



The role of sprouts in human nutrition. A review

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Abstract. Based on the data of the literature it can be stated that the original composition of the seeds essentially changes during germination. The quantity of the protein fractions changes, the proportion of the nitrogen containing fractions shifts towards the smaller protein fractions, oligopeptides and free amino acids. Beyond this changes the quantity of the amino acids (some of them increase, others decrease or do not alter) during germination, and nonprotein amino acids also are produced. In consequence of these changes, the biological value of the sprout protein increase, and greater digestibility was established in animal experiments. The composition of the triglycerides also changes, owing to hydrolysis free fatty acids originate, that can be considered as a certain kind of predigestion. Generally, the ratio of the saturated fatty acids increases compared

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to unsaturated fatty acids, and the ratio within the unsaturated fatty acids shifts to the essential linoleic acid. The quantity of the antinutritive materials decreases, and the utilization of the macro and micro elements is increased owing to germination. Furthermore the sprouts contain many such materials (sulphoraphane, sulphoraphene, isothiocyanates, glucosinolates, enzymes, antioxidants, vitamins) that are proved to be effective in the prevention of cancer, or in the therapy against cancer.

1 Introduction

Sprouts are forming from seeds during sprouting. The sprouts are outstanding sources of protein, vitamins and minerals and they contain such in the respect of health-maintaining important nutrients like glucosinolates, phenolic and selenium-containing components in the Brassica plants or isoflavones in the soyabean. As the sprouts are consumed at the beginning of the growing phase, their nutrient concentration remains very high.

In the sprouts besides the nutrients phytochemicals, vitamins, minerals, enzymes and amino acids are of the most importance as these are the most useful in the respect of the human health (*AACR.*, 2005; *Schenker*, 2002; *Finley*, 2005; *Webb*, 2006).

In the last decades of the passed century the attention of experts dealing with the healthy nutrition turned more and more towards the determination of the biological value of the nutritional sprouts (*Penas et al.*, 2008). In this period the consumption of the germinated seeds became common also in Western Europe as the sprouts meet the requirements of the modern nutrition. Compared to the seeds it was established that the sprout due to its transformed protein content which is of higher biological value, the higher polyunsaturated fatty acid content, higher vitamin content and the better utilization of the minerals has a higher nutritional value. During the germination the polysaccharides degrade into oligo- and monosaccharides, the fats into free fatty acids, whereas the proteins into oligopeptides and free amino acids, which processes support the biochemical mechanisms in our organism. They improve the efficiency of both the protein-decomposing and the carbohydrate- and fatty acid-decomposing enzymes therefore germination can be considered as one kind of predigestion that helps to break down the high-molecular complex materials into their building blocks.

During the germination the amount of the antinutritive materials (trypsin inhibitor, phytic acid, pentosan, tannin) decreases and after the germination also compounds with health-maintaining effects and phytochemical properties

(glucosinolates, natural antioxidants) could be detected that can have a considerable role among others also in the prevention of cancer. Thus, germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health (*Sangronis and Machado, 2007*).

During germination from the seed at rest a new plant is developing if the moisture content is favourable, the temperature is appropriate and oxygen is available for the respiration. These processes were dealt with mainly in the Far-Eastern countries but important researches were conducted also in Europe (*Martinez-Villaluenga et al., 2008*). Due to this both at the European and the Far-Eastern markets a varied supply of sprouts has been developed from which the most popular are the sprouts of adzuki bean, alfalfa, broccoli, buckwheat, clover, mungo bean, mustard, radish, red cabbage and soybean. In Japan the sprouts are classified into different categories depending on they were grown in artificial or natural light or in dark, out of which the sprouts produced in light are consumed raw, whereas those produced in dark are consumed heat-treated.

In Transylvania no examinations have been carried out related to the sprouts and also in Hungary there has been a few results on the nutritional value and its change during germination. We set therefore as aim to determine the nutritional value of the most common commercially obtainable sprouts, wheat, lentil, sunflower and radish seed, and to monitor the change in the nutritional value during germination. We would like to examine the fat content and fatty acid composition, protein content and amino acid composition as well as the amount of the free amino acids, the vitamin content and the possibility of production of selenium-enriched sprouts, by which we could contribute to the better selenium status of the population.

In our first communication we work up the freshest literature, then later we would like to report our new scientific results.

Anti-cancer effect of the sprouts

In the recent years the prevention of diseases in natural ways gained more and more attention. The potential protective effect of the consumable sprouts and their active components against cancer was studied in several in vivo and in vitro model experiments. The results show a positive correlation between the prevention from cancer of several organs and the consumption of the vegetable or its active components. Despite this, the effect and mechanism of these chemopreventive phytochemicals have not been clarified yet (*Murillo and Mehta, 2001; Munday and Munday, 2002*).

During investigation of the biological activity of the phytoactive substances can be found in the Brassica sprouts their effect was studied as regards the biotransforming enzymes taking part in the carcinogenic metabolism, the antioxidant status and the chemically induced cancer (*Moreno et al.*, 2006; *Lee and Lee*, 2006; *Pereira et al.*, 2002; *Fahey et al.*, 1997; *Shapiro et al.*, 2001; *Fahey and Talalay*, 1999).

Consumption of Brassica plants especially broccoli is inversely proportional to the development of breast cancer in case of premenopausal women, whereas in case of postmenopausal women only a very little effect or no effect at all was observed, and even the type of the glutathione-S-transferase did not influence the course of the disease. These results emphasize the role of the Brassicaceae in the decrease of the risk of the premenopausal breast cancer (*Gill et al.*, 2004; *Ambrosone et al.*, 2004).

Some health-protecting phytochemicals can be found in the sprout in a much higher concentration than in the developed plant (*Harrison*, 1994; *Fernández-Orozco et al.*, 2006). These have significant antigenotoxic effect against damage to DNA induced by H_2O_2 as in those people who consumed for 14 days 113 g of cabbage and leguminous sprouts compared to the control diet the risk of cancer reduced. The experiment supports the theory that consumption of sprouts of the Brassica plants can be brought into connection with the reduction of cancerous diseases (*Gill et al.*, 2004; *Haddad et al.*, 2005). It is continuously necessary to develop such new kind of foodstuffs in an amount that makes the marketing in the foodstuff supplying systems possible (*Webb*, 2006; *Linnemann et al.*, 2006). These products have considerable added value which promotes the healthy nutrition. The application of foodstuffs containing bioactive components can lead to the improvement of the food technologies and to healthy nutrition (*Schneeman*, 2004; *Ubbink and Mezzenga*, 2006).

The nutritional sprouts are new foodstuffs rich in nutrients and phytonutrients, that can be produced and consumed without special product development, new appliances or expensive marketing. Protection against cancer by the means of foods is very attractive, especially when taking into consideration that in many kinds of cancer (eg. lung cancer) very little progress was achieved by the medicine (*Ferlay et al.*, 2004). So the consumer is interested in a higher consumption of the functional foodstuffs which contain also physiologically useful components (*Linnemann et al.*, 2006; *International Food Information Council Foundation*, 2006). At the same time some people have aversion to the establishment and accumulation of the bioactive components in the foodstuffs (*Finley*, 2005; *Brandt et al.*, 2004). Despite this the consumption demand on the sprouts has been increased that requires the optimization

of the quality, the consumability and bioactivity. In the first part of our work we deal with the chemical properties of the nutritional sprouts and with the evaluation of the in the respect of health-maintaining important components, bioactive substances.

Sulforaphane and isothiocyanate content of nutritional sprouts

The bioactive components of the sprouts of the Brassica plants are the glucosinolates and their products the isothiocyanates as well as the phenols, vitamins and minerals. To the vegetables of the Brassica plants consumed by the humans belong the broccoli, cabbage, Brussels sprouts, cauliflower, chinese cabbage and radish. The Brassica plants contain carotenoids, vitamin C, fiber, flavonoids and such health-protecting substances as the glucosinolates (*Jeffery and Jarrell, 2001; Holst and Williamson, 2004*). In the broccoli sprouts the most important glucosinolate is the glucoraphanine that is hydrolyzed by the microflora of the intestine into isothiocyanate and sulforaphane. In the plants the myrosinase enzyme hydrolyzes the glucosinolates mainly into isothiocyanates. These isothiocyanates have different biological effects: some of them damage the liver or are goitrogen, the others have antibacterial, fungicide and anticancer effect (*Moreno et al., 2006; Heaney and Fenwick, 1987; Shikita et al., 1999; Gamet-Payraastre, 2006*).

The primary *in vivo* metabolic pathway of the isothiocyanates is the mercaptane acid pathway which is the elimination pathway of most of the xenobiotics. The conjugation with glutathione resulting in thiol derivatives catalysed by the glutathione-S-transferase is followed by a successive glutamine and glycine cleavage resulting in L-cysteine isothiocyanates that are acylated to N-acetyl-L-cystein isothiocyanate derivatives (mercaptane acids) that empty from the organism via the urine. Based on this the GST plays an important role in the formation of the isothiocyanates (ITC) in the human organism. Number of isothiocyanates forming in the reactions can reach several hundreds. Generally it can be established that the ITC produced is determined by the type and amount of the consumed vegetable, by the way of processing and the quality of the chewing, as well as the nature of GST (*Munday and Munday, 2002; Lampe and Peterson, 2002; Ambrosone et al., 2004*).

The non-germinated seeds have the highest glucosinolate content that decreases in the sprouts. The Brassica sprouts at age of 3 days contain 10-100 times more glucoraphanine than the matching ripe plant (*Pereira et al., 2002; Perez-Balibrea et al., 2006*) due to which even a small amount of cabbage sprout reduces the risk of cancer, and is equally effective like a higher amount

of the same plant (*Fahey et al.*, 2006; *Fahey et al.*, 1997; *Shapiro et al.*, 2001; *Lee and Lee*, 2006).

Sulforaphane in different experimental models both *in vivo* in animals and *in vitro* in various cell cultures reduced the different forms of cellular proliferation, maybe by the activation of the enzymes that detoxicate the compounds causing cancer (*Bertelli et al.*, 1998; *Barillari et al.*, 2005a; *Barillari et al.*, 2005b; *Kensler et al.*, 2005). The broccoli sprouts and also the plant itself are considered a very good source of sulforaphane that occurs in the broccoli sprouts in a concentration of above 105 mg/100 g whereas in the broccoli plant in a concentration of 40–171 mg/100 g in the dry matter (*Bertelli et al.*, 1998; *Nakagawa et al.*, 2006; *Perocco et al.*, 2006).

Different researchers studying the beneficial effects of the broccoli sprouts and sulforaphane claim that due to its indirect antioxidant properties it strengthens the enzymes taking part in the antioxidant defence of the cells and detoxicates the carcinogen ones reducing by this the possibility of development of a cancer in the body (*Shapiro et al.*, 2001; *Perocco et al.*, 2006; *Fahey et al.*, 1997; *Shapiro et al.*, 2001; *Fahey and Talalay*, 1999). In the literature several reports confirm the anticarcinogenic effect of sulforaphane, however, there is a lack of information as to the safe applicability of its natural precursor glucoraphanine. In an *in vivo* experiment by the examination of the absorption and metabolism of glucoraphanine during consumption of Brassica plants it was established that glucoraphanine in the intestine metabolizes partly due to the effect of the microflora into sulforaphane in humans (*Conaway et al.*, 2000) and in the rodents (*Perocco et al.*, 2006). The dosage used in the experiment was set based on the glucoraphanine-sulforaphane content of the broccoli sprout which was used previously in cancer chemotherapeutic studies (*Fahey et al.*, 1997; *Lee and Lee*, 2006; *Fahey and Talalay*, 1999).

Liang et al. (2005) established that sulforaphane is an isothiocyanate that naturally occurs in the family of Brassica plants in a higher amount, and by its consumption the formation of tumors can be prevented. Sulforaphane content of five representatives of the Brassica family (broccoli, cabbage, cauliflower, savoy, Brussels sprouts) was determined by reversed-phase HPLC, using acetonitrile-water linear gradient. The raw sulforaphane was extracted first with ethyl acetate, 10% ethyl alcohol and hexane then the obtained extract was purified by low-pressure column chromatography on silica gel. The yield and purity of sulforaphane was higher than 90% using the gradient elution.

Perocco et al. (2006) examining the increase of the free-radicals by completing the food with glucoraphanine, found only a slight induction in case of the enzyme glutathione-S-transferase. These results are in contradiction with the

previous findings. They suggest that long-time consumption of glucoraphanine rather increases than decreases the risk of cancer by inducing the enzymes causing cancer by establishing oxidative stress. They claim that uncontrolled glucoraphanine consumption over a long time is a potential source of danger, despite this they recognize the advantageous effect of nutrition rich in fruits and vegetables on health-maintaining.

The broccoli sprouts were earlier proven to be a rich source of such chemopreventive materials as isothiocyanates. The isothiocyanate extract of broccoli prevents the cancerous cells of the bile from growing, due to its antiproliferative activity owing to the isothiocyanates it is good for the prevention and treatment of cancer (*Gamet-Payraastre*, 2006; *Lee and Lee*, 2006). Sulforaphane has antibacterial effect against the helicobacter pylori that causes chronic gastritis and ulcer of the small intestine, thus this material in the Brassicae is a potential medicine against helicobacter pylori. Furthermore, one week consumption of broccoli sprout improved the cholesterol metabolism and reduced the oxidative stress markers such as plasma amino acid content and various enzymes (*Murashima et al.*, 2004).

Clarke et al. (2008) examined the anticancer effect of sulforaphane in case of broccoli, cabbage, Brussels sprouts and cauliflower. It was established that sulforaphane occurs in an especially high concentration in the broccoli and broccoli sprout and due to its high isothiocyanate content reduces the risk of cancer including intestine and prostate cancer. Earlier also the enzyme inhibitor effect of sulforaphane was examined studying such enzymes that can be made responsible for cancerous lesions. The authors studied the effect of sulforaphane on the renewal of the cells and on the mechanism of the death of the cells during which they dealt with the anticancer properties of sulforaphane focusing on the different chemopreventive mechanisms. When they treat the effect of sulforaphane on humans they describe its chemistry, metabolism, absorption and they studied those factors influencing the biological availability of sulforaphane.

Glucosinolate content of nutritional sprouts

The two kinds of methionine glucosinolate have an extra sulfur atom in a different oxidation state in the side chain. These are forming a redox system (glucoraphenine, glucoraphasatine), which differs from the glucoerucin-glucoraphanine system in one double bond only. There is a difference in the radical-capturing capacity of the two systems (*Barillari et al.*, 2005a; *Barillari et al.*, 2005b). *Lepidium sativum* sprouts grown in light contain during the first

week of the germination high concentration of benzylglucosinolate, and only in traces 2-phenethyl glucosinolate which finding involves a further vegetable with its bioactive compounds into the circle of vegetables with health-maintaining effect (*Gil and Macloed, 1980; Glendening and Poulton, 1988*).

White mustard is commonly consumed fresh worldwide due to its special spicy taste. These vegetables contain several health-protecting compounds such as carotenoids, vitamin C, fibres, flavonoids and glucosinolates (*Barillari et al., 2005a; Martinez-Sánchez et al., 2006*). In the white mustard seeds and in the lyophilized sprout among the glucosinolates the glucoerucin is the main component. In contrast to other glucosinolates such as glucoraphanine, glucoerucin has both direct and indirect antioxidant effect due to which consumption of the white mustard and its sprouts is very useful for the human health (*Barillari et al., 2005a; Barillari et al., 2005b*).

Interesting members of the glucosinolate-containing Brassica family are the wild mustard and Turkish mustard, both of them are rich in such bioactive phytochemicals as phenols, flavonoids and vitamin C, each of them are present in the seed, the root and in the three, five and seven days old sprouts (*Martinez-Sánchez et al., 2006; Bennett et al., 2006*). Methanolic extract of the radish sprout has a very high antioxidative activity owing to the different sinapic acid esters and flavonoids with very high radical-capturing capacity as the basis of their biological activity (*Takaya et al., 2003*). Dichloromethanic fraction of the sprouts obtained from the methanolic extract contains nicotinamide adenine dinucleotide and chinon-reductase that are playing a significant role in the defence of the liver cells against chemically carcinogenic and other compounds. These results indicate that the radish sprouts can be considered a safe, useful, new source of food that reduce the risk of cancer development (*Lee and Lee, 2006*).

A new OH-containing jasmonic acid methyl ester stimulates the biosynthesis of the vegetable secondary metabolites, the cell oxidation, the L-phenylalanine-ammonia-lyase activity and takes a powerful effect on the biosynthesis of the secondary metabolites in vegetable cell cultures. Co-ordinated activation of the metabolic pathways by jasmonate helps in the development of resistance against environmental stress including the synthesis of indole glucosinolates in the Brassica family (*Bennett and Wallsgrove, 1994; Liang et al., 2006*).

One of the striking and typical features of the Brassica plants is the high glucosinolate content often up to 1% of the dry-matter (*Pereira et al., 2002; Fahey et al., 1997*). There has been a few attempt for the determination of the human glucosinolate consumption which can reach according to some sources the value of 300 mg/day ($\approx 660 \mu\text{mol/day}$). Clarification of the applicability,

transport and metabolism of these glucosinolates is the precondition of the understanding of the mechanism of the protective effect on the human organism (Moreno *et al.*, 2006; Gill *et al.*, 2004; Murillo and Mehta, 2001; Munday and Munday, 2002; Lampe and Peterson, 2002; Ye *et al.*, 2002).

If mirosinase of vegetable origin is present in the diet, the glucosinolates hydrolyze in the intestine. If the mirosinase is inactivated by heat prior to the consumption, the ionic feature of the glucosinolates prevents them from entering the intestine where they are metabolized by bacterial enzymes (Moreno *et al.*, 2006). Due to mirosinase glucose and other products e.g. isothiocyanates are forming. The glucosinolates decompose due to the mirosinase of vegetable origin in the small intestine or due to bacterial enzymes in the large intestine and their metabolites can be detected in the urine 2-3 hours after the consumption of the Brassica plants. The first step of the clarification of the positive effect on the human health and the prophylactic activity is the monitoring of the chemistry and metabolism of the glucosinolates in the food chain from the growing to the consumption (Ferlay *et al.*, 2004; Jeffery and Jarrell, 2001; Pereira *et al.*, 2002; Fahey *et al.*, 1997; Shapiro *et al.*, 2001; Fahey and Talalay, 1999).

According to Bellostas *et al.* (2007) the sprouts of the Brassica plants contain the glucosinolate in a high concentration therefore these plants can be very well used for the chemical defence in case of cancers. In their experiments they examined the glucosinolate content of the ripe seed, the sprout and the sprouted plant of five cabbage species (white cabbage, red cabbage, savoy, broccoli and cauliflower). The concentration of the individual glucosinolates highly varied among the cabbage plants. Concentration of the alkyl glucosinolates decreased whereas the indole-3-methylglucosinolate content increased during the germination period. During the germination of four and seven days the root of the sprouted plant contained the glucosinolate in the highest amount both for the four and seven day-sprouting.

Flavonoid content of the nutritional sprouts

The different conditions of the seed sprouting have effect on the flavonol content. The highest miricetin, merin, quercetin and camphorol content in the radish and lucerne sprouts was measured when the sprouting was done in dark at 20°C. Neither an increase of the germination temperature up to 30°C nor a decrease of that down to 10°C affected the efficiency of the flavonol synthesis. Similarly, neither a UV nor an IR radiation for between 20 min 24 hours increased significantly the flavonol content of the sprouts compared to the seed

(*Janicki et al.*, 2005).

The economical importance of the family of the leguminous plant is obvious as many plants of this family are used as food and feeding stuff. Very precious vegetables both in the animal and human nutrition are the broad bean, mungobean, pea, chick pea, lupine and the lentil sprouts. Soybean is one of the most important food seed in the Asian countries, beneficial effect of foodstuffs made of it is known (*Xu et al.*, 2005; *Kim et al.*, 2006). It was also reported that the phenolic components in the sprouts vary according to the growing conditions, and it was also established that the light can stimulate the production of the phytochemicals including the higher isoflavon content in the soya sprouts (*Kim et al.*, 2006).

Kim et al. (2007) sprouted buckwheat for a period of 1-10 days in a glass house under low light conditions and determined the chlorogen acid and flavonoid content including the C-glucoside flavons (orientin, isoorientin, vitexin, isovitexin) as well as rutin and quercetin. Rutin content of one meal portion (on average 20-30 mg/g) was 30 times higher than in the root and pericarp. By analyzing the radical-capturing capacity of the sprouts by the 2,2-diphenyl-1-picryl-hydrazil method it was established that it increased significantly for six to ten days in the portion from 1.52 to 2.33 μmol for the one buckwheat variety whereas for the other it increased from 1.46 to 2.09 μmol but the difference between the two sorts of buckwheat was not significant. On the basis of their investigations they recommend the consumption of the buckwheat sprouts during the everyday meals.

Other curative effects of the nutritional sprouts

Secondary metabolites of the vegetables are the unique sources of medicines, food additives and taste materials and other industrial products. In the passed hundred years certain vegetables became important sources of the new medicines, various vegetable drugs. As soon as the hydroponical growing of plants became selective and reproducible, the production of the bioactive components increased dramatically, many of which showed in vitro activity against bacteria, fungi and the cancer (*Poulev et al.*, 2003; *Zhao et al.*, 2005).

Plants are excellent sources of the phenolic phytochemicals out of which especially the antioxidants have outstanding role in the therapeutic applications as functional food components. Based on these facts the system of phytochemicals in the leguminous plants was developed by natural controlling means during which the pentose-phosphate cycle shifted into the direction of the phenolic phytochemicals (*Shetty and McCue*, 2003).

Antibiotic effect of nutritional sprouts

Many of the secondary vegetable metabolites have role in the fight against living creatures causing damage to the plant and the pathogenic microbes. Many of those components (cyanogen glucosides, glucosinolates, phenols, terpenes and sterols) originate from sikiminic acid or from aromatic hydrocarbons, that have important roles in those protecting mechanisms induced by infection or parasites. Accumulation of these metabolites occurs in the cells exposed to stress including phytoalexins after an infection by pathogen microorganisms (Zhao *et al.*, 2005). Similar abiotic stress can be triggered also by the UV light, yet the concentration of the flavonoids (morin, mircetin, quercetin and camphorol) in case of the radish and alfalfa sprouts was higher also when they were kept in dark than when they were treated with UV or IR radiation (Janicki *et al.*, 2005).

The pea can produce such phenolic phytochemicals that act as inhibitors on the pathogenic microorganisms and by which the helicobacter can be contolled like by a medicine. The pea sprouts combined with acetylsalicylic acid make possible the development of such a phenolic functional foodstuff that is suitable against the helicobacter pylori (Ho *et al.*, 2006).

Phytic acid and phytase content of the nutritional sprouts

Seeds and four-day-old sprouts of four Brassica varieties (little radish, radish, white mustard and rape) were established to contain inositol hexaphosphate that is called phytic acid or phytate in the salt form. This component proved to be biologically active and potentially useful in the respect of health as it reduced the blood sugar level, the amount of cholesterol and triglycerols, reduced the risk of cancer development and heart diseases (Frias *et al.*, 2005b). These contain high amount of tiamin, riboflavin, Ca, Mg, Cu, Mn, Fe and Zn as well as dietary fibres, that makes possible the development of a new potential foodstuff (Fernández-Orozco *et al.*, 2006; Zielinski *et al.*, 2005).

Sung *et al.* (2005) examined the effect of the germination temperature at 10, 20 and 25°C, in a 6–10-day interval for barley seeds on the phytase enzyme production. The growing rate and protein production of the barley plants increased with increasing temperature. Using SDS PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) it was established that during the germination period the proteins transformed, some of them disappeared, some of them appeared on the electrophoretogram. At the beginning of the germination the phytase activity was practically null, and showed a significant

increase during the sprouting. In the first couple of days it increased to the eightfold value then reduced. The utilizable phosphate content in connection with the activity of the phytase enzyme increased rapidly at the beginning of the sprouting. The protein and phytase production reached their maximum in two days. Partial purification of the raw enzyme extract by hydrophobic chromatography resulted in two phytase fractions. Molecular weight of the first fraction was 62 and 123 kDa, whereas that of the second fraction was 96 kDa. The ideal temperature for the production of the first fraction was 55°C, while for the second fraction it was 50°C. The optimal pH of the first fraction was 6.0, and 5.0 for the second fraction.

Biogenic amine content of the nutritional sprouts

Frias et al. (2007) examined biogenic amine content and cytotoxicity of alfalfa and fenugreek sprouts. The biogenic amines of both the alfalfa and the fenugreek affects the glucose and cholesterol content of the blood, therefore it is very important to have information on them in the respect of healthy nutrition. As the flours made of alfalfa and fenugreek sprouts can be considered as new types of functional foodstuffs, therefore it is important to study the biogenic amines and cytotoxicity of the sprouts. The sprouts of both plants were produced during four days at 20 and 30°C under light and dark conditions. The non-sprouted seeds contained putrescine, cadaverine, histamine, tyramine, spermidine and spermine. Bioactive amine content of the alfalfa sprouts was two times higher than that of the original seeds, and the sprouting at 20°C without light produced the lowest biogenic amine content. In the fenugreek sprouts only cadaverine and putrescine increased during the germination; the temperature and light had only a slight effect on the biogenic amine content. Biogenic amine content of the sprouted seeds always remained below the yet acceptable healthy level. Based on the experiment regarding the apoptosis and proliferation of cells it was established that the sprouts did not affect the processes of the cells.

Nutritional value of the sprout protein

Wanasundara et al. (1999) examined the change in nitrogen-containing components of linseed sprouts during the sprouting. The linseed sprouts were germinated in an eight-day-period during which the dry-matter content decreased by 35%. During the germination period the decrease in the total nitrogen content was relatively minimal, the nonprotein nitrogen content in-

creased from 9% to 33.5% in the percentage of the total protein, however. For the free amino acids an increase was observed. Among the amino acids the glutamine exhibited the greatest change in the germination period as this amino acid amide is an amide group donor, contributing to the development of the sprout. During sprouting the water-soluble protein content increased, the salt-soluble protein fractions decreased, however. Polyamine content, ie agmatine, spermidine and putrescine that are very important in the regulation of the cell metabolism and the growth, also increased during the germination period. During eight days of sprouting the amount of the cyan-containing glucosides, the linustatin and neo-linustatin decreased by around 40–70%. The trypsin inhibitor content of the linseed is quite minimal and also in the sprouts it can be detected in traces after eight days of sprouting.

Mbithi-Mwikya et al. (2000) studied the nutrients in finger-millet and the change in the antinutritive materials during sprouting. They examined beyond this the in vitro protein digestibility during a germination of 96 hours in sampling periods of 12 hours. The antinutritive materials were reported to decrease, during which tannins and phytates decreased below the detection limit. The trypsin inhibitor activity reduced to its one-third. During 48 hours a considerable decrease was obtained in the starch content which was in relation with the high increase of the sugar content. The protein digestibility increased significantly, however, 13.3% of the dry-matter was lost during the 96-hour sprouting period. The authors came to the conclusion that it was not necessary to apply a germination time longer than 48 hours since the longer germination time reduces considerably the dry-matter content due to the respiration without any significant improvement in the nutritional value.

Rozan et al. (2001) examined the amino acid composition of various seeds and four days old sprouts in case of five different lentil varieties. The free proteinous amino acid content increased considerably after the sprouting, with asparagine in the highest concentration. Also the amount of the non-proteinic amino acids differs substantially in the seeds and in the sprout. γ -OH-arginine, γ -OH-ornithine, α -amino-adipic acid and taurine could be found both in the seeds and the sprouts, whereas γ -aminobutyric acid, α -aminoadipic acid, 3-isoxazolinone derivative and the 2-carboxylic-methyl-isoxazoline-5-pyrimidine occurred only in the sprouts. These latter compounds were identified first in the lentil varieties. Various combinations of the non-proteinic amino acids can give information on the genetic distance of the different varieties and perhaps explain also the cross-breeding of varieties.

Urbano et al. (2005a) germinated green peas both in dark and in light for two, four and six days and examined the proteolytic activity of the obtained

sprouts, the soluble protein content and the non-protein nitrogen content, and the utilization of starch in growing rats. During their experiments the food-intake significantly increased when two- and four-day-old pea sprouts were eaten which was in a connection with a considerable decrease of the factors causing puffing-up. The nitrogen digestibility was fully identical for the sprout flours compared to the original green pea flour. The nitrogen balance, the percentage of the absorbed retained nitrogen, the protein efficiency ratio and the utilizable carbohydrate index were significantly higher for those animals that consumed green pea sprouted for two to four days than for those consumed raw green pea or green pea sprouted for six days. The authors came to the conclusion that two days of germination is sufficient for the digestibility of protein and carbohydrate content of the green pea to improve significantly. Sprouting in light or in dark did not influenced the nutritional value.

Urbano et al. (2005b) in another experiment examined the digestibility of protein and carbohydrate content of pea sprouts and the raw pea by *in vitro* and *in vivo* methods. In their experiments the pea was germinated for 3 to 6 days then *in vivo* protein and carbohydrate balance of rats was investigated. The germination considerably decreased the γ -galactosidase content and significantly increased the sucrose, glucose and fructose content. The ratio of the utilizable starch to the total starch increased due to the technological intervention. The vitamin B₂ content increased significantly, at the same time there was no significant change in the vitamin B₁ content in the sprouted pea. Examining the protein digestibility it was established that it significantly increased during the germination. The daily feeding stuff uptake, the nitrogen absorption and nitrogen balance, the ratio of the retained nitrogen to the absorbed nitrogen, the protein efficiency ratio and the utilizable carbohydrate index improved significantly during three days of sprouting, then each value decreased significantly to the day 6 of sprouting. It was established that sprouting of the pea for three days improved significantly the utilization of both the protein and carbohydrate.

Nutritional value of the fat of the sprouts

Kim et al. (2004) examined the change in the fatty acid composition due to sprouting. It was found that in the most sprouts the fatty acid present in the highest concentration was linolenic acid, its concentration increased in seven days up to 52.1%, and the total amount of unsaturated fatty acids was higher than 83%, that is, the unsaturated fatty acids dominated over the saturated ones. The amount of oleic acid was 36.8%, that of linoleic acid 38.1%

and that of linolenic acid was 2.7% in the original seed. During sprouting the concentration of the saturated fatty acids decreased rapidly and myristic acid and stearic acid disappeared from the sample after one day of sprouting. Out of the unsaturated fatty acids oleic acid decreased to a greater extent, whereas linoleic acid and linolenic acid increased during the germination. This is very important because linoleic acid, linolenic acid and arachidonic acid are essential to the human organism. Linoleic acid is capable of transporting bioactive compounds and it can be converted into arachidonic acid from which hormone-like compounds are forming. Summarized, it was established that the majority of the fatty acids of buckwheat are the unsaturated fatty acids, out of which linoleic acid occurred in the highest amount.

Tokiko and Koji (2006) examining fat content and fatty acid composition of various sprouts established that the fat content ranged from 0.4 to 1.6%. During the examination of the fatty acid content it was found that linolenic acid was present in the highest concentration, in 23% in case of buckwheat, in 48% in the soyabean, in 47.7% in the clover and in 40.6% in the pea.

Carbohydrate content of nutritional sprouts

Nodaa et al. (2004) examined the physical and chemical properties of the partially degraded starch of wheat sprout. γ -Amylase present in the sprout degrades partially the starch therefore the examinations targeted determination of physical and chemical properties of the starch degraded this way. By determining the swelling ability and viscosity it was found that they considerably decreased, at the same time the digestibility of starch increased due to the glucoamylase activity, which was due to the extremely late harvest. There are also such varieties that are not especially sensitive to the sprouting and that did not show any change even when harvested very late. In case of certain wheat varieties the extremely late harvest did not cause any significant change in the amylase content, in the average particle size, in the behaviour against heat and the length of the amylopectin chains. However, using electron microscope it was established that the late harvest can result in small sized and porous starch particles.

Nutritional value of soybean

The nutritional value of soybean sprouts changes during the germination; the free amino acid content increases and the vitamin C content increases approx. to its 200-fold value compared to the non-germinated seed, at the same time

the phytic acid content and the trypsin inhibitor activity decrease (*Kim et al.*, 1993). Chitosane (polymer of 2-amino-2-deoxy-D-glucose) is accepted as natural food supplement that increases the growth and yield of seeds such as soybean, potato, tomato, cabbage, improves the quality of the vegetables and increases the lifetime of fruits after harvest (*Kim*, 1998). Wetting the soybean seeds before sprouting with a chitosane solution with a molecular weight higher than 1000 enhances the productivity of the soybean sprouts without any kind of side effect. Due to the different dilution effects and to the molecular weight the vitamin C content decreased somewhat (*Lee et al.*, 2005).

Change in the lipoxygenase, phytic acid and trypsin inhibitor activity of nutritional sprouts

Frias et al. (2005b) germinated seeds of four Brassica plants (dwarf radish, radish, white mustard and rape) in order to study the presence of inositol hexaphosphate and the change in the trypsin inhibitor activity. It was established that the decrease in the phytic acid content was in a close relation with the germination time. After four days of sprouting the phytic acid content was less by 50% in three of the four analyzed samples. A strong decrease could be observed in the phytic acid content due to heat treatment (pasteurization and sterilization) both in the radish and rape sprouts. Due to heat treatment the amount of inositol hexaphosphate, that transformed into penta-, tetra and triphosphate. The trypsin inhibitor content in case of the radish and rape sprouts was decreased only by the heat treatment to a more significant extent.

In a study, phytate and phenolic components of African millet, the pH, viscosity, in vitro solubility of Fe and Zn and their change were examined during the swelling, germination and fermentation. The germination is recommended combined with fermentation to the developing countries especially for the feeding of children (*Kayodé et al.*, 2006).

Kumar et al. (2006) investigated the change in the lipoxygenase isoenzymes and the trypsin inhibitor activity during the sprouting of soybean at different temperatures. Two sorts of soybean were incubated for 144 hours at 25 and 30°C in a germination equipment and the activity of the lipoxygenase isoenzymes and the trypsin inhibitor was determined in every 24 hours of the germination. The lipoxygenase 1 as well as lipoxygenase 2 and 3 decreased gradually during the 144 hours, the rate of decrease for both lipoxygenase classes and for both soybean varieties was at 35°C the fastest. The trypsin inhibitor also gradually decreased during the germination, however, the rate of the decrease was higher at higher temperature. By polyacrylamide gel elec-

trophoresis analysis of the protein content of the sprouts it was found that the original Kunitz inhibitor decreased continuously at both temperatures for both of the genotypes in the course of sprouting, however, a new trypsin inhibitor could be detected during 48 hours at 35°C. The early appearance of the modified Kunitz inhibitor at 35°C compared to 25°C supports the theory that at higher temperature the decomposition of the Kunitz inhibitor takes place more rapidly.

Antioxidant, polyphenol and vitamin C content of the nutritional sprouts

Giberenic acid and indole-3-acetic acid have positive effect on the biosynthesis of vitamin C therefore during the sprouting of soybean the vitamin C content of the sprouts increases (Kim, 1988). The effect of a weak lighting on the ascorbic acid content and the growth of the soybean sprouts was also examined during which the lighting of 12 hours of ultraviolet and 12 hours of red light enhanced the phytochemical quality of the soybean sprouts (*Xu et al.*, 2005). In the course of two, three, four, five, six and nine days of sprouting the nutritional value of the lupine sprouts increased significantly owing to the increase of the vitamin C and polyphenol content, at the same time the amount of such antinutritive materials as the trypsin inhibitor and phytic acid decreased. The sprouting of lupine appears therefore to be a good method in the respect of the increase of the antioxidant capacity (*Fernández-Orozco et al.*, 2006).

Vitamin C takes part as antioxidant, cell marking modulator in the physiological processes of plants including the biosynthesis of the cell wall. It supports the phytohormone synthesis, the establishment of the stress resistance, the cell-division and the growth (*Wolucka et al.*, 2005).

Gill et al. (2004) in their experiments examining extract prepared of sprouts of Brassica plants and leguminous plants ten men and ten women consumed 113 g of sprouts of Brassica plants and leguminous plants for 14 days. The effect of the sprouts was examined based on the damage to the DNA, the change of activity of glutathione-S-transferase, glutathione peroxidase and superoxide dismutase detoxifying enzymes. Determination of the antioxidant status based on the plasma Fe reducing ability as well as antioxidant and blood fat content of the plasma, and the lutein and lycopene content of the plasma. There was a significant antigenotoxic effect in case of the by hydrogen peroxide induced damage to DNA for the peripheral blood lymphocytes for those persons who consumed the sprouts compared to the persons being on the control diet. No significant changes were found in case of the detoxifying enzymes by measur-

ing the antioxidant level of the plasma and its activity. The results confirm that consumption of the Brassica plants is in association with a lower risk of cancer via a lower damage to DNA.

Doblado et al. (2007) examining the change in the vitamin C content and antioxidant capacity of the raw and the sprouted horse-bean applied a 300, 400 and 500 MPa pressure for 15 minutes at room temperature. In the raw seeds no vitamin C content could be detected, whereas the horse-bean sprouts contained considerable amount of vitamin C. The antioxidant capacity in the germinated seeds increased by around 58–67%. The high-pressure treatment modified somewhat the vitamin C content and also the antioxidant capacity and beyond a pressure of 500 MPa the decrease was significant. Although the treatment of the sprouts at high pressure resulted in a high (15–17 mg/100 g) vitamin C content and also the antioxidant capacity was by around 26–59% higher than for the non-sprouted horse-bean, the high-pressure treatment had only a slight effect on the quality of the freshly consumed sprouts.

Hsu et al. (2008) studied the improvement of the antioxidant activity of buckwheat sprout using trace element containing water. Trace element containing water of 100–500 mg/kg concentration was applied in order to find out whether the trace elements have any kind of favourable effect on the increase of the antioxidant activity. Trace element containing water of 300 mg/kg concentration increased significantly the Cu, Zn and Fe content of the sprouts, but it did not affect their Se and Mn content. Rutin, quercitrin and quercetin content of the sprouts did not differ whether the germination was carried out in micro element containing water or in deionized water. Ethanolic extract of the buckwheat sprout, germinated in a 300 mg/kg concentration trace element containing water, exhibited a higher radical capturing activity, iron ion chelate activity, superion anion radical capturing activity and also a higher inhibitor activity prior to the lipid peroxidation. The extract of the sprouts prepared in a trace element containing water increased also the intracellular superoxide dismutase activity which resulted in compounds containing lower level of active oxygen in the examined human cells.

Fernandez-Orozco et al. (2008) examined the change of the antioxidant capacity of mungo bean and two sorts of soybean during sprouting. The mungo bean was germinated for 2, 3, 4, 5 and 7 days, the sort jutra of soybean for 2, 3, 4 days and the variety merit for 2, 3, 4, 5 and 6 days. Based on their examinations the vitamin C and E content and the reduced glutathione activity vary depending on the leguminous plants used and the sprouting conditions. The mungo bean and soybean sprouts contain much more phenolic components than the original raw bean. The superoxide dismutase activity in the mungo

bean increased to 308% during seven days, there was no increase in the variety jutra while for the sort merid an increase of 20% could be observed between day five and day six of the germination. The peroxide radical-capturing capacity and the antioxidant capacity increased by around 28–70% and 11–14%, respectively, in case of the soybean, which values were for the mungo bean 248 and 61% at the end of the sprouting. Inhibition of the lipid peroxidase increased between day 5 and day 7 of the germination by 359% for the mungo bean, by 67% for the soybean merid, and it was practically unchanged for the variety jutra. It is established that the germination of mungo bean and soybean is a good technology for producing a functional foodstuff with a higher antioxidant capacity.

Amici et al. (2008) during their experiments with wheat sprout found that it contained high amount of organic phosphates and it was a powerful mixture of molecules such as enzymes, reducing glucosides and polyphenols. Antioxidant compounds of wheat sprout are capable of protecting the deoxyribonucleic acid from the oxidative damages caused by free radicals. They reported that polyphenols like e.g. epigallocatechin-3-gallate had antioxidant and protease effect in the cancerous cell. In the course of their examinations they could identified five different phenolic derivatives as follows: gallic acid, epigallocatechin-3-gallate, epigallocatechin, epikatechin and catechin. They established that the wheat sprout extract reduced the growth of the cancerous cells and increased the amount of the intracellular oxidative proteins.

Randhir et al. (2008) examined the effect of the heat treatment in autoclave on the total phenol content and the antioxidant activity, in case of barley, buckwheat, wheat and oat sprouts as well as sprouted plants. α -Amilase and α -glucosidase inhibition and the levo-dihydroxyphenylalanine content as well as angiotensine enzyme inhibition being in association with high blood pressure and inhibition associated with gastric ulcer were *in vitro* evaluated. Due to heat treatment the total phenol content and the antioxidant activity associated with the capture of free radicals were generally increased. Activity of the α -amilase inhibitor increased in case of buckwheat and oat, at the same time it decreased for the barley and maize sprout and sprouted plant. Glucosidase inhibitor activity increased in wheat, buckwheat and oat, but decreased in the maize sprout. In all of the examined sprouts and sprouted plants the levo-dihydroxyphenylalanine content decreased. The angiotensine enzyme activity increased in the buckwheat and oat, whereas decreased in the wheat and maize sprout. Each sprout and sprouted plant increased the inhibitor activity associated with gastric ulcer. From these changes it can be concluded that due to heat treatment the phenolic compounds and the

phenolic oxidation and polymerisation change, therefore in case of foods being used in treatment of chronic diseases, modifications related to heat treatment are required in order to produce the biologically active components.

Lopez-Amoros et al. (2006) studying phenolic components and antioxidant activity of leguminous plants examined the effect of the different germination conditions in case of bean, lentil and pea on such bioactive components as the flavonoid and non-flavonoid phenolic compounds, and beyond this also analyzed the free radical capturing capacity of the samples. The analyzed leguminous plants contained in different amount the hydroxybenzoic acids and aldehydes, hydroxycinnamic acid and its derivatives, the flavonoglucosides and flavon-3-ols as well as procyanidines. It was established that in case of the leguminous plants the germination alters the quality and quantity of the phenolic components, the actual changes depend on the leguminous plant itself and the germination conditions. The changes affect the functional properties of the leguminous plants and consequently the antioxidant activity. Antioxidant activity of beans and peas during the sprouting increased extremely, whereas that of the lentil showed a decrease.

Vitamin B content of the nutritional sprouts

Sato et al. (2004) by examining the vitamin B₁₂ content of the Japanese radish looked for the answer to how the plant can absorb it from a vitamin B₁₂-containing solution and incorporate into their cells. It was established that the B₁₂ content of the raw sprout of the Japanese radish can increase up to 1.5 µg/g when a solution with 0–200 µg/ml vitamin B₁₂ content was used during the germination. Vitamin B₁₂ content can be extracted due to heat treatment from the sample compared to the control for which no heat-treatment was applied.

Oestrogen content of nutritional sprouts

Vegetable components with oestrogen activity (daidzein, genistein, kumestrol, formononetin and biochanin) can play a role in the cancer prevention, in the improvement of the symptoms of menopause and they can have also other health-protecting effects. The most important sources of the phytoestrogens isoflavon and kumestan are the sprouts and the leguminous plants (*Reinli and Block, 1996*). In experiments with Australian postmenopausal women who consumed traditional foods with linseed, soybean flour and alfalfa sprouts it was concluded that there was a relationship between the consumption of foods

with low oestrogen content and the development of hormone-dependent cancer (*Morton et al.*, 1994).

Resveratrol content of nutritional sprouts

Resveratrol is one of the phytoalexins that were widely analyzed and that are considered as potentially bioactive phytochemicals in the prevention of cardiovascular diseases, inflammations, ageing and cancer (*Alarcón and Villegas*, 2005; *Vitaglione et al.*, 2005; *González-Barrio et al.*, 2006; *Valenzano and Cellerino*, (2006). Sprouting three sorts of peanut for 9 days at 25°C in 95% humidity the resveratrol content increased significantly. Sucrose, glucose and total free amino acid content of the sprout also increased significantly. The taste and flavour of the sprouts were also improved therefore the peanut sprouts can be considered as functional vegetables (*Wang et al.*, 2005).

King et al. (2006) during their researches proved the positive effect of resveratrol on health-maintaining. Despite this, further information is required regarding the applicability, metabolism and effect of resveratrol in the cells (*Wang et al.*, 2005; *King et al.*, 2006).

Utilization of the macro and micro element content of nutritional sprouts

Nutritional importance of pea is owing to the high protein content, polysaccharides, vitamins, minerals, dietary fiber and the antioxidants (*Ho et al.*, 2006). Soaking prior to the germination is responsible for the loss of Mg and Zn that continuously emptying from the seed during the sprouting. Absence or presence of light did not affect in the four days old pea sprouts the Zn and Mg content during the sprouting, at the same time sprouting for two and four days improved the biological availability of Zn Mg (*Urbano et al.*, 2006).

Selenium-enriched nutritional sprouts

During sprouting the developing vegetable organism can enrich from the appropriately conditioned nutrient soil various macro and micro elements in its tissues. A part of the population of Hungary and Romania consumes bread made of wheat grown on selenium-defficient areas due to which the selenium status of a good part of the population is not sufficient. By producing the sprouted plants it is also targeted to investigate how the selenium content of the sprouted plant can be increased. Plants with increased selenium content can be produced only by growing on a high-selenium soil. For the increase of

the selenium content of the soil selenite and selenate are used that can cause environmental pollution. It appears therefore to be the best to produce these plants with high-selenium content in a closed system for which an outstanding approach applied by Japanese researchers for sprouts (*Sugihara et al.*, 2004; *Yoshida et al.*, 2007a; 2007b; *Hama et al.*, 2008; *Li et al.*, 2008). In a closed system it is relatively easy to raise sprouts with increased selenium content and there are no environmental concerns.

In animal organisms selenium is present in the form of selenomethionine that can be converted by the plants into selenocystein. These amino acids and their monomethylated derivatives could be detected in some vegetables with increased selenium content (*Sugihara et al.*, 2004). The outstanding anticancer effect of these vegetables was proved and it was found to be higher than that of the inorganic selenite. These results made us to begin to deal with the increase of the selenium content of the sprouts, hoping that by this we can contribute to the optimal selenium status of the population.

Selenium supplementation in the form of selenomethyl-selenocystein (Se-MSc) received much scientific attention as a chemopreventive compound. Se-MSc and its derivatives occur mainly in selenium-enriched vegetables. Selenium-enriched broccoli sprouts decreased significantly the incidence of the abnormal epithelic protrusion in the colon in rats when the feeding stuff contained 2 µg/g selenium (*Finley et al.*, 2005), which demonstrates well the protecting effect of broccoli and broccoli sprouts against colon cancer. Beside Selenomethionine also Se-methyl-selenocystein and seleno-2-propenyl-selenocystein were detected in alfalfa sprouts (*Gergely et al.*, 2006).

Sugihara et al. (2004) germinated seeds of special Japanese radish on a high-selenium soil and experienced that 5–10 µg/ml selenite acted as growth inhibitor. Major part of the selenium taken up (69–98%) could be extracted with 0.2 M HCl solution, and selenomethyl-selenocystein was found to be the main selenium component by high-performance liquid chromatographic analysis. In the high-selenium sprouts also selenomethionine, non-utilized selenite, γ-glutamyl-Se-methyl-selenocystein and an unknown selenium-containing component could be detected. As monomethylated selenoamino acids were proven to have anticancer effect, the sprouts enriched in selenium are thought to be useful in the prevention of cancer.

Yoshida et al. (2007a) examined the utilizability of selenium-enriched radish sprout and measured how selenium influenced the glutathione peroxidase activity. In male rats intestinal tumour was caused by 1,2-dimethyl-hydrazine and the anticarcinogen activity of the utilized selenium was evaluated. It was established that selenium supplementation independently of the dose increased

the selenium concentration of the serum and liver and the glutathione peroxidase activity, with higher values obtained in the groups receiving selenite supplementation than in case of selenium-enriched sprouts. The availability of selenium in the sprout ranged between 33 and 65%. When the amount of selenium was increased to 2 µg/g both the selenite form and selenium taken in with the radish sprout prevented the tumour cells from growing. These results indicate that although selenium in the form of radish sprout had a lower nutritional biological value but a significantly higher antitumour activity than selenite.

Yoshida *et al.* (2007a,b) examined the nutritional availability of the selenium content of selenium-enriched pumpkin and radish sprouts in male mice kept on a Torula yeast selenium-deficient diet. After a three-week-feeding the mice were divided into seven groups, some of the mice consumed the basic diet, the others received a selenium supplementation of 0.05 and 0.25 µg/g in the form of Na-selenite, selenium-enriched pumpkin and selenium-enriched radish sprout for an additional one week. The supplementation increased depending on the selenium content the selenium concentration of the serum and liver and the glutathione peroxidase activity. Selenium content and glutathione peroxidase activity of the serum were not affected significantly by the source of selenium, however, both selenium content and glutathione peroxidase activity of the liver were increased significantly by the Na selenite supplementation compared to the selenium-enriched pumpkin and selenium-enriched radish sprout. There was also a difference between the selenium-enriched pumpkin and selenium-enriched radish sprout in the increase of the selenium content of the liver as the selenium-enriched pumpkin increased significantly more the selenium content of the liver than the selenium-enriched sprout. Based on the analysis of the liver it was found that selenium was utilized both from the selenium-enriched pumpkin and the selenium-enriched radish sprout in 97%, whereas in case of selenite the utilization is only 65%. However, when the selenium utilization was examined on the basis of the glutathione peroxidase it was experienced that from both the selenium-enriched pumpkin and the selenium-enriched radish sprout it was only 50% compared to Na selenite.

Hama *et al.* (2008) examined the effect of the selenium-enriched Japanese radish on the activity of glutathione peroxidase and glutathione S-transferase in rats. Based on their examinations the selenium-enriched Japanese radish sprout having 80% of the total selenium content in the form of Se-methyl-selenocystein, hindered the formation of mammary cancer induced by 7,12-dimethyl-benz(a)anthracene in rats. Examining the effect of selenium-enriched Japanese radish sprout on the oxidative stress 344 female rats were involved

into the experiments during which 0; 2.4; 5.0; 8.8 and 12.5 mg/kg selenium was fed with selenium-enriched sprout for three weeks, which was added to a commercially obtainable rat feed. Glutathione peroxidase and glutathione S-transferase activity of the liver, kidney and lungs of the rats were measured. When the highest selenium dose (12.5 mg/kg) was fed, the blood had the highest selenium content, followed by the liver, and finally by the lungs. The selenium diet of 12.5 mg/kg reduced the increase of the body mass but increased the mass of the liver.

Li et al. (2008) analyzed the synergism of the broccoli sprout extract and selenium in human hepatocytes by examining the thioredoxin reductase activity. Isothiocyanates in foods have a regulating effect on the thioredoxin reductase activity of the human cell cultures. The synergism between sulforaphane and selenium induces the thioredoxin reductase activity by modifying both the transcription and translation. Sulforaphane, erucine and ibarane regulate the expression of thioredoxin reductase in the human cells both on the protein and the mRNA level. The effect of the broccoli extract on the dying hepatocytes rich in isothiocyanates, sulforaphane, iberine and induces selenium synergically. Isothiocyanates content of the broccoli sprout extract was 1.6; 4 and 8 μmol which was tested by mRNA induction during protein synthesis. Induction of the broccoli sprout was 1.7–2.2 times higher than that of the control, due to a joint treatment with 0.2–1 μmol of selenium the expression increased to a 3.0–3.3-fold value. Furthermore, broccoli sprout extract stimulated the activity of the cellular enzymes which induction was associated with the selenium addition. In the knowledge of these facts it can be stated that broccoli sprout extract of 8 μmol and the selenium addition increased the amount and activity of the enzymes in the cells to the 3.7–5-fold value. Selenium or the broccoli sprout extract alone resulted in only an around twofold increase. These data suggest that the broccoli sprout, by the application of the physiologically appropriate concentrations between isothiocyanates contained and selenium, can have an important role in the protection against the oxidative stress.

Microbiological safety of nutritional sprouts

Several studies dealt with the food safety of nutritional sprouts especially focusing on the microbiological quality, but also their physical and chemical properties and the polluting materials were examined (*Thomas et al.*, 2003; *Gabriel*, 2005). It was found that organoleptic examinations should be carried out in order to estimate the efficiency of the presprouting procedures also in

respect of inactivity of the pathogenic sprouts (*Gabriel, 2005; Fahey et al., 2006*).

Penas et al. (2008) examined the microbiological safety of wheat, mungo bean and alfalfa sprouts due high pressure treatment. Using different time, pressure and temperature combinations the germinating capacity of mungo bean and alfalfa seeds as well as the improvement of the native microbiological state were examined. In case of mungo bean the sprouting capacity was not affected by the increasing temperature and pressure up to 250 MPa. When the temperature was increased from 10 to 40°C it had a positive effect on the vitality of the alfalfa sprouts, that was, however, decreased by the pressure when increased from 100 to 400 MPa. The number of the aerobic mesophyl and fecal coliform bacteria, as well as yeast and mould populations decreased when the pressure and temperature were increased. It was found that the optimal treatment conditions without any loss in the germination capacity were 48°C and 100 MPa for the alfalfa, and 250 MPa in the case of the mungo bean.

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References

- [1] AACR, Broccoli sprouts, cabbage, Ginkgo biloba and garlic: a grocery list for cancer prevention. American Association for Cancer Research. Public & Media: News. <http://www.aacr.org/default.aspx?p=1275&d=553> (Access on June 14, 2006) (2005).
- [2] C.A. Alarcón de la Lastra, I. Villegas, Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications, *Molecular Nutrition and Food Research*, 49 (2005) 405-430.
- [3] C.B. Ambrosone, S.E. McCann, J.L. Freudenheim, J.R. Marshall, Y. Zhang, P.G. Shields, Breast cancer risk in premenopausal women is

- inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype, *Journal of Nutrition*, 134 (2004) 1134-1138.
- [4] M. Amici, L. Bonfili, M. Spina, V. Cecarini, I. Calzuola, V. Marsili, M. Angeletti, E. Fioretti, R. Tacconi, G.L. Gianfranceschi, A.M. Eleuteri, Wheat sprout extract induces changes on 20S proteasomes functionality, *Biochimie*, 90 (2008) 790-801.
- [5] J. Barillari, D. Canistro, M. Paolini, F. Ferroni, G.F. Pedulli, P. Iori, L. Valgimigli, Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite container in rocket (*Eruca sativa* Mill.) seeds and sprouts, *Journal of Agriculture and Food Chemistry*, 53 (2005a) 2475-2482.
- [6] J. Barillari, R. Cervellati, M. Paolini, A. Tatibouët, P. Rollin, R. Iori, Isolation of 4-methylthio-3-butenyl glucosinolate from *Raphanus sativus* sprouts (Kaiware-daikon) and its redox properties, *Journal of Agriculture and Food Chemistry*, 53 (2005b) 9890-9896.
- [7] N. Bellostas, P. Kachlicki, H. Sorensen, J.C. Sorensen, Glucosinolate profiling of seeds and sprouts of *B. Oleracea* varieties used for food, *Scientia Horticulturae*, 114 (2007) 234-242.
- [8] R.N. Bennett, E.A.S. Rosa, F.A. Mellon, P.A. Kroon, Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in *Eruca sativa* (salad (rocket). *Diplotaxes eruroides* (Wall rocket), *Diplotaxis tenuifolia* (wild rocket) and *Bunias orientalis* (Turkish rocket), *Journal of Agriculture and Food Chemistry*, 54 (2006) 4005-4015.
- [9] R.N. Bennett, R.M. Wallsgrove, Secondary metabolites in plant defence mechanisms Tansley, *The New Phytologist*, 127 (1994) 617-633.
- [10] D. Bertelli, M. Plessi, D. Braghiroli, A. Monzani, Separation by solid phase extraction and quantification by reverse phase HPLC of sulforaphane in broccoli, *Food Chemistry*, 63 (1998) 417-421.
- [11] K. Brandt, L.P. Christensen, J. Hansen-Moller, S.L. Hansen, J. Haraldsdottir, L. Jespersen, S. Purup, A. Kharazmi, V. Barkholt, H. Frokiar, M. Kobak-Larsen, Health promoting compounds in vegetables and fruits: a systematic approach for identifying plant components with impact on

- human health, *Trends in Food Science and Technology*, 15 (2004) 384-393.
- [12] J. Clarke, R.H. Dashwood, E. Hoa, Multi-targeted prevention of cancer by sulforaphane, *Cancer Letters*, 269 (2008) 291-304.
- [13] C.C. Conaway, S.M. Getahun, L.L. Liebes, D. Pusateri, M. Botero-Omary, F.L. Chung, Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli, *Nutrition and Cancer*, 38 (2000) 168-178.
- [14] R. Doblado, J. Frias, C. Vidal-Valverde, Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. carilla) seeds induced by high pressure treatment, *Food Chemistry*, 101 (2007) 918-923.
- [15] J.W. Fahey, P. Talalay, Antioxidant functions of sulforaphane: a potent inducer of Phase-II detoxication enzymes, *Food Chemical Toxicology* 37 (1999) 973-979.
- [16] J.W. Fahey, P.J. Ourisson, F.H. Degnan, Pathogen detection, testing, and control in fresh broccoli sprouts, *Nutrition Journal*. 5 (2006) 13, <http://www.nutritionj.com/content/5/1/13>.
- [17] J.W. Fahey, Y. Zhang, P. Talalay, Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens, *Proceedings of the National Academy of Sciences*, 94 (1997) 10367-10372.
- [18] J. Ferlay, F. Bray, P. Pisani, D. Parkin, GLOBOCAN: 2002 Cancer Incidence, Mortality and Prevalence Worldwide. IARC Press, Lyon, France, (2004).
- [19] R. Fernandez-Orozco, J. Frias, H. Zielinski, M.K. Piskula, H. Kozłowska, C. Vidal-Valverde, Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emerald, *Glycine max* cv. Jutro and *Glycine max* cv. Merit, *Food Chemistry*, 111 (2008) 622-630.
- [20] R. Fernández-Orozco, M.K. Piskula, H. Zielinski, H., Kozłowska, J. Frias, C. Vidal-Valverde, Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton, *European Food Research and Technology*, 223 (2006) 495-502.

- [21] J.W. Finley, Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds, *Annals of Botany*, 95 (2005) 1075-1096.
- [22] J. Frias, C. Martinez-Villaluenga, P. Gulewicz, A. Perez-Romero, R. Pilarski, K. Gulewicz, C. Vidal-Valverde, Biogenic amines and HL60 cytotoxicity of alfalfa and fenugreek sprouts, *Food Chemistry*, 105 (2007) 959-967.
- [23] J. Frias, M.L. Miranda, R. Doblado, C. Vidal-Valverde, Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. Multolupa, *Food Chemistry*, 92 (2005a) 211-220.
- [24] J. Frias, H. Zielinski, M.K. Piskula, H. Kozłowska, C. Vidal-Valverde, Inositol phosphate content and trypsin inhibitor activity in ready-to-eat cruciferous sprouts, *Food Chemistry*, 93 (2005b) 331-336.
- [25] A.A. Gabriel, Microbial quality of chlorine soaked mung bean seeds and sprouts, *Food Science and Technology Research*, 11 (2005) 95-100.
- [26] L. Gamet-Payraastre, Signaling pathways and intracellular targets of sulforaphane mediating cell cycle arrest and apoptosis, *Current Cancer Drug Targets*, 6 (2006) 135-145.
- [27] V. Gergely, M. Montes-Bayón, P. Fodor, A. Sanz-Medel, Selenium species in aqueous extracts of alfalfa sprouts by two-dimensional liquid chromatography coupled to inductively coupled plasma mass spectrometry and electrospray mass spectrometry detection, *Journal of Agriculture and Food Chemistry*, 54 (2006) 4524-4530.
- [28] V. Gil, A.J. Macloed, Benzylglucosinolate degradation in *Lepidium sativum*: effects of plant age and time of autolysis, *Phytochemistry*, 19 (1980) 1365-1368.
- [29] C.I.R. Gill, S. Halder, S. Porter, S., Matthews, S., Sullivan, J. Coulter, H. McGlynn, I. Rowland, The effect of cruciferous and leguminous sprouts on genotoxicity, in vitro and in vivo, *Cancer Epidemiology Biomarkers and Prevention*, 13 (2004) 1199-1205.
- [30] T.M. Glendening, J.E. Poulton, Glucosinolate biosynthesis. Sulfation of desulfobenzylglucosinolate by cell-free extracts of cress (*Lepidium sativum* L.) seedlings, *Plant Physiology*, 86 (1988) 319-321.

-
- [31] R. González-Barrio, D. Beltrán, E. Cantos, M.I. Gil, J.C. Espin, F.A. Tomás-Barberán, Comparison of ozone and UV-C treatments on the postharvest silbenoid monomer, dimer and trimer induction in var. "Superior" white table grapes, *Journal of Agriculture and Food Chemistry*, 54 (2006) 4222-4228.
- [32] P.S. Haddad, G.A. Azar, S. Groom, M. Boivin, Natural health products, modulation of immune function and prevention of chronic diseases, *Evidence-Based Research in Complementary and Alternative Medicine*, 2 (2005) 512-520.
- [33] H. Hama, O. Jamanoshita, M. Chiba, I. Takeda, T. Nakajima, Selenium-enriched Japanese radish sprouts influence glutathione peroxidase and glutathione S-transferase in an organ-specific manner in rats, *Journal of Occupational Health*, 50 (2008) 147-154.
- [34] H.C. Harrison, Growing Edible Sprouts at Home (A3385). University of Wisconsin-Extension (UWEX), Cooperative Extension Publications RP-04-94-1.5M-20-MS. Madison, Wisconsin, USA (1994)
- [35] R.K. Heaney, G.R. Fenwick, In: Natural Toxicants in Foods: Progress and Prospects. Ellis Horwood Series in Food Science and Technology. (Ed. Watson, H.) Ellis Horwood, Chichester, UK, (1987) 76-109.
- [36] C.Y. Ho, Y.T. Lin, R.G. Labbe, K. Shetty, Inhibition of *Helicobacter pylori* by phenolic extracts of sprouted peas (*Pisum sativum* L.), *Journal of Food Biochemistry*, 30 (2006) 21-34.
- [37] B. Holst, G. Williamson, A critical review of the bioavailability of glucosinolates and related compounds, *Natural Product Reports*, 21 (2004) 425-447.
- [38] C.K. Hsu, B.H. Chiang, Y.S. Chen, J.H. Yang, C.L. Liu, Improving the antioxidant activity of buckwheat (*Fagopyrum tataricum* Gaertn) sprout with trace element water, *Food Chemistry*, 108 (2008) 633-641.
- [39] International Food Information Council Foundation, Functional foods fact sheet: antioxidants (2006).
<http://www.ific.org/publications/factsheets/antioxidantfs.cfm>
- [40] B. Janicki, B. Kupcewicz, A. Napierala, A. Madzielewska, Effect of temperature and light (UV, IR) on flavonol content in radish and alfalfa sprouts, *Folia Biologica*, 53 (2005) 121-125.

-
- [41] E.H. Jeffery, V. Jarrell, *Cruciferous vegetables and cancer prevention*, In: Handbook of Nutraceuticals and Functional Foods. (Ed. Wildman REC.) CRC Press: Boca Raton FL, (2001) 169-192.
- [42] P.A.P. Kayodé, M.J.R. Nout, E.J. Bakker, M.A.J.S. Van Boekel, Evaluation of the simultaneous effects of processing parameters on the iron and zinc solubility of infant sorghum porridge by response surface methodology, *Journal of Agriculture and Food Chemistry*, 54 (2006) 4253-4259.
- [43] T.W. Kensler, L.P. Jacobson, J.B. Wiang, J.W. Fahey, L. Ye, J.G. Chen, P.A. Egner, K.K. Stephenson, J.L. Coady, Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China, *Cancer Epidemiology Biomarkers and Prevention*, 14 (2005) 2605-2613.
- [44] E.H. Kim, S.H. Kim, J.I. Chung, H.Y. Chi, J.A. Kim, I.M. Chung, Analysis of phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merrill) and sprouts grown under different conditions, *European Food Research and Technology*, 222 (2006) 201-208.
- [45] S.J. Kim, I.S.M. Zaidul, T. Maeda, T. Suzuki, N. Hashimoto, S. Takigawa, T. Noda, C. Matsuura-Endo, H. Yamauchi, A time-course study of flavonoids in the sprouts of tartary (*Fagopyrum tataricum* Gaertn.) buckwheats, *Scientia Horticulturae*, 115 (2007) 13-18.
- [46] S.L. Kim, S.K. Kim, C.H. Park, Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable, *Food Research International*, 37 (2004) 319-327.
- [47] S.D. Kim, S.H. Kim, E.H. Hong, Composition of soybean sprout and its nutritional value, *Korean Soybean Digest*, 10 (1993) 1-9.
- [48] S.K. Kim, Application of chitin and chitosan in agriculture, *Journal of Chitin Chitosan*, 3 (1998) 327-342.
- [49] S.O. Kim, Effect of growth regulators on growth and vitamin C biosynthesis during germination of soybeans, *Journal Korean Society Food and Nutrition*, 17 (1988) 115-124.
- [50] R.E. King, J.A. Bomser, D.B. Min, Bioactivity of resveratrol, *Comprehensive Reviews in Food Science and Food Safety*, 5 (2006) 65-70.

-
- [51] V. Kumar, A. Rani, V. Pandey, G.S. Chauhan, Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures, *Food Chemistry*, 99 (2006) 563-566.
- [52] Y.H. Kuo, P. Rozan, F. Lambein, J. Frias, C. Vidal-Valverde, Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes, *Food Chemistry*, 86 (2004) 537-545.
- [53] J.W. Lampe, S. Peterson, Brassica, biotransformation and cancer risk: genetic polymorphism alter the preventive effects of cruciferous vegetables, *Journal of Nutrition*, 132 (2002) 2991-2994.
- [54] S.O. Lee, I.S. Lee, Induction of quinone reductase, the phase 2 anticarcinogenic marker enzyme, cells by radish sprouts, *Raphanus sativus* L, *Journal of Food Sciences*, 71 (2006) S144-S148.
- [55] Y.S. Lee, Y.H. Kim, S.B. Kim, Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights, *HortScience*, 40 (2005) 1333-1335.
- [56] D. Li, K. Wub, A. Forbes Howie, G.F. Beckett, W. Wang, Y. Bao, Synergy between broccoli sprout extract and selenium in the upregulation of thioredoxin reductase in human hepatocytes, *Food Chemistry*, 110 (2008) 193-198.
- [57] H. Liang, Q. Yuan, Q. Xiao, Purification of sulforaphane from Brassica oleracea seed meal using low-pressure column chromatography, *Journal of Chromatography*, 828 (2005) 91-96.
- [58] Y.S. Liang, H.K. Kim, A.W.M. Lefeber, C. Erkelens, Y.H. Choi, R. Verpoorte, Identification of phenylpropanoids in methyl jasmonate treated Brassica rapa leaves using two-dimensional nuclear magnetic resonance spectroscopy, *Journal of Chromatography*, 1112 (2006) 148-155.
- [59] A.R. Linnemann, M. Benner, R. Verkerk, M.A.J.S. van Boekel, Consumer-driven food product development, *Trends in Food Science and Technology*, 17 (2006) 184-190.
- [60] J. Lintschinger, N. Fuchs, H. Moser, R. Jager, T. Hlebeina, G. Markolion, W. Gössler, Uptake of various trace elements during germination of wheat, buckwheat and quinoa, *Plant Foods for Human Nutrition*, 50 (1997) 223-237.

-
- [61] M.L. Lopez-Amoros, T. Hernandez, I. Estrella, Effect of germination on legume phenolic compounds and their antioxidant activity, *Journal of Food Composition and Analysis*, 19 (2006) 277-283.
 - [62] A. Martínez-Sánchez, A. Allende, R.N. Bennett, F. Ferreres, M.I. Gil, Microbial, nutritional and sensory quality of Rocket leaves as affected by different sanitizers, *Postharvest Biology and Technology*, 42 (2006) 86-97.
 - [63] C. Martinez-Villaluenga, J. Frias, P. Gulewicz, K. Gulewicz, C. Vidal-Valverde, Food safety evaluation of broccoli and radish sprouts, *Food and chemical Toxicology*, 46 (2008) 1635-1644.
 - [64] S. Mbithi-Mwikya, J. Van Camp, Y. Yiru, A. Huyghebaert, Nutrient and antinutrient changes in finger millet (*Eleusine coracana*) during sprouting, *Lebensm. Wiss. Technol.*, 33 (2000) 9-14.
 - [65] D.A. Moreno, M. Carvajal, C. López-Berenguer, C. García-Viguera, Chemical and biological characterisation of nutraceutical compounds of broccoli, *Journal of Pharmaceutical and Biomedical Analysis*, 41 (2006) 1508-1522.
 - [66] M.S. Morton, K. Griffiths, G. Wilcox, M.L. Wahlqvist, Determination of lignans and isoflavonoids in human female plasma following dietary supplementation, *Journal of Endocrinology*, 142 (1994) 251-259.
 - [67] R. Munday, C.M. Munday, Selective induction of phase II enzymes in the urinary bladder of rats by allyl isothiocyanate, a compound derived from Brassica vegetables, *Nutrition and Cancer*, 44 (2002) 52-59.
 - [68] M. Murashima, S. Watanabe, X.G. Zhuo, M. Uehara, A. Kurashige, Phase 1 study of multiple biomarkers for metabolism and oxidative stress alter one-week intake of broccoli sprouts, *BioFactors*, 22 (2004) 271-275.
 - [69] G. Murillo, R.G. Mehta, Cruciferous vegetables and cancer prevention, *Nutrition and Cancer*, 41 (2001) 17-28.
 - [70] K. Nakagawa, T. Umeda, O. Higuchi, T. Tsuzuki, T. Miyazawa, Evaporative light-scattering analysis of sulforaphane in broccoli samples: quality of broccoli products regarding sulforaphane contents, *Journal of Agriculture and Food Chemistry*, 54 (2006) 2479-2483.

-
- [71] T. Nodaa, S. Takigawaa, C. Matsuura-Endoa, K. Saitoa, K. Takataa, T. Tabikia, T.A.M. Wickramasingheb, The physicochemical properties of partially digested starch from sprouted wheat grain, *Carbohydrate Polymers*, 56 (2004) 271-277.
- [72] L. Nugon-Baudon, O. Szytit, P. Raibaud, Production of toxic glucosinolate derivatives from rapeseed meal by intestinal microflora of rat and chicken, *Jornal of the Science Food and Agriculture*, 43 (1988) 299-308.
- [73] E. Penas, R. Gomez, H. Frias, C. Vidal-Valverde, Efficacy of combinations of high pressure treatment, temperature and antimicrobial compounds to improve the microbiological quality of alfalfa seeds for sprout production, *Food Control*, 20 (2009) 31-39.
- [74] E. Penas, R. Gomez, J. Frias, C. Vidal-Valverde, Application of high-pressure on alfalfa (*Medicago sativa*) and mung bean (*Vigna radiata*) seeds to enhance the microbiological safety of their sprouts, *Food Control*, 19 (2008) 698-705.
- [75] F.M.V. Pereira, E. Rosa, J.W. Fahey, K.K. Stephenson, R. Carvalho, A. Aires, Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* var. *Italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes, *Journal of Agriculture and Food Chemistry*, 50 (2002) 6239-6244.
- [76] S. Perez-Balibrea, D.A. Moreno, C. García-Viguera, Determination of the health-promoting compounds of broccoli sprouts grown under two different light conditions: In: *Future Trends in Phytochemistry. A young Scientists Symposium*. Palacký University & Institute of Experimental Botany AS and the Phytochemical Society of Europe (Olomouc, Czech Republic) (2006).
- [77] P. Perocco, G. Bronzetti, D. Canistro, L. Valgimigli, A. Sapone, A., Affatato, G.F. Pedulli, L. Pozzetti, M. Broccoli, R. Iori, J. Barillari, V. Sblendorio, M.S. Legator, M. Paolini, S.Z. Abdel-Rahman, Glucoraphamin, the bioprecursor of the widely extolled chemopreventive agent sulforaphane in humans after ingestion of steamed and fresh broccoli, *Nutrition and Cancer*, 38 (2006) 168-178.
- [78] A. Poulev, J.M. O'Neal, S. Logendra, R. Pouleva, V. Tineva, A.S. Garvey, D. Gleba, I.S. Jenkins, B.T. Halpern, R. Kneer, G.M. Cragg, I.

- Raskin, Elicitation, a new window into plant chemodiversity and phytochemical drug discovery, *Journal of Medicinal Chemistry*, 46 (2003) 2542-2547.
- [79] R. Randhir, Y.I. Kwon, K. Shetty, Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings, *Innovative Food Science and Emerging Technologies*, 9 (2008) 355-364.
- [80] K. Reinli, G. Block, Phytoestrogen content of foods - a compendium of literature values, *Nutrition and Cancer*, 26 (1996) 123-148.
- [81] P. Rozan, Y.H. Kuo, F. Lambein, Amino acids in seeds and seedlings of the genus *Lens*, *Phytochemistry*, 58 (2001) 281-289.
- [82] E. Sangronis, C.J. Machado, Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*, *LWT*, 40 (2007) 116-120.
- [83] K. Sato, Y. Kudo, K. Muramatsu, Incorporation of a high level of vitamin B₁₂ into a vegetable, kaiwaredaikon (Japanese radish sprout), by the absorption from its seeds, *Biochimica et Biophysica Acta*, 1672 (2004) 135-137.
- [84] S. Schenker, Facts behind the headlines, Broccoli, British Nutrition Foundation - *Nutrition Bulletin*, 27 (2002) 159-160.
- [85] B.O. Schneeman, Emerging food technology and world health, *Journal of Food Sciences*, 69 (2004) C123-C126.
- [86] T.A. Shapiro, J.W. Fahey, K.L. Wade, K.K. Stephenson, P. Talalay, Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans, *Cancer Epidemiology Biomarkers and Prevention*, 10 (2001) 501-508.
- [87] K. Shetty, P. McCue, Phenolic antioxidant biosynthesis in plants for functional food application: integration of systems biology and biotechnological approaches, *Food Biotechnology*, 17 (2003) 67-97.
- [88] M. Shikita, J.W. Fahey, T.R. Goleen, W.D. Holtzclaw, P. Talalay, An unusual case of 'uncompetitive activation' by ascorbic acid: purification and kinetic properties of a myrosinase from *Raphanus sativus* seedlings, *Biochemical Journal*, 341 (1999) 725-732.

-
- [89] G. Sripriya, U. Antony, T.S. Chandra, Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*), *Food Chemistry*, 58-4 (1997) 3455-3501.
- [90] S. Sugihara, M. Kondu, Y. Chihara, M. Yuji, H. Hattori, M. Yoshida, Preparation of Selenium-enriched Sprouts and Identification of their Selenium Species by High-performance Liquid Chromatography- Inductively Coupled plasma Mass Spectrometry, *Biosci. Biotechnol. Biochem.*, 68 (2004) 193-199.
- [91] H.G. Sung, H.T. Shin, J.K. Ha, H.L. Lai, K.J. Cheng, J.H. Lee, Effect of germination temperature on characteristics of phytase production from barley, *Bioresource Technology*, 96 (2005) 1297-1303.
- [92] Y. Takaya, Y., Kondo, T. Furukawa, M. Niwa, Antioxidant constituents of radish sprout (Kaiware-daikon), *Raphanus sativus* L., *Journal Agric. Food Chem.*, 51 (2003) 8061-8066.
- [93] J.L. Thomas, M.S. Palumbo, J.A. Farrar, T.B. Farver, D.O. Cliver, Industry practices and compliance with U.S. Food and Drug Administration Guidelines among California sprout firms, *Journal of Food Protection*, 66 (2003) 1253-1259.
- [94] M. Tokiko, Y. Koji, Proximate composition, fatty acid composition and free amino acid composition of sprouts, *Journal for the Integrated Study of Dietary Habits*, 16 (2006) 369-375.
- [95] J. Ubbink, R. Mezzenga, Delivery of functionality in complex food systems: introduction, *Trends in Food Science and Technology*, 17 (2006) 194-195.
- [96] G. Urbano, P. Aranda, A. Vilchez, C. Aranda, L. Cabrera, J. Porres, M. Lopez-Jurado, Effects of germination on the composition and nutritive value of proteins in *Pisum Sativum*, *L. Food Chemistry*, 93 (2005a) 671-679.
- [97] G. Urbano, M. López-Jurado, S. Frejnagel, E. Gómez-Villalva, J.M. Porres, H. Frías, C. Vidal-Valverde, P. Aranda, Nutritional assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by in vitro and in vivo techniques, *Nutrition*, 21 (2005b) 230-239.

-
- [98] G. Urbano, M. López-Jurado, C. Aranda, A. Vilchez, L. Cabrera, J.M. Porres, P. Aranda, Evaluation of zinc and magnesium bioavailability from pea (*Pisum sativum* L.) sprouts. Effect of illumination and different germination periods, *International Journal of Food Science and Technology*, 41 (2006) 618-626.
- [99] D.R. Valenzano, A. Cellerino, Resveratrol and the pharmacology of aging: a new vertebrate model to validate and old molecule, *Cell Cycle*, 5 (2006) 1027-1032.
- [100] P. Vitaglione, S. Sforza, G. Galaverna, C. Ghidini, N. Caporaso, P.P. Vescovi, V. Fogliano, R. Marchelli, Bioavailability of transresveratrol from red wine in humans, *Molecular Nutrition and Food Research*, 49 (2005) 495-504.
- [101] P.K.J.P.D. Wanasundara, F. Shahidi, M.E. Brosnan, Changes in flax (*Linum usitatissimum*) seed nitrogenous compounds during germination, *Food Chemistry*, 65 (1999) 289-295.
- [102] K.H. Wang, Y.H. Lai, J.C. Chang, T.K. Ko, S.L. Shyu, R.Y.Y. Chiou, Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable, *Journal of Agriculture and Food Chemistry*, 53 (2005) 242-246.
- [103] G.P. Webb, *Dietary Supplements and Functional Foods*, Blackwell Publishing Ltd., Oxford, (2006) 1-120.
- [104] B.A. Wolucka, A. Goossens, D. Inzé, Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions, *Journal of Experimental Botany*, 56 (2005) 2527-2538.
- [105] M.J. Xu, J.F. Dong, M.Y. Zhu, Effects of germination conditions on ascorbic acid level and yield of soybean sprouts, *Journal of the Science of Food Agriculture*, 85 (2005) 943-947.
- [106] L. Ye, A.T. Dinkova-Kostova, K.L. Wade, Y. Zhang, T.A. Shapiro, P. Talalay, Quantitative determination of dithiocarbamates in human plasma, serum erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans, *Clinica Chimica Acta*, 316 (2002) 43-53.

-
- [107] M. Yoshida, T. Okada, Y. Namikawa, Y. Matsuzaki, T. Nishiyama, K. Fukunaga, Evaluation of nutritional availability and anti-tumor activity of selenium contained in selenium-enriched kaiware radish sprouts, *Biosci. Biotechnol. Biochem.*, 71 (2007a) 2198-2205.
- [108] M. Yoshida, K. Sano, E. Ishiyuki, T. Nishiyama, K. Fukunaga, Assessment of nutritional availability of selenium-enriched pumpkin, *Biomed Res Trace Elements*, 18 (2007b) 391-394.
- [109] J. Zhao, L.C. Davis, R. Verpoorte, Elicitor signal transduction leading to production of plant secondary metabolites, *Biotechnology Advances*, 23 (2005) 283-333.
- [110] H. Zielinski, J. Frias, M.K. Piskula, H. Kozłowska, C. Vidal-Valverde, Vitamin B₁ and B₂ dietary fiber and minerals content of Crucifera sprouts, *European Food Research and Technology*, 221 (2005) 78-63.

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