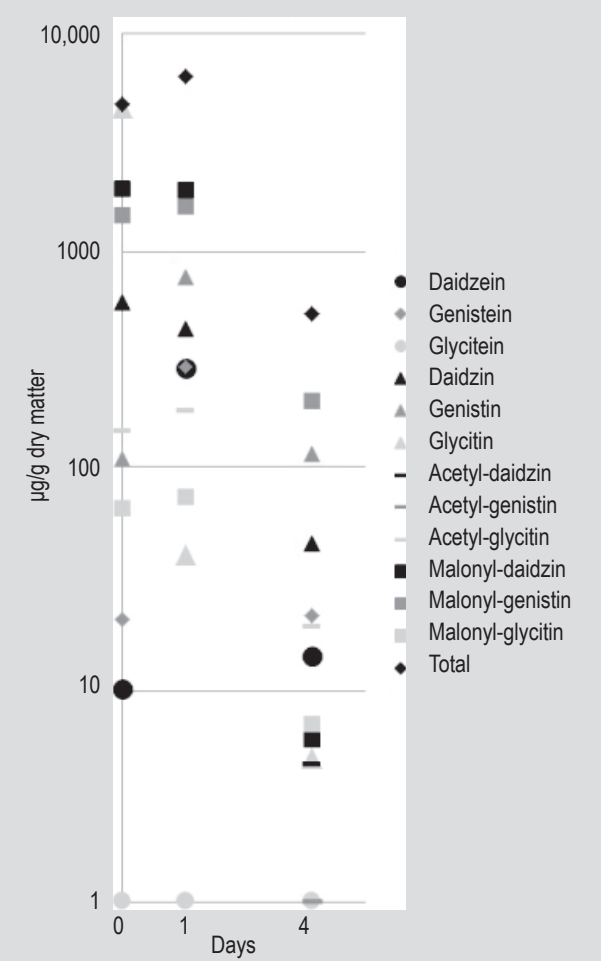

Figure 2. The isoflavone composition of soybean (Lin and Lai, 2006).

from the glucosides and the other is that isoflavones are created by biosynthesis. Levels of both the aglycons and the total isoflavones fall significantly in the seeds germinated for four days (Lin and Lai, 2006).

Kim *et al.* (2013) measured the changes in the isoflavone profile of the sprouted soy sprouts during germination, using HPLC (Figure 3). The soybean seed consists of an outer seed coat, two cotyledons and the embryo (Liu, 1996). Kim *et al.* (2013) called the embryo soy germ and conducted an examination of it. Generally, 2% of the total seed is the embryo. This so-called germ is fairly compact, but if it is separated from the rest of the seed then the valuable nutrients can easily be extracted. Presumably the soy germ contains bioactive compounds in a relatively larger amount than the cotyledons, which form the bulk of the seed (Schryver, 2002).

In the ungerminated whole soybean genistein is present in the largest amount, followed by daidzein and finally glycitein. However, glycitein is present in the largest amount in the soy germ, followed by daidzein and finally by genistein. The extent of biosynthesis of isoflavones is

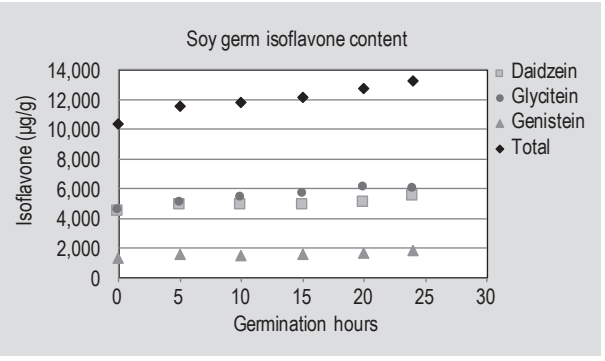

Figure 3. Changes in soy germ isoflavone content during germination (Kim *et al.*, 2013).

probably different in the cotyledons and in the germ, because of this different isoflavone profile that can be formed. In the dormant phase the total isoflavone content of the soy germ is approximately 7.5 times higher than that of the whole seed. The research team continued the germination for 24 h. During this time the total isoflavone content grew continuously reaching 127.9% of the starting amount. The amount of genistein increased by 39.4%, glycitein by 31.1% and daidzein by 21.4%. The total isoflavone content of germinated soy germ was approximately 9.6 times higher after 24 h than its content in the whole soybean (Kim *et al.*, 2013).

The chemical structures of isoflavones affect their bioavailability and biological activity. During an *in vivo* experiment Hutchins *et al.* (1995) observed that the aglycon form of daidzein and genistein were more bioavailable, meaning that this form can be better utilised than the conjugated form of those molecules. The glucoside forms are very poorly absorbed in the human body due to their high molecular weight and hydrophilic nature (Brown, 1988). Based on a number of assumptions, glucosides should be converted into their aglycon form in order to be absorbed in the human intestine. The conversion is carried out either by the bacterial flora found in the human intestine or by the hydrolytic enzymes in the digestion tract. The biological accessibility of the isoflavones in the human body is significantly affected by deglycosylation (Albertazzi and Purdie, 2002).

Cooking, baking and other technologies do not affect the total isoflavone content of soybean. Only the ratio of the conjugated and unconjugated forms is changed. However, the alcoholic solution, which is used for the extraction of soybean meal and the other processing steps like fermentation, can reduce the total isoflavone content. Isoflavones are bound to proteins in soybean (Coward *et al.*, 1993).

Phytic acid content

Germination is considered a suitable procedure for improving the nutritional value of legume seeds by reducing levels of anti-nutritional factors. Phytic acid, also known as myo-inositol-hexakisphosphate (IP6), is an important phosphoric acid storage form present in many plant tissues. Its hydrolysis to inositol and phosphoric acid is catalysed by the enzyme phytase through several intermediate products. Phytic acid inhibits the absorption of some minerals, for example iron, zinc, calcium and magnesium. It binds with the ions thereby preventing their absorption in the intestine. It is present as a salt in nature in a form known as phytate. (Reddy *et al.,* 1982; Sandberg, 2002).

The amount of phytic acid in food is slightly reduced as a result of simple cooking processes. The phytic acid content of food can be reduced efficiently by soaking in acidic medium or by lactic acid fermentation or germination (Reddy *et al.,* 1989). The seedling utilises the reserved nutrients for its own growth during the germination process. The degradation products derived from the hydrolysed phytic acid are also used as nutrients for the developing plant.

The phytate content of soybean is not uniformly distributed in the seed. As phytic acid binds strongly to glycinin it is present as a protein-bound form in the protein-bodies (Tombs, 1967). Maga (1982) pointed out that when the amount of protein increased, the amount of phytic acid also increases. The mobilisation of the reserved nutrients found in the protein-bodies happens at the beginning of germination. Since the level of the reserved proteins accumulated during seed maturation decreases, the level of phytic acid also decreases.

Lestienne *et al.* (2005) examined the changes that took place in the phytate content of legume seeds due to soaking. Soaking was carried out in water at 30 °C and with slow shaking for 24 h. The phytate content of soybean decreased significantly, by approximately 23%, due to the treatment. At the end of the process the soaking water did not contain any phytic acid, indicating that the enzymatic hydrolysis took place either already in the seed or in the soaking water.

Egli *et al.* (2006) measured the phytase activity and the phytic acid content of some cereals, some legumes and two oilseeds during germination. The enzyme and the phytic acid are mostly accumulated in the cotyledons of the legume seeds. They began the biological process with 16 h soaking and examined the germinated samples after 24, 48 and 72 h. The phytase activity of the soybean decreased slightly as a result of the soaking conditions (Figure 4).

The phytase activity increased during germination in all the investigated legumes and oilseeds. Only a slight increase was observed in the first 24 h, but this became more significant when germination continued. In case of soybean the enzyme activity more than doubled compared to the initial value after 72 h germination (Egli *et al.,* 2006). Some researchers assume there is a latent period of a few days before the increased phytase activity can be observed. For example, the maximum enzyme activity was measured in the case of peas after 6-7 day of germination (Guardiola and Sutcliffe, 1971), while in the case of beans maximum enzyme activity was measured after 6 day of germination (Eskin and Wiebe, 1983).

Egli *et al.* (2006) determined the phytic acid content as the sum of inositol hexaphosphate and inositol pentaphosphate (Figure 5). The lower order inositol phosphates do not form strong complexes with minerals and trace elements and were therefore omitted from the measurements.

Chen and Pan (1977) examined the reduction in the phytate content of peas and soybeans during germination. They measured an increase in phytate activity in soybeans after five days of germination. The increase was approx. 227% compared to the untreated samples. They observed a significantly higher increase in the case of peas.

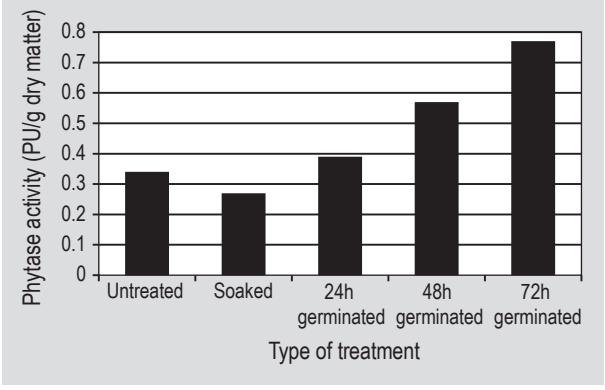

Figure 4. Changes in the phytase activity of soybean during germination (Egli *et al.*, 2006).

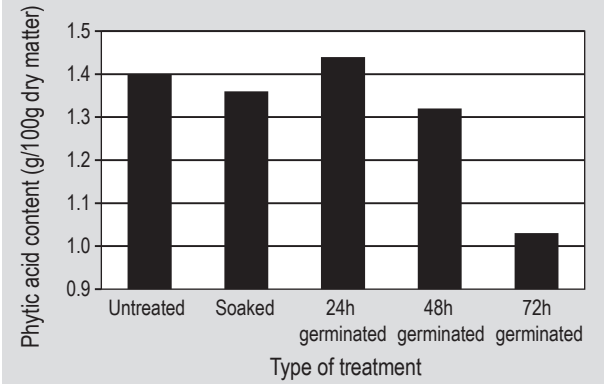

Figure 5. Changes in the phytic acid content of soybean during germination (Egli *et al.*, 2006).

Folate content

Folate's important biological functions are as follows: it acts as a C1 donor in enzyme complexes; it is involved in the degradation of amino acids; it is necessary for the synthesis of the building blocks of nucleic acids, for DNA and RNA error-correcting and for the operation of these macromolecules (Bassett and Sammán, 2010). Therefore, it plays an important role in the formation of new cells, in cell-systems with intensive cell proliferation such as the formation of blood cells, and it is responsible for the integrity of mucosa of the oral, intestinal and urogenital tract. In periods of rapid growth, for instance in childhood or during pregnancy, it is extremely important to ensure adequate folate intake. Since folate helps to prevent damage caused in the DNA, some evidence supports its preventive role in some forms of cancer, particularly in colon cancer (Duthie *et al.,* 2004).

In natural form it can be found for example in liver, egg yolk, citrus fruits, bananas, legumes, many vegetables (especially in those with green leaves), nuts (hazelnut, walnut, almond), yeast, wheat germ and whole grain products. Bassett and Sammán (2010) determined the folate content of soybean. Their quantitative results refer to 100 g of fresh (non-treated) soybean. They measured 101 μg total folate content in the raw soybean and 77 μg total folate in the once-boiled soybean (Bassett and Sammán, 2010). Previous studies measuring raw soybean found higher values of folate (Souci *et al.,* 2008; Yon and Hyun, 2003). Rychlik *et al.* (2007) investigated the folate content of 14 legumes and discovered that the folate content of soybean was the highest.

Rapid cell division is typical during the germination processes of plants. To ensure the transmission of C1 group, folate is necessary for cell metabolism and nucleotide synthesis thus the folate synthesis speeds up with the growth of the seedling (Jabrin *et al.,* 2003). So the folate content can be increased to 1.7-4.3 fold of the content of the non-germinated soybean (Hefni and Witthoft, 2011; Kariluoto, 2008). Shohag *et al.* (2012) examined the changes in the folate content of two soybean varieties during germination. In Figure 6 the total folate content is plotted against the time of germination. It clearly shows that the total folate content of the soybean increases significantly after a short-term germination. In the case of the two soybean varieties tested, the total folate content reaches the maximum level after four days of germination, which is 3.5-3.7 times the initial value. After germination, the folate content decreases significantly. However, the folate content measured after 10 days of germination was still higher than that in non-germinated soybean (Shohag *et al.,* 2012).

In both non-germinated seeds examined 5-CH_3-H_4-folate was present in the biggest amount, followed by the H4-folate. However, the amount of H4-folate was higher throughout the germination process. The levels of these two forms of folate were also the highest in the seeds germinated for four days, corresponding to the amount of total folate. H4-folate is one of the first intermediate molecules of folate biosynthesis and is also a central molecule in the metabolism of folate (Shohag *et al.,* 2012).

Hefni and Witthöft (2012) checked the effect of germination on various varieties of wheat and rice. Depending on the species, a 3-4 fold increase was observed in the folate content after 48-72 h of germination. The folate content of the seedling increased more slowly thereafter. After 96 h of germination a 4-6 fold increase was measured.

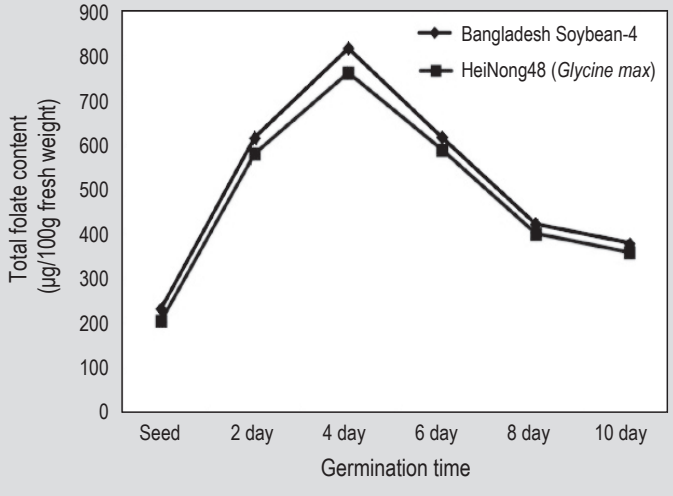

Figure 6. Changes in the folate content of soybean during germination (Shohag *et al.*, 2012).

γ-aminobutyric-acid content

γ-aminobutyric-acid (GABA) is an amino acid derivative formed by α-decarboxylation of glutamic acid. In plants it acts as the nitrogen storage form and plays a part in metabolism (Selman and Cooper, 1977). In animals and humans it functions as a neurotransmitter with an inhibitory effect and it can be found in both the central nervous system and the periphery. GABA enhances the production of growth hormones, the mobilisation of fat, the building of muscles; it has an appetite-controlling effect and is involved in the regulation of sleep. It plays an important role in the regulation of the operation of the cardiovascular system. Furthermore, it has been shown in experiments involving animals and humans that it reduces blood pressure after both central or systemic introduction (Hayakawa *et al.,* 2002). GABA strengthens the immune system (Abdou *et al.,* 2006) and increases insulin secretion by the pancreas. This latter effect makes it important in the prevention of diabetes (Adeghate and Ponery, 2002).

The free amino acid content of soybean, including GABA content, increased gradually during germination. Martínez-Villaluenga *et al.* (2006) examined two soybean cultivars (var. Merit and var. Jutro). The changes in the GABA content during soybean germination are shown in Figure 7.

A remarkable, almost four-times bigger GABA content was observed when the initial and the 4- or 6-day germinated samples were compared. The amount of GABA increased to approximately 1 mg/g dry matter during the long biological process. In the case of both soybean varieties the relationship between GABA content in soybean sprouts vs soybean seeds was illustrated, and can be seen in Figure 8 (Martínez-Villaluenga *et al.,* 2006).

Kim *et al.* (2013) germinated soybean germs for 24 h at room temperature. They investigated among other things the changes in free amino acids including GABA during germination (Figure 9). The total free amino acid content gradually increases as germination progresses. Before germination the soy germ contained 4,290.1 mg/100 g free amino acids and this value increased to 6,089.6 mg/100 g after 24 h germination. The ratio of growth was almost 42%. The level of GABA was more than 27 times higher after one day of germination (Kim *et al.,* 2013).

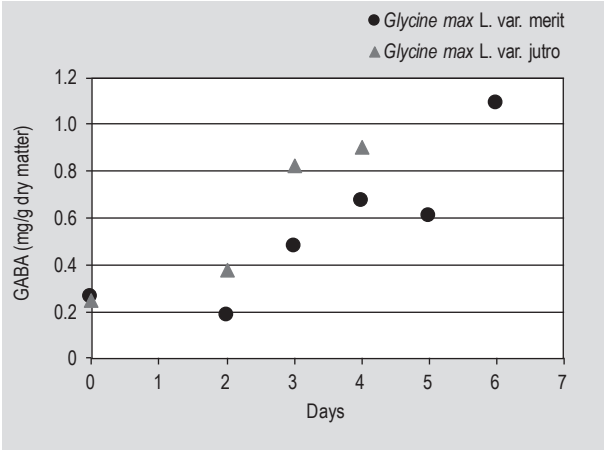

Figure 7. γ-aminobutyric-acid (GABA) content of germinated soybean (Martínez-Villaluenga *et al.*, 2006).

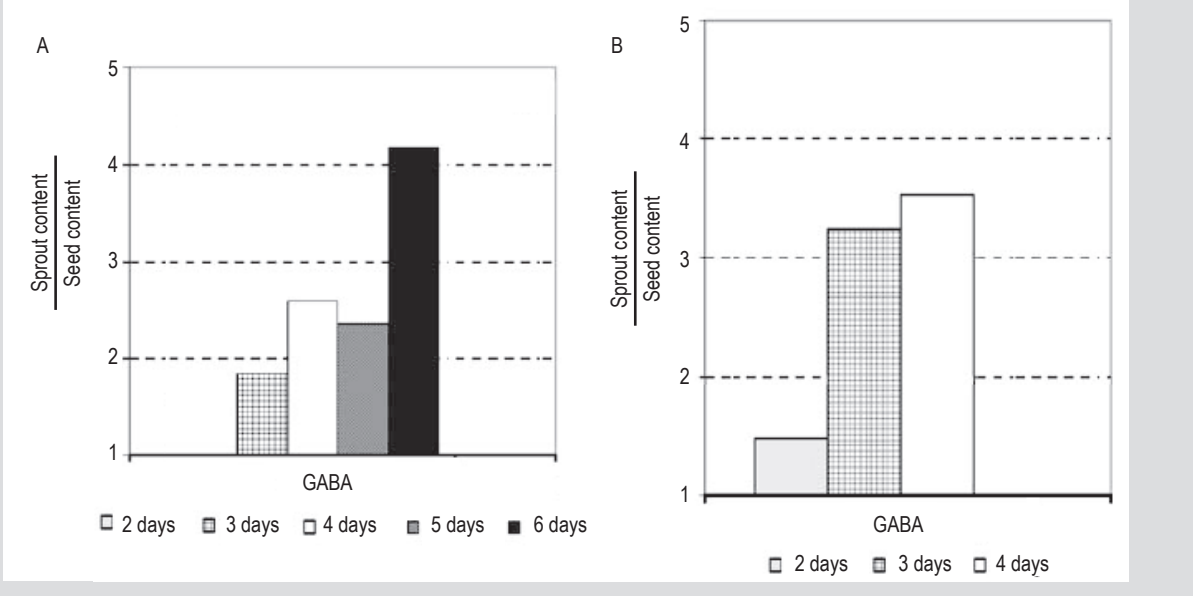

Figure 8. Proportion of the γ-aminobutyric-acid (GABA) content of germinated/non-germinated soybean varieties Merit (A) and Jutro (B) (Martínez-Villaluenga *et al.*, 2006).

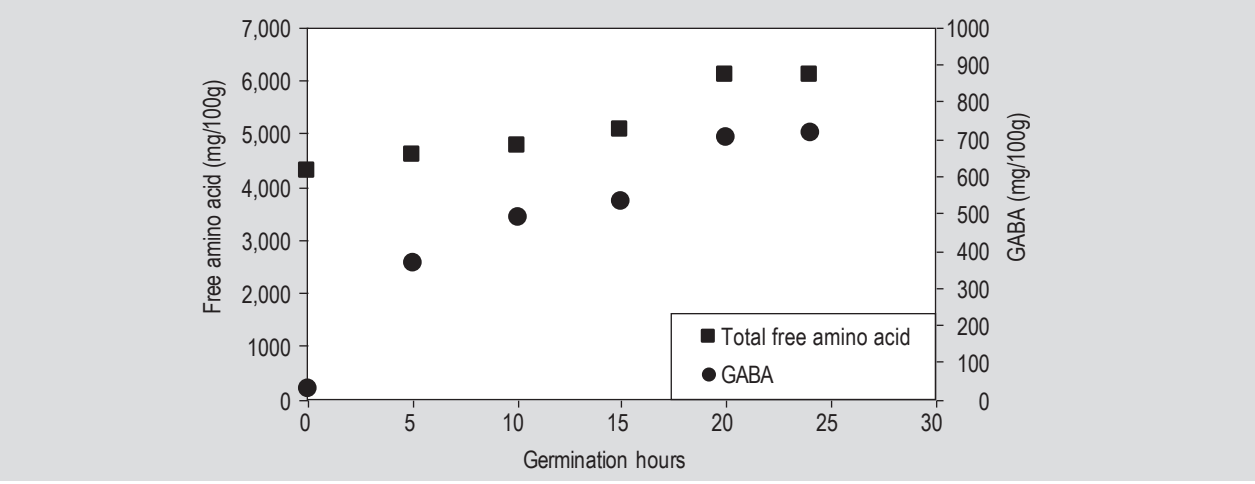

Figure 9. Changes in the total free amino acid and γ-aminobutyric-acid (GABA) content of soy germ during germination (Kim *et al.*, 2013).

Tocopherol content

The tocopherols are fat-soluble phenolic compounds with methyl groups. They protect the body from the damaging effects of free radicals, acting as antioxidants. Natural vitamin E is the comprehensive name for four different tocopherols. These four different tocopherols are the α-, β-, γ- and δ-tocopherols; they differ only in the location and number of the methyl substituents found on the chromanol ring. Their biological properties also differ slightly. For example, it has been shown that the γ-tocopherols have anti-cancer and anti-inflammatory effects (Elson and Haas, 2013). The accumulation of oxidative free radicals in our body plays a role in the formation of several diseases that can be prevented by the consumption of these compounds. Vitamin E inhibits the formation of diabetes, liver diseases and atherosclerosis as well (Moon and Shibamoto, 2009). It inhibits atherosclerosis by preventing LDL-cholesterol oxidation. Furthermore, vitamin E contributes to the extended lifetime of red-blood cells and promotes the absorption of iron by maintaining it in a more easily absorbable form. It is involved in the transmission of genetic information, in protein, carbohydrate and fat metabolism, as well as in the liquid household of the organism. It is an essential component of the cell's respiration, and is required to slow down the aging process and help maintain the biological activity of vitamin A. In cosmetic products vitamin E is used to increase skin hydration and reduce the harmful effects of UV-rays (Elson and Haas, 2013).

Soybean contains tocopherols in a significant amount. Of α-, β-, γ- and δ-tocopherols, the γ-isomer is found in the largest concentration (Seguin *et al.,* 2009). Soybean contains only small amounts of β-tocopherols (Yoshida *et al.,* 1998) while δ-tocopherols are present in the second largest amount. In most soybean varieties 60% of total tocopherols are γ-isomer and 25% of them are δ-isomer (Ujiie *et al.,* 2005). Seguin *et al.* (2009) examined 20 soybeans with different genotypes. The seeds were grown in six different locations in the Quebec province. The tocopherol concentration changed significantly among different genotypes. The biggest fluctuation was shown by α-tocopherols, ranging from 8.7 to 33.2 μg/g. There was only a small difference among the same genotypes grown in different environments. Carrao-Panizzi and Erhan (2007) studied the tocopherol content of soybeans with different genotypes and in different growing conditions. They examined a total of 89 different varieties grown in the south and north of Brazil. The particular tocopherol isomers and the total tocopherol content also showed significant variability. Their levels fluctuate according to the genetic and environmental conditions between the following values:

- α-tocopherol: 11-191 mg/kg
- β-tocopherol: 6-64 mg/kg
- γ-tocopherol: 304-1,333 mg/kg
- δ-tocopherol: 174-580 mg/kg
- total tocopherol: 561-1,983 mg/kg

Shi *et al.* (2010) germinated soybeans for 7 days. The germination was carried out at 25 °C and they examined the changes in several macro- and micro components during the biological process. They did not find any differences in tocopherols during germination with/without the presence of light. The tocopherol content of the germinated soybeans fluctuated between 150 and 299 μg/g. γ-isomer was present in the highest amount throughout the whole biological process, followed by the δ- and then by α-tocopherol. The amount of γ- and δ-tocopherols increased from the beginning till the third day of germination and then in the following days it decreased gradually. The amount of α-tocopherol was at a low level throughout the entire biological process with no notable fluctuations. Figure 10 shows the measurement results of 7-day germination in the presence of light and without light.

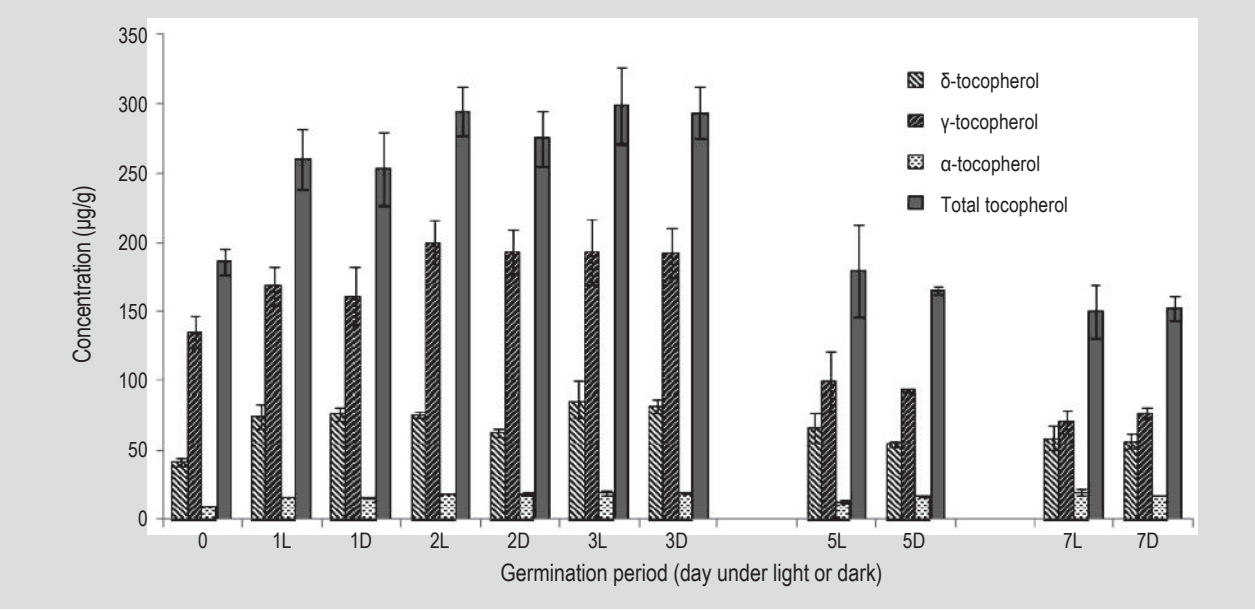

Figure 10. Tocopherol content of soybean during germination (L) with or (D) without light. Numbers represent the days of germination (Shi *et al.*, 2010).

Kim *et al.* (2013) investigated the tocopherol content of soy germ germinated at room temperature for 24 h. They measured the tocopherol content of the whole soybean used initially and that of the germ. The total tocopherol content of the germ was approximately twice that in the whole soybean. The quantitative distribution of tocopherol isomers is also different in the embryo compared to the whole seed. The soy germ contains 59.5% γ-tocopherol, 35.0% α-tocopherol, 4.8% δ-tocopherol and 0.61% β-tocopherol, while the whole seed contains 67.4% γ-tocopherol, 25.5% δ-tocopherol, 6.6% α-tocopherol and 0.49% β-tocopherol. It is likely that the α- and γ-isomers are concentrated mainly in the embryo, while β- and δ-isomers are in the cotyledons. The tocopherol content of the soy germ increased rapidly as germination progressed. The amount of α- and γ-tocopherols increased most significantly among the four isomers, thus germination promotes the increase of tocopherol content in soy germ, but the relative proportion of the different isomers does not change significantly.

Zielinski (2003) investigated the changes in tocopherols and antioxidants with low molecular weight also during the germination of soybean. The germination was carried out at 25 °C for 7 days. After the first day of germination the concentration of each tocopherol isomers had already started to increase. The amount of α-isomer increased the most and γ- the least. The total level of tocopherols decreased thereafter; then, from the fourth day it began to increase again and it reached its maximum value on the seventh day. The concentration of α- and β-isomers reached 4-5 times the initial value, while the amount of the two other isomers decreased by a smaller amount until the 7th day of germination.

Dietary fibre content

The non-digestible vegetable polysaccharides in mammalian food and lignin belong to dietary fibres. Crude fibres are the residues of the vegetable food after acidic and alkaline treatment, while dietary fibres are the residues resistant to digestive enzymes. These are not utilised carbohydrates according to their composition, with the exception of the cell wall builder lignin. They are not digested and not absorbed in the small intestine, but fermented completely or partially by the colonic bacterial flora (AACC, 2001). The dietary fibres can be divided into water-insoluble (cellulose, hemicellulose, lignin, water-unextractable arabinoxylans) and water soluble (pectin, fructans, rubbers, water-extractable arabinoxylans) groups. Water-insoluble fibres have only a very limited ability to bind water, while water-soluble fibres are able to bind significant amounts of water and thus are able to swell (Kalač and Mika, 1997).

The dietary fibres increase bowel movement so excreta and carcinogenic compounds pass through the intestinal tract more quickly. They have several beneficial physiological effects, for example they reduce the cholesterol, triglycerides, and glucose levels in blood (AACC, 2001). As they absorb water in the stomach, they swell and cause satiety. Dietary fibres help defecation and reduce the risk of colon diseases. Several studies demonstrate that high fibre consumption reduces the risk of cardiovascular disease, obesity and diabetes as well as helping the operation of the immune system (Slavin, 2013).

The total dietary fibre of six different (*Glycine max* L.) soybean varieties fluctuated between 22.5 and 29.3 g/100 g dry matter (Lin and Lai, 2006). A significant part of the total

dietary fibre, approximately 86.5-93.5%, is insoluble dietary fibre. The total dietary fibre content of whole and peeled soybean 100 g dry matter is equal but their proportion differs. Peeled beans contain more soluble fibres and fewer insoluble fibres because the seed coat and the cell wall of cotyledons contain the soluble and insoluble fibres in different ratios (Písaríková and Zraly, 2009).

The dietary fibre content of the seed is highly influenced by the germination process. The following observations were made in peas germinated in 0, 2, 4 and 6 days. The temperature of the germination was 20 °C and the germination was carried out both in constant light and in constant darkness. The amount of total dietary fibre increased in all cases compared to the initial, non-germinated seed. The increase in the soluble and insoluble dietary fibres was more significant in the dark than in the light. The amount of insoluble dietary fibre was highest after 6 days of germination in the dark. Its value reached 136% of the initial amount. In the presence of light the amount of soluble dietary fibre had increased already by approximately 65% after two days of germination and it maintained this level after the 4- and 6-day biological process. In contrast, in the dark the amount of soluble fibre increased continuously as germination progressed, so after six days germination it was 2.5 times the initial value. This coincides with observations of other leguminous plants, e.g. soybean and lupine. As the volume of both fibre fractions increases, so the germinated peas contain significantly more dietary fibres than the non-germinated ones. The ratio of the two fibre fractions, defining the core nutritional and functional properties can be changed by varying the conditions of germination (Martín-Cabrejas *et al.,* 2003).

Donangelo *et al.* (1995) examined the changes in the chemical composition of three leguminous plants, including soybean under germination process. The germination was carried out in the dark at 28 °C for 48 h. The amount of each chemical component was measured before germination and after the two-day germination process. In the case of soybean the amount of total dietary fibre and also the soluble and insoluble dietary fibre increased due to germination. According to their measurements the total dietary fibre content of the 48-h germinated soybean was 14.5% higher than the same value of the initial soybean. Of the soluble and insoluble fibre fractions, the amount of insoluble fibre increased the most. On the other hand some researchers observed a decrease in the dietary fibre content during soybean germination (Chitra *et al.,* 1996; Martín-Cabrejas *et al.,* 2003).

5. The short-term germination process

Soybean has a wide range of industrial applications. It is often used in cattle forage and for catering, as well as in the pharmaceutical, cosmetic and biotechnology industry. Many industrial food products are produced from the mature soybean due to its protein composition, which has a complete and balanced amino acid pattern. In addition to the favourable nutritional properties, soybean also has outstanding functional properties for technological purposes, including emulsifying ability, consistence and structure configuration, fat and water binding capacity and viscosity-increasing properties. The versatility of soy-based food products increases significantly when sprouted beans are used as raw material. Germination is a holistic, inexpensive and effective technology to improve the nutritional value of soybeans.

The time required for the technological process increases when germination lasts longer, i.e. 4-6 days, which is disadvantageous from an economical point of view. Also loss in nutrients occurs as the biological sprouting process has progressed when seeds are germinated for a longer time. After a few days' germination (36-48 h) of soybean under suitable conditions the radicle and plumule appear on the seed. The germ uses the earlier released nutrients for its own growth at this time point. The seed goes through internal-structural and chemical conversions, resulting in partial consumption of the original values of soybean. These disadvantageous effects can all be avoided with short-term germination; this way we get a valuable food component from a nutritional point of view compared to the widely used and consumed seeds (Fitorex, 2008).

Anti-nutritive substances, such as enzyme inhibitors and stachyose which inhibits digestion, are partly degraded after short-term germination. The subunit structure of protein is partially decomposed due to the mobilisation effects of germination and therefore the nutritional value and the availability (digestibility) of the compounds increases. The amount of methionine increases (Barcelos *et al.,* 2002), phytases are activated and the amount of phytic acid decreases during the process (Suparmo and Markakis, 1987). Soybeans contain insoluble carbohydrates that are decomposed into sugar during the natural hydrolytic process, i.e. the carbohydrate content of the beans decreases. The amount of isoflavones, β-carotene, vitamins and dietary fibre increases significantly already in the initial stage of germination (Fitorex, 2014).

A special raw material has been produced with short-term germination of soybean for the food industry. This product (YASO) is produced by germinating the soybean for 48 h till the appearance of 5-8 mm long sprouts. Continuous access to oxygen as well as sufficient moisture is provided for the seeds during the procedure. The process is stopped by heat treatment and the germinated soy is cooked until it is soft, if so desired. The final product is precooked and pasteurised so it can be used without any further heat treatment (Fitorex, 2008).

6. YASO, a unique quality food/raw material

YASO is a totally new, soy-based industrial food raw material or additive. It retains all the favourable properties of intact soybean and some of them can be better exploited. Furthermore, all substances with disadvantageous properties (inhibitors, stachyose, allergens) are reduced to a minimum level or are degraded completely. These changes are the result of a natural process, i.e. germination carried out under controlled conditions. YASO production is carried out in a completely natural way; the product does not contain any additives. Only certified non-GM soybean is used as a raw material (Fitorex, 2014). This unique product is widely recognised and the Hungarian company has won several prestigious, international awards in recent years with YASO (Fitorex, 2014). The composition of YASO is summarised in Table 2.

YASO is rich in vitamins and minerals. For example, the E vitamin content is higher than 3.0 mg/g dry material. The calcium and phosphorus content of YASO is also significant and plays an important role in the prevention of osteoporosis. YASO has a higher amount of magnesium, potassium compared to the initial soybean and iron utilisable by the human body. The genistein content of each variety increases to 2-6 times of the air-dried soybean. As a source of highly digestible complete protein and a good source of dietary fibre, omega-3 and omega-6 fatty acids combined with no cholesterol and low carbohydrate, it is extremely suitable for special diets, particularly for diabetics, weight loss management, different hospital diets and healthy aging. Due to its beneficial composition it is a top quality meat substitute. Thanks to its high levels of unsaturated fatty acids and lecithin, YASO is recommended for people suffering from heart and cardiovascular disease or hypercholesterolemia. It may help to control the blood glucose level and has antioxidant properties (Fitorex, 2014).

Table 2. Composition of YASO (Fitorex, 2014).

Component		Quantity
Moisture (m/m%)		62.44
Protein (m/m%)		16.3
Total fat (m/m%)		8.7
of which ω-3, ω-6 (m/m%)		5.7
Dietary fibre (m/m%)		8.3
Carbohydrates (m/m%)		2.9
Minerals (m/m%)	calcium	0.09
	magnesium	0.08
	phosphorus	0.19
	potassium	0.26
	sodium	0.07
Vitamins (µg/g)	vitamin C	140
	vitamin E	32
	vitamin B3	5.1

The taste and smell of products made from soy are mainly determined by the lipid content of soybean. The seed is relatively rich in lipoxygenases. These are compounds that play a significant role in the expression of the characteristic, and generally disliked, taste and smell of soybean and its products. The taste and smell is greatly improved by the short-term germination because the activity of lipoxygenase is decreasing during germination (Suberbie *et al.,* 1981). The unpleasant taste of soybean is absent in YASO due to the enzyme activation and enzymatic hydrolysis processes. This results in a pleasant, almost neutral-tasting product somewhat reminiscent of peanut flavour (Fitorex, 2014).

The mature soybean seed contains α-galactoside carbohydrates, mainly in the form of raffinose and stachyose; these compounds cause flatulence, bloating and loss of appetite. The human digestive system cannot hydrolyse and utilise these compounds, therefore the bacteria in the large intestine start to ferment them. The process causes gas formation resulting in unpleasant side effects. The tetra-saccharide stachyose and the tri-saccharide raffinose degrade into monosaccharides and disappear during the germination process as well as part of the formed monosaccharides (Baltes, 1989). In the case of YASO, both the stachyose, and raffinose content is reduced to a small fraction of the initial air dried values. For example, from the initial 24.0-28.0 mg/g dry matter of stachyose only 2.2-2.3 mg/g dry matter remains in the product. Therefore, the consumption of YASO does not cause bloating (Fitorex, 2008).

The use of YASO in the food industry has several favourable properties, similar to other processed soy products. On the one hand it improves the nutritional properties of the end-product; on the other hand its functional properties can simplify or even speed up the technological process in many cases. During the manufacturing steps of various additive-containing food products the following properties of YASO may be important: emulsifying ability, favourable consistence, structure-configuring ability, fat and water-binding capacity and viscosity-increasing property (Fitorex, 2008).

This unique raw material offers a wide range of product development opportunities within the healthy and functional food segment. YASO is an ideal ingredient for 'something-free' foods (e.g. gluten-free, lactose-free, cholesterol-free and chemical-free); it improves the physiological effect of products, because it is rich in vitamins and omega-3, -6 fatty acids and has low carbohydrate content. Furthermore, it is a totally natural product (Fitorex, 2014).

The product is produced in whole bean, minced and pureed form. Any form of YASO can be consumed as is, however,

it is remarkably easy to add to other food products. The advantage of the pureed form is that it does not require a rehydration step, and can easily be mixed into sauces or dispersed in other mushy food. This makes YASO the perfect food source for patients suffering from swallowing difficulties. Unlike other soy products, this product does not require any special preparation in the kitchen. The usual food preparation methods can be applied, and if it is used as a food additive the commonly used recipes and procedures can be adopted without any change (Fitorex, 2008). It can be widely used in the food industry (bakery, confectionery, snack, semi-finished or ready-made meals and meat industry), in households and in public and hospital catering as well. YASO may become a particularly important component of vegetarian or meat-free diets as an easily digestible vegetable protein source (Fitorex, 2014).

Although the low environmental effect of the manufactured products (and technology) is not a nutritional issue it is still very important. Sustainable development has come to the forefront in the food industry, due to the earth's continually growing population and the intensive utilisation of our natural resources. Production of soy protein is extremely efficient concerning the efficient cultivation of land and the reduction of water usage.

7. Conclusions

Different stages of seed germination result in different tissues in soybean seed from a chemical and morphological point of view. Furthermore, the short-term period of sprouting could provide a unique quality of seed with significant nutritional benefits. In the early phase, i.e. the first 48 h of sprouting the germ, the plant hormones and the preformed enzyme pattern are activated. The start of initiated mobilisation of reserves led by the activated and *de novo* synthesised enzymes results in a decrease in compactness of tissue structure, while the degree of polymerisation of reserves are reduced, thus improving the availability of macronutrients. Because of the elevated hydrolytic activities, the level of bioactive components liberated from their different complexes is significantly increased. After the short-term germination and heat treatment the water soluble and heat sensitive anti-nutritive factors are inactivated and levels of allergens are radically decreased. Overall the main advantages of this processing, besides the abovementioned characteristics, are that the fully rehydrated, mild pre-digested, meat-analogue material can be directly applied for food industrial purposes as well as in the manufacturing of functional food products. The short-term germinated and heat-treated raw material or food additive (seed / ground material / pulp) can be used in dedicated diets like gluten-free, vegetarian, low-carb, weight control, healthy-ageing, hospital catering, etc.

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Conflict of interest

A. Jednákovits and J. Szilbereky are employed by Fitorex Ltd., the producer of YASO.

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