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The role of selenium content of wheat in the human nutrition. A literature review

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Abstract. The authors discuss the importance of the selenium content of wheat in the human nutrition during which they deal with selenium as a component of enzymes, selenium defficiency of domestic animals, the role of selenium in the nutrition, in this connection the consequences of selenium defficiency andtoxicity of selenium. Thereafter, analyzing the selenium content of wheat and its utilization, the contribution of cereals to the human's selenium demand, selenium content of wheat cultivated on different soils, effect of technology (grinding) on the selenium content of flour, selenium species occuring in wheat, bioavailability of selenium content of various foods as well as the effect of selenium on the enzyme activity in wheat are treated. In the second part of the study availability of selenium content of soil is analyzed in different plants, mainly in

Key words and phrases: selenium, selenium species, selenium deficiency, selenium toxicity, selenium content of wheat, selenium content of soil, bioavailability of selenium content, possibilities for increasing the selenium content of wheat.

wheat, possibilities for increasing selenium content in foods by increasing selenium content of soil is investigated, and finally incorporation of selenium in wheat is treated. It is established that selenium is an essential element for the human organism as it is co-factor of many enzymes. It also discussed that certain parts of the world can be extremely selenium deficient, and there are also such areas where selenium content of the soil reaches a toxic level. Different selenium species are utilized differently in the humans and in animals, therefore it is not enough to analyze only the total selenium content but also the selenium species should be analyzed. Finally, it is established that by increasing the selenium content of soil the selenium content of wheat can be considerably increased, which as being the most important cereals, can considerably contribute to the satisfaction of the human selenium requirements.

1 The role of selenium in the human nutrition

Selenium as enzyme component

Selenium can be present in foods as essential nutrient or toxic material, as selenium known earlier merely as carcinogenic and toxic material (*Whanger*, 2002) found even if in small amount to be essential for animals at the end of the 1950's (*Schwarz and Foltz*, 1957), since selenium is an essential component of more than 30 selenoproteins and selenoenzymes, in mammals (*Brown and Arthur*, 2001; *Rayman*, 2002). Properties and biological functions of around 15 selenoenzymes including the antioxidant glutathione-peroxidases (GPx) were discovered, three forms of thioredoxin reductases playing important role in regeneration of the antioxidant system of the organism and contributing to the establishment of the intracellular redox status.

The properties of three iodothyronine deiodinase enzymes that are playing a role in the formation of the thyroid hormone were also described (*Brown and Arthur*, 2001; *FAO*, *WHO*, 2001; *Rayman*, 2002). In these selenoproteins selenium is present in the form of selenocysteine (Se-Cys) determined by the codon UGA, typically a stop codon, during the ribosomic protein synthesis (*Low and Berry*, 1996; *Stadtman*, 1996). Under normal physiological conditions selenium occurs in Se-Cys almost fully ionized which provide an especially effective biological catalysis for the selenoproteins (*Brown and Arthur*, 2001; *Stadtman*, 1996). In plants, selenium has no known functional effect. Incorporation of the selenoamino acids into the plants happens by replacement of cysteine and methionine which is usually associated with harmful consequences for the plants.

Selenium deficiency of domestic animals

Selenium defficiency of domestic animals is well-known, white-muscle disease of calves and sheeps is induced by selenium deficiency. Based on the literature it appears that out of the domestic animals the sheep, mainly the young lamb is the most sensitive to the selenium deficiency, but it is very important to satisfy the selenium need of all the other animals as well. *Serdaru et al.* (2003) examined the selenium status of 185 feeding stuff samples (hays, green plants and feedstuff concentrates) cultivated in south-west Romania (Dobrudja). Selenium content of the samples was determined spectrofluorometrically after derivatization with 2,3-diamminenaphthalene. Only 6.5% of the samples contained an appropriate selenium content (0.15-0.30 mg/kg), while 93.5% of the analyzed samples had a selenium content ranging 0.001-0.150 mg/kg that is, these feedstuffs were selenium deficient. Based on the selenium content the samples were divided into three groups: 3.2% very deficient (selenium content below 0.01 mg/kg), 84.9% critical (selenium content between 0.01-0.1 mg/kg), and 5.4% borderline case with a selenium content of 0.1-0.15 mg/kg. Summarized, it can be said that the feeding stuffs cultivated in Dobrudja are in general selenium deficient and no question about that actions should be taken in order to eliminate selenium deficiency of animals.

Vignola et al. (2009) examined and evaluated the performance, the quality and oxidative stability of meat, the total Se and specific selenoamino-acids content of muscle of lambs that were fed diets supplemented from different Se sources and at different levels. Forty-eight Apennine lambs 30 day old (12.78 $\pm$ 0.94 kg) received, during a 63 day period, a total mixed ration (TMR) which was either Se unsupplemented (Control group – background only – 0.13 mg/kg Se) or supplemented with Na selenite (0.30 mg/kg Se as sodium selenite) or selenium enriched yeast (0.30 mg/kg and 0.45 mg/kg Se as Se-yeast). Growth performance, feed to gain ratio, carcass and meat quality (pH, drip and cooking losses, colour, GSH-Px activity and chemical analysis) did not show any difference between the treatments. Meat colour and oxidative stability during 9 days of refrigerated storage were unaffected by dietary supplementation, suggesting that, at the levels of Se used in this experiment, dietary Se, even from an organic source, had limited potential for reducing lipid oxidation. Selenium supplementation raised the Se content in muscle ($P < 0.001$) with the greatest increase when Se-yeast was fed. Although selenite increased total Se, it did not influence total or specific selenoamino-acids in this tissue. On the contrary, Se-yeast supplementation led to an increase in muscle Se-methionine content. It was concluded that Se supplementation can increase significantly

muscle Se levels and produce, particularly when Se-yeast is fed, a source of Se enriched meat as Se-methionine.

Juniper et al. (2009a) examined the effect of feeding stuff supplementation with selenium-enriched yeast and sodium selenite on the quality of lamb meat. Total selenium content, selenomethionine and selenocysteine content and oxidative stability of meat after slaughtering were examined due to consumption of selenium-enriched yeast and sodium selenite. During the 112-day-experiment done with 50 lambs, the feeding stuff was supplemented with different amount of selenium-yeast and sodium selenite. At the beginning of the experiment and at day 28, 56, 84 and 112 blood sample was taken and the amount of selenium as well as various selenium-containing compounds and glutathione peroxidase activity were measured. At the end of the experiment the animals were slaughtered, then determination of selenium and various selenium species were carried out in heart, liver, kidney and skeletal muscle. As the lambs received feedstuff supplemented with selenium yeast total selenium level, selenomethionine level of the blood and glutathione peroxidase activity of the erythrocytes increased, however, no change was experienced during the supplementation with sodium selenite. With the exception of kidney tissue, all the other tissues showed a dose-dependent change regarding both total selenium content and selenomethionine content during supplementation with selenium-containing yeast. Based on a longer term examination, selenium content was higher for animals fed with selenium yeast than for those that were fed with sodium selenite, which means a better utilization for selenium from the yeast. Selenium or selenocysteine supplementation did not affect the glutathione peroxidase activity and thiobarbituric acid reactive substances. Despite this, the oxidative stability was slightly higher due to selenium supplementation.

Juniper et al. (2009b) examined the effect of high-dosage selenium yeast supplementation on selenium content of lamb issues and on different selenium compounds. 32 lambs weighing 6.87 kg were fed with milk-replacing feed preparation supplemented with 6.3 mg/kg selenium on dry-matter basis. Selenium content of feed of the control lambs that received no selenium supplementation was 0.13 mg/kg. The experiment was carried out during 91 days, and blood samples were taken on days 28, 56 and 91. At the end of each treatment five lambs were slaughtered and selenium content of the heart, liver and skeletal muscles was examined. Total selenium content of blood of group treated with selenium yeast was 815 ng/ml, this value for the control group was 217.8 ng/ml. Total selenium content of the tissues in the groups received selenium supplementation was significantly higher compared to the

control group (26 times higher in the skeletal muscle, 16 times higher in the liver, 8 times higher in the heart and 3 times higher in the kidney). Total selenium content as well as selenomethionine and selenocysteine content differed considerably in the individual tissues. Selenocysteine was the dominant amino acid in the liver and kidney. It was established that due to the selenium supplementation total selenium content of tissues increased and also that total amount of selenium and selenoamino acid was different due to the treatments.

Kumar et al. (2009) studied the effect of inorganic and organic selenium supplementation on 18 lambs of weight of 24.68 kg and aged 8-9 months. The lambs were divided randomly into six different groups. Feeding stuff of the control group consisted of maize groats, soybean flour, wheat bran, mineral supplementation without selenium, common kitchen salt and wheat straw. Beyond this, the experimental group received 15 mg/kg selenium in the form of sodium selenite, the other the same quantity but in the form of organic selenium supplementation. The experiment was conducted 90 days long. In order to trigger the humoral immune response the animals were vaccinated with Haemorrhagic septicaemia vaccine, and at the beginning of the experiment, on day 30, 60 and 90 blood sample was taken. Selenium supplementation had no effect whatsoever on the total cholesterol, total protein, albumin and globulin amount of the serum, the ratio of albumin and globulin, Ca and P level of the serum as well as activity of the measured enzymes (glutamate-oxalacetate-trans-amilase, glutamate-pyruvate-trans-amilase). In opposition to it, compared to the control group selenium level of the serum significantly increased as well as the number of the red blood cells and the humoral immune response, for both groups received selenium supplementation. The daily weight gain was the highest for the group received organic selenium supplementation, followed by the group received inorganic selenium supplementation, and the lowest value was measured for the control groups. The supplementation with the organic and inorganic selenium improved the growth indices. Regarding the humoral immune response and antioxidant status of the lambs the organic selenium proved to be more effective.

Hall et al. (2009) examined selenium status of sheeps due to short term high selenium feeding and mineral supplementation, respectively. Sheep grazed in Se-deficient areas without access to Se supplements may be Se deficient by the end of the grazing season. One limitation to feeding mineral mixes and feeds containing inorganic Se-supplements is the short duration of Se storage in the animal. Another is that Se supplementation may be limited by country-specific regulations. However, the use of feedstuffs naturally high in Se to deliver supranutritional levels of Se is not regulated. The purpose of this

study was to evaluate Se status in sheep after short-term exposure to high-Se-fertilized forage vs. a commonly used mineral supplement containing inorganic sodium selenite. Selcote Ultra® was mixed with nitrogen fertilizer in the form of urea and applied to pasture at a rate of 3.4 kg Selcote Ultra®/ha and 67.4 kg nitrogen/ha. Thirty ewes were randomly divided into two groups. One group of 15 ewes grazed Se-fertilized pasture for 40 days and had no mineral supplementation. The other group of 15 ewes grazed a non-Se-fertilized pasture and received a custom made mineral supplement containing 200 mg/kg sodium selenite for 40 days. Subsequently the two groups of ewes were combined and grazed a non-Se-fertilized pasture and had no mineral supplementation. Sheep were bled pre- and post-treatment and then every 2-4 weeks thereafter for approximately 9 months to assess whole-blood Se concentrations. Whole-blood Se concentrations were higher ($P < 0.0001$) immediately post-treatment in sheep grazing Se-fertilized forage (573 ± 20 ng/ml) compared to sheep receiving mineral supplement containing Se (286 ± 20 ng/ml), and were still higher ($P < 0.0001$) at the end of 9 months (97 ± 7 ng/ml vs. 61 ± 7 ng/ml, respectively). Whole-blood Se concentrations were within the normal reference interval (150-500 ng/ml) for a longer period of time in sheep grazing Se-fertilized forage (7 months) compared to sheep receiving mineral supplement containing inorganic Se (4 months). No sheep showed clinical signs of ill-effects from Se supplementation. In conclusion, short-term exposure of sheep to Se-fertilized forage results in whole-blood Se concentrations sufficient to maintain adequate concentrations throughout grazing periods when there is limited access to Se supplements.

The role of selenium in the human nutrition – consequences of selenium deficiency

In humans two diseases have been associated with severe selenium deficiency: the Keshan disease (endemic cardiomyopathy) and Kaschin-Beck disease (an osteoarthropathy). Keshan disease is endemic in children and in women in childbearing age, and occurs from north-east to south-west China. These areas are characterised by very low selenium availability in soil and extremely low selenium concentrations in crops (*Combs*, 2001; *FAO*, *WHO*, 2001; *Tan and Huang*, 1991).

According to *Boldery et al.* (2006) lack of vitamins and minerals was brought into connection with cardiomyopathic diseases already in the 30's of the last century. (Cardiomyopathy (CMP) is a collective term for heart diseases leading to weakening of pump functions of the heart, attributed to the own disease

of the muscle.) Dilatative cardiomyopathic diseases caused by selenium deficiency were first reported in 1935 in China's Keshan county. In their publication a cardiomyopathic disease is reported that can cause also loss of weight and problems of pancreas. The patient who was treated for heart complaints, lost weight after surgical treatment. After he received selenium supplementation his health condition and heart functions considerably improved. This case draws the attention that selenium deficiency can be a cause of cardio-vascular diseases.

Keshan disease is caused probably not by selenium deficiency alone but also infection with a coxsackie virus which is initially not virulent but after being in contact with a selenium deficient patient, becomes active (*Beck et al.*, 2004; *FAO*, *WHO*, 2001). The Kaschin-Beck disease was detected in China in children aged 5-13 years, and less extensively in south-east Siberia (*FAO*, *WHO*, 2001). Apart from selenium deficiency this disease can be associated with mycotoxins in foods and fulvic acids in drinking water.

It appears to be evident that even a less apparent selenium deficiency can affect human health in various ways because it affects the immune functions, viral infections, male fertility, thyroid function, asthma and inflammatory diseases (*Rayman*, 2000, 2002). Selenium may play a role in the prevention of cardiovascular diseases, however, this has not been proved to be unequivocal (*Rayman*, 2000, *Stranges et al.*, 2006).

Increasing number of evidence has shown anticarcinogenic effect of selenium (*Combs*, 2005; *Combs et al.*, 2001; *Whanger*, 2004). Epidemiological studies showed a significant negative correlation between cancer mortality rates and selenium content of forage crops in some US counties (*Clark et al.*, 1991). Several clinical trials with humans have shown beneficial effect of selenium on reduction of cancer (*Combs*, 2005; *Whanger*, 2004). In an experiment the participants received a daily dose of 200 μg of selenium as selenium-enriched yeast it was found that selenium supplementation had no significant effect on non-melanoma skin cancer, but it reduced significantly the total cancer incidence, total cancer mortality and the incidences of prostate, colon and lung cancers (*Clark et al.*, 1996, 1998). These clinical results are consistent with studies on small animals where selenium was shown to have antitumour effect (Whanger, 2004).

Hartikainen (2005) investigated biogeochemistry of selenium and its impact on food chain quality and human health. In areas where soils are low in bioavailable selenium (Se), potential Se deficiencies cause health risks for humans. Though higher plants have been considered not to require this element, the experience with low-Se soils in Finland has provided evidence that the

supplementation of commercial fertilizers with sodium selenate affects positively not only the nutritive value of the whole food chain from soil to plants, animals and humans but also the quantity of plant yields. The level of Se addition has been optimal, and no abnormally high concentrations in plants or in foods of animal origin have been observed. Se levels in serum and human milk indicate that the average daily intake has been within limits considered to be safe and adequate. In fact, plants act as effective buffers, because their growth is reduced at high Se levels. They also tend to synthesize volatile compounds in order to reduce excess Se. On the other hand, when added at low concentrations, Se exerts a beneficial effect on plant growth via several mechanisms. As in humans and animals, Se strengthens the capacity of plants to counteract oxidative stress caused by oxygen radicals produced by internal metabolic or external factors. At proper levels it also delays some of the effects of senescence and may improve the utilization of short-wavelength light by plants. High additions are toxic and may trigger pro-oxidative reactions. Thus, the present supplementation of fertilizers with Se can be considered a very effective and readily controlled way to increase the average daily Se intake nationwide.

Selenium intake of humans ranges between very wide limits due to the consumption of foods with diffent selenium content. *Combs* (2001) showed in his study that in the different parts of the world there can be a difference of orders of magnitude in the selenium intake of people. In China for example in the Keshan area the selenium intake varies between 7-11 μg/day, whereas in the Enshi county of central China it can reach several thousands μg per day. In Europe selenium consumption of adults is around 30-100 μg per day, in North America 60-220 μg/ and in New Zealand's selenium deficient areas in some populations the daily selenium intake ranges between 19-80 μg/day. In some European countries selenium intake has decreased significantly in recent decades (*Rayman*, 2002). For example, average selenium intake in UK for adults has decreased from 60-63 μg/day measured in the 1970s to 29-39 μg in 1995 (*Ministry of Agriculture Fisheries and Food*, 1997; *Rayman*, 2002). The main reason for this is the decreased import of breadmaking wheat from North America which contains generally much more selenium than the wheat grown in the UK.

It appears that there is no general agreement as regards the sufficient selenium intake for humans (*Thomson*, 2004). The minimum selenium intake for the prevention of the Keshan disease was found to be around 17 μg/day (*Yang and Xia*, 1995), however, selenium intake for the maximum plasma GPx activity is estimated to be about 45 μg/day (*Thomson*, 2004). In the United

States and Canada the recommended daily dietary allowance is 55 μg/day. The European population reference intake is also set at 55 μg/day. In Australia and New Zealand the recommended dietary intakes for male and female adults are 70 and 60 μg/day, respectively. In the UK the reference nutrient intake is set at 75 μg and 60 μg/day for male and female adults, respectively. In contrast, the WHO and FAO normative requirement estimates are 40 μg and 30 μg/day for men and women, respectively. Based on the FAO and WHO normatives it is not possible to reach the maximal GPx activity. Further selenium intake is recommended for e.g. cancer prevention (*Rayman*, 2002). *Combs* (2001) suggested that a plasma selenium level above 120 μg/l may be useful for minimizing the risk of cancer. To achieve this, the dietary selenium intake should be at least 1.5 μg/kg body weight per day which is equivalent to 90-120 μg/day for a 60-80 kg person. Further research is needed to determine the minimum selenium intakes for a protective effect. Based on surveys of plasma or serum selenium levels *Combs* (2001) estimated that worldwide 0.5 and 1 billion people may be selenium deficient. In many European countries current plasma or serum selenium concentrations are below the level required for the maximum activity of plasma GPx.

Rasmussen et al. (2009) examined in Denmark the change in serum total selenium and selenomethionine content during eight years with special respect to the effect associated with selenium status. Blood samples were taken from 817 randomly chosen people, and and information on smoking habits, alcohol consumption and sporting activity was collected by questionnaire. For men the average serum selenium level was 98.7 μg/l, the selenoprotein level was 2.72 mg/l. Both selenium and selenoprotein level of the serum increased with the age and the selenoprotein level was higher for men than for women. Selenium level of the serum decreased by around 5% between 1997-2005, at the same time the selenoprotein level significantly increased. Fish consumption had only a very slight effect on the selenium level and did not influence the selenoprotein level at all. Smoking, alcohol consumption, physical exercises or drug consumption did not affect selenium status. It was found that selenium status of the Danish population was appropriate. No groups could be found where according to age, sex and lifestyle special attention should be paid to the selenium deficiency.

Toxicity of selenium

Excessive selenium intakes can lead to chronic toxicity (selenosis) with health damages as loss of hair and nails, skin lesions, hepatomegaly, polyneuritis

and gastrointestinal disturbances. Chronic selenosis was reported from Enshi county in central China where soil, locally produced foods, and water contain extremely high levels of selenium (*Combs*, 2001; *Yang and Xia*, 1995). Studies in this county showed that the toxic dietary selenium intake that would maintain the characteristic selenosis symptoms (fingernail changes) was approximately 1600 μg/day (*Yang and Xia*, 1995). A reduction of the selenium intake to 819 ± 126 μg/day enabled five selenosis patients to recover from fingernail lesions. Based on these studies *Yang and Xia* (1995) suggested that 600 μg/day was the individual daily maximum safe intake and recommended a maximum dietary selenium intake of 400 μg/day.

2 Selenium content in wheat and bioavailability of the selenium content

Contribution of the cereals to the selenium need of humans

Cereals, meats and fish are the main sources of selenium in the human diets (*Combs*, 2001). Cereals and cereal products contribute around 70% to the total dietary selenium intake in the low selenium areas of China, and 40-50% in the low-income population in India. In the UK a survey carried out in 1995 estimated that cereals and cereal products accounted for 18-24% of the total selenium intake. A survey in 27 regions of Russia showed a highly significant correlation between serum selenium and selenium content in wheat flour which indicates that wheat is an important source of selenium for the Russian population (*Golubkina and Alfthan*, 1999).

Selenium content of wheat grown on different soils

Cereals and cereal products contain a wide range of selenium concentration, most being between 10-550 μg/kg on fresh weight basis (*FAO*, *WHO*, 2001). More extreme values have also been reported, e.g. cereal grains produced in the Keshan county in China contain 3-7 μg/kg selenium, whereas wheat grain produced in North and South Dakota in the US may contain more than 2000 μg Se/kg. The nutritional minimum level both for animals and humans is about 50-100 μg Se/kg in dry fodder/food, and intake below that may cause selenium deficiency (*Gissel-Nielsen et al.*, 1984).

Based on previous studies in China, *Tan* (1989) proposed the following ranges of grain selenium concentrations: below 25 μg/kg deficient, 25-40 μg/kg marginal, 40-1000 μg/kg moderate to high, and above 1000 μg/kg excessive.

In general, European wheats contain lower levels of selenium than North American wheats. Low selenium concentrations have been reported in Scandinavian countries with concentration in wheat ranging between 7-18 μg/kg (*Gissel-Nielsen et al.*, 1984).

Murphy and Casman (2001) examined selenium content of foods consumed in Ireland. In the last 20 years in the UK and in other countries in Europe the selenium intake decreased due to the reduced importation of wheat with high selenium and protein content from North America and Canada. There are no results about selenium content of the Irish flour, bread and other foods, therefore it is difficult to estimate the daily selenium intake in Ireland. For this reason selenium content of various Irish foods, especially bread and flour were measured, after acidic digestion by hydride generation atomic absorption spectrophotometry. Less fine wheat flour had a higher selenium content (7.7-9.9 μg/100 g) than the finest wheat flour with a selenium content ranging between 6.0-6.9 μg/100 g. Selenium content of the Irish brown bread (8.6-12.9 μg/100 g) was higher than that of the white bread (6.6 μg/100 g). It was found that the Irish flours and breads did not contain so much selenium as those from North America or Canada, and contained only a little more selenium that those currently being consumed in the UK.

British wheat also has low selenium status (*Barclay and Macpherson*, 1986). *Adams et al.* (2002) conducted a survey of 452 grain samples of breadmaking wheat produced in the UK in the 1982, 1992 and 1998 seasons and reported a range of 6-858 μg Se/kg dry weight with mean and median values of 32 and 22 μg/kg, respectively. On a fresh weight basis and assuming a 15% moisture content, the mean and median values were 27 and 18 μg Se/kg, respectively. 91% of the samples contained less than 50 μg Se/kg fresh weight. In comparison, *Wolnik et al.* (1983) reported a range of 10-5300 μg Se/kg fresh weight with mean and median values of 160 and 370 μg Se/kg, respectively, for 290 wheat samples collected from major growing areas in US. Wheat samples from Manitoba of Canada had a mean selenium concentration of 760 μg/kg (*Boila et al.*, 1993). These surveys indicate that North American wheats contain on average more than 10-fold larger concentrations of selenium than British wheats. Similarly, selenium concentrations in wheats produced in New Zealand are considerably lower than those produced in Australia, and the importation of Australian wheat was found to be an important beneficial factor affecting the blood selenium status in the residents of Hamilton area of New Zealand (*Watkinson*, 1981). A recent surveys of South Australian wheats reported a range of 5-720 μg Se/kg, with a mean value of 155 μg/kg (*Lyons et al.*, 2005b).

Lyons et al. (2005a) examined selenium status of wheat in Australia and found that wheat (Triticum aestivum L.) contributed to the greatest extent to the average plasma selenium concentration of 103 μg/l. By the analysis of selenium content of 834 blood plasma in six experiments was obtained that the selenium content was higher in males and increased with the age. This study showed that many South Australians consume inadequate amount of selenium to maximise selenoenzyme expression and cancer protection, and indicated that levels declined around 20% from the 1970's. No significant genotypic variability for grain selenium concentration was observed in modern wheat cultivars, but the diploid wheat was higher. Grain selenium concentration ranged between 5-720 μg/kg, and it was apparent that this variation was determined mostly by available selenium content of the soil. In both glasshouse and field trials selenium applied as sodium selenate at rates of 4-120 g Se/ha increased grain selenium concentration progressively up to 133-fold value when sprayed on soil at seeding and up to 20-fold when applied as a foliar spray after flowering. A treshold of toxicity of around 325 mg Se/kg in leaves of young wheat plants was observed, a level that would not be normally reached with selenium fertilisation. On the other hand sulphur applied at the low rate of 30 kg/ha at seeding reduced grain selenium concentration by 16%. It was established that agronomic biofortification could be used by food companies as a cost-effective method to produce high-selenium wheat products that contain most selenium in the desirable selenomethionine form. Further studies are needed to assess the functionality of high-selenium wheat, e.g. short-term clinical trials that measure changes in genome stability, lipid peroxidation and immunocompetence. Increasing selenium content of wheat is a food system strategy that could increase the selenium intake of whole population.

Lyons et al. (2005b) analyzed selenium content of various wheats grown in Mexico and Australia and other commercial cultivars. Cultivars were also grown under the same conditions to assess genotypic variation in selenium density. Selenium content of the grains varied between 5-720 μg Se/kg and this variation could be associated with the selenium content of the soil. On identical soils no significant genotypic variation could be found among modern commercial bread and durum wheat, triticale or barley. However, the diploid wheat and rye were 42% and 35%, respectively, higher in grain selenium concentration than other cereals in separate field trials, and in a hydroponic trial rye was 40% higher in foliar selenium content than two wheat landraces. While genotypic differences may exist in modern wheat varieties, they are likely to be small in comparison with background soil variation, at least in South Australia and Mexico.

Effect of the technology (milling) on the selenium content of flour

Milling of wheat has only a small effect on the selenium concentration of flour fractions. A study by *Eurola et al.* (1991) showed that wheat bran and flour fractions contained slightly more selenium than other flours, whereas selenium levels of breads were somewhat lower than those of the corresponding flours. Similarly, *Lyons et al.* (2005a) found selenium to be fairly evenly distributed throughout in the wheat grain, except that the embryo tended to have a higher concentration than the other milled fractions. It was established that further processing did not affect selenium content of wheat products.

Selenium forms in foodstuffs and in wheat

Selenium exists in foods in different chemical forms. *Guo and Wu* (1998) examined the free selenoamino acids in the plant tissues and their distribution in high-selenium soils. Accumulation of the selenoamino acids in the vegetable tissues is associated with not only with the tolerance against selenium of the plants but also selenium poisoning of animals. Selenoamino acid content of the selenium-tolerant plants was examined by high-resolution gas chromatography coupled with mass spectometer. Five selenoamino acids (selenocystine, selenomethionine, selenocysteine, Se-methyl-selenocysteine and γ-glutamyl-Se-methyl-selenocysteine) were identified in the vegetable tissue concentrates. The amount of Se-methyl-selenocysteine was 15.3 μmol/kg in the plants grown on low-selenium soils and 109.8 μmol/kg in the plants grown on high-selenium soils. Also γ-glutamyl-Se-methyl-selenocysteine was detected although in a very low concentration. Selenium-accumulation experiments showed that concentration of selenocysteine increased to 5-fold in the vegetal tissues, while the total amount of selenium ranged from 5.07 to 22.02 mg/kg, but no further increase in the selenocysteine concentration was found with the further increase of the selenium concentration from 22.0 mg/kg to 117.5 mg/kg. It was found that selenomethionine in the plants represented more than 50% of total selenium. Further investigations are necessary in order to find out what mechanism affects the accumulation of selenocysteine in plants.

A range of selenocompounds have been identified in plants such as selenate, selenite, selenocysteine (Se-Cys), selenomethionine (Se-Met), selenohomocysteine, Se-methyl-selenocysteine (MeSeCys), γ-glutamyl-Se-methyl-selenocysteine, selenocysteineselenic acid, Se-propionylselenocysteine selenoxide, Se-methylselenomethionine (SeMM), seleno-cystathionine, dimethyl diselenide, selenosinigrin, selenopeptide and selenovax (*Whanger*, 2002).

Se-Met is the predominant form of Se in the wheat grain (56-83%), with other selenocompounds existing in smaller proportions: selenate (12-19%), Se-Cys (4-12%), Se-methyl-selenocysteine (1-4%), and others 4-26% (*Whanger*, 2002). In contrast, over 50% of the Se in wheat straw is selenate. An enzymatic hydrolysis released 70% of the Se in a wheat flour sample, of which Se-Met and Se-Cys accounted for 73% and 27% of the released selenium, respectively (*Moreno et al.*, 2004). *Stadlober et al.* (2001) reported that the enzymatic hydrolysis of wheat, barley and rye flour samples released 80-95% of the total Se, with 62-86% being Se-Met.

3 Bioavailability of selenium content of different foods

The bioavailability of selenium varies between different foods. SeMet (in plant and animal sources) and SeCys (mainly in animal sources) have high bioavailability (more than 90%), whereas the bioavailability of the inorganic selenate and selenite exceeds 50% (*Thomson*, 2004). Selenium in wheat grain has high bioavailability. In a feeding trial with rats, wheat Se had a bioavailability of 83%, compared to mushrooms (5%), tuna (57%) and beef kidney (97%) (*Thomson*, 2004). A study in humans showed that the inclusion of Se-enriched wheat in the diet for six weeks increased serum selenium significantly, whereas the consumption of selenium-enriched fish gave no significant effect (*Meltzer et al.*, 1993). *Fox et al.* (2005) compared the efficiency of selenium absorption in three food sources by humans using intrinsic labelling with the stabile isotopes 77Se and 82Se. They found that Se-absorption was significantly higher from wheat (81%) and garlic (78%) compared to fish (56%). Due to the high bioavailability of selenium, wheat would be a good choice for biofortification in order to enhance selenium intake by humans.

Effect of selenium on the activity of the enzymes of wheat

Nowak et al. (2004) examined the effect of selenium on the activity of oxido-reductase enzymes in soil and plants. In glasshouse trials the effect of 0.015; 0.15; 0.45 mmol/kg hydrogen selenide concentration was examined on the activity of the oxido-reductase enzymes of in wheat. Hydrogen selenide increased in each concentration the activity of nitrate reductase, but in plants the polyphenol oxidase activity was increased by the two lower concentrations only. The highest selenium concentration inhibited both the polyphenol

oxidase and peroxidase. In the plants the catalase activity reduced in the concentration range of 0.15-0.45 mmol/kg. It was found that the peroxidase activity in soil decreased due to the uptakable selenium content. The lowest selenium dose significantly (by 10%) increased the catalase activity, however, the effect of the higher doses on the enzyme activity is uncertain.

Bioavailability of selenium in soils to plants

If no direct selenium supplementation is applied, the selenium status in humans is determined by the supply of selenium in the soil to plants, which is governed largely by the underlying geology. The Earth's crust has an average selenium concentration of about 0.05 mg/kg (*McNeal and Balistrieri*, 1989). Magmatic rocks generally contain less selenium than sedimentary rocks especially shales (*Mayland et al.*, 1989). The concentration of selenium in most soils lies within the range of 0.01-2 mg/kg (*Kabata-Pendias and Pendias*, 1992). Selenium content of the soil in some parts of the world is low, including Nordic European countries, New Zealand, eastern and central Siberia, and the Keshan disease belt in China. These areas are notable also for having low selenium status in forage and food crops, humans and animals. Studies in New Zealand showed a high incidence of selenium-responsive disease in sheep in areas with soils containing less than 0.5 mg Se/kg (*Oldfield*, 1999). *Tan* (1989) defined the Se status for human nutrition according to the concentration of total selenium in soil as: deficient if less than 0.125 mg/kg; marginal 0.125-0.175 mg/kg; 0.175-3 mg/kg moderate-high; and excessive if above 3 mg/kg, respectively. Soils in England and Scotland tend to have relatively low selenium contents (*Oldfield*, 1999). A geochemical survey of the UK showed a range of total selenium in soils from 0.1 to 4 mg/kg, with more than 95% of the samples containing less than 1 mg/kg (*Broadley et al.*, 2006).

In contrast to the low Se soils, some areas in the world (e.g. the Great Plains of the US and Canada, Enshi county of China, parts of Ireland, Colombia and Venezuela) are seleniferous (*Combs*, 2001), with the soils having developed mainly from Se-enriched shales (*Mayland et al.*, 1989). Seleniferous soils are generally defined as those bearing vegetation containing more than 5 mg Se/kg, and are associated with Se poisoning of livestock and wild life (*Gupta and Gupta*, 1998; *Oldfield*, 1999). The total Se concentrations in seleniferous soils are usually in the range of 5-1200 mg/kg (*Mayland et al.*, 1989). Selenium deficient and selenium-toxic environments have been shown to occur within 20 km of each other in the Enshi county of China as a result of the variation in the underlying geology (*Fordyce et al.*, 2000a).

Soil conditions such as pH, soil texture and the contents of iron oxide/hydroxides and organic matter have a significant influence on the bioavailability of Se to plant uptake (*Gissel-Nielsen et al.*, 1984; *Mikkelsen et al.*, 1989). Soil pH and Eh affect the chemical species of Se presentin soil (*Elrashidi et al.*, 1987). Selenate is the predominant form in alkaline and well-oxidised soils, whereas in well-drained mineral soils with pH from acidic to neutral selenium exists predominantly as selenite. Under strongly reduced soil conditions selenide becomes the dominant form. Selenite is much more strongly adsorbed by the adsorbing surfaces of soils than selenate, and the adsorption of both decreases markedly with increasing pH (*Barrow and Whelan*, 1989). Selenate is only weakly adsorbed through a non-specific mechanism based on electrostatic forces, similar to the adsorption of sulphate, whereas the mechanism of selenite adsorption appears to be an innersphere surface complexation, similar to that for phosphate adsorption (*Barrow and Whelan*, 1989; *Neal et al.*, 1987). *Neal and Sposito* (1989) found no adsorption of selenate in alluvial soils from California over the pH range of 5.5-9. This means that selenate is more soluble and mobile than selenite in soil, and is therefore more bioavailable to plants but also more prone to leaching. Selenium bioavailability to plants generally decreases with decreasing pH and with increasing contents of clay, iron oxides and hydroxides and organic matter (*Gissel-Nielsen et al.*, 1984; *Johnsson*, 1991; *Mikkelsen et al.*, 1989). High contents of iron oxides and hydroxides and of organic matter, and low pH in soil have been identified as important factors contributing to the incidence of Keshan disease in China (*FAO*, *WHO*, 2001; *Fordyce et al.*, 2000b; *Johnson et al.*, 2000). Soil compaction and irrigation influence selenium concentration in wheat grain. Irrigation resulted in a 10-fold decrease in selenium concentration, possibly due to increased leaching losses of selenium, an antagonistic effect of sulphur in the irrigation water and a dilution effect from a higher grain yield (*Zhao et al.*, 2007). Soil compaction also led to a significant reduction in grain selenium concentration.

Zhao et al. (2006) investigated the effect of soil compaction and irrigation on grain selenium concentration. Grain selenium concentration ranged between 10-115 μg/kg which decreased by 30-75% due to irrigation. Significant selenium reducing effect of soil compaction has been explained by different mobility of the element due to different ionic transport from soil to the root. The observed effects in grain selenium content are considerable both in the respect of human nutrition and animal feeding because the concentration can vary from the sufficient to the very low level. It was found that the physical condition of the soil was always to be considered when the bioavailability of selenium is assessed.

Fan et al. (2008) examined the change in selenium content of soil and wheat grain in the last 160 years in the UK where the daily selenium consumption has been considerably decreased since the 1970's. In order to decide whether this was caused by the change in wheat grain selenium concentration or by a change in the environment, cereal grains, wheat grains and soil samples taken from areas differently fertilized were retrospectively analyzed back in the passed 160 years. Grain selenium content ranged between 11 and 236 ng/g. Grain samples collected from non-fertilized areas contained significantly more selenium than those from fertilized soils. No significant difference were found when short-straw wheat samples from the 1960's were analyzed. Wheat samples before 1920 and after 1970, respectively, contained more selenium than those between 1920 and 1970, although soil selenium content increased continuously during the passed 160 years. Based on the obtained results it was established that wheat grain selenium content affected by the sulphur emission into the atmosphere and the transfer from the atmosphere into the soil, respectively, but the yield increased by the plant breeding did not reduce significantly selenium content in the fertilized samples.

Zhao et al. (2009) examined selenium content of 150 bread wheat varieties cultivated at different places and in different seasons. No considerable difference was found in the selenium content among the expressly bread wheat varieties, which was chiefly affected by the type of soil. Selenium content was not dependent on which part of the wheat grain was sampled.

Possibilities of increasing selenium content of foods by increasing selenium content of the soil

Selenium content in food and forage crops can be increased by the addition of selenium to the soil-crop systems, a practice termed agronomic biofortification. The best example is the Finnish practice of adding sodium selenate to all multi-element fertilizers, which has occurred since 1984 (*Eurola et al.*, 1991; *Hartikainen*, 2005; *Yläranta*, 1990). Initially, 6 mg Se/kg fertilizer for grass and hay crops and 16 mg Se/kg fertilizer for cereals were used. These levels of addition provided approx. 3 and 8 g Se/ha for grass and cereals, respectively (*Eurola et al.*, 1990). Selenium concentrations in plants, animal products, soils, water and human sera have been monitored regularly, and the results have been used to adjust the amount of selenium addition. Between 1991-1997 the lower level (6 mg Se/kg fertilizer) was used for all crops. Since 1998 the selenium concentration has been raised to 10 mg Se/kg fertilizer (*Hartikainen*, 2005). This practice has substantially increased Se concentra-

tions in crops, vegetables, fruits and animal products. For example, the mean Se concentrations in all Finnish cereal grains were below 10 μg/kg dry weight before Se fertilization, and were increased to 250 μg/kg for spring wheat, 50 μg/kg for winter wheat, 40 μg/kg for rye, 170 μg/kg for wheat flour and 180 μg/kg for wheat bread, respectively, in the first three growing seasons after Se fertilization (*Eurola et al.*, 1990). As a result, the average Se intake in Finland increased from 25 μg/day before Se fertilization to around 110 μg/day (*Eurola et al.*, 1991), and the serum Se level increased from 60-70 μg/l to more than 100 μg/l (*Varo et al.*, 1988). The contribution of cereals to the total Se intakes also increased from 9% to 26% (*Eurola et al.*, 1991). Compared to direct selenium supplementation, agronomic biofortification is considered to be advantageous in that inorganic Se is assimilated by plants into organic forms (e.g. SeMet) which are more bioavailable to humans. In addition, plants act as an effective buffer that can prevent accidental excessive Se intakes by human that may occur with direct supplementation (*Hartikainen*, 2005).

Selenium fertilization has also been practiced in other regions of the world, such as for pasture in New Zealand and crops in the Keshan disease area in China. There have been numerous reports of Se-enriched plant products obtained by Se fertilization, including Se-enriched broccoli, garlic, onions, potatoes, mushrooms and tea. Both pot and field studies have shown that the addition of selenate increases plant selenium concentrations much more effectively than the addition of selenite (*Gissel-Nielsen et al.*, 1984; *Shand et al.*, 1992; *Singh*, 1991; *Cartes et al.*, 2005), therefore selenate is more widely used in Se fertilization and is available in a range of commercial Se fertilizers (*Broadley et al.*, 2006). It is important that field experiments are conducted under different cropping systems and climatic conditions to obtain reliable information on the optimum rate of Se fertilization. Field studies in Canada showed that the addition of 10 g Se/ha was necessary to ensure adequate selenium concentration (above 100 μg/kg) in barley grain (*Gupta*, 1995). *Tveitnes et al.* (1996) applied calcium nitrate enriched with 25 mg Se/kg as a top dressing for spring wheat in a Norwegian field trial. This provided 6.5 g Se/ha and increased the Se concentration in wheat grain to the desired level. In general, there is a little residual value to subsequent crops from appropriate additions of Se applied to previous crops, suggesting that the applied selenium which is not taken up by the plants is either fixed in the soil or lost to the environment. Monitoring of the environment is also required to ensure that agronomic biofortification of selenium does not lead to significant enrichment in water bodies. Finnish data show little evidence of Se enrichment in lake ecosystems (*Mäkelä et al.*, 1995; *Wang et al.*, 1995), although some ground water samples showed simultaneous

increases in total N, P and Se concentrations that may have resulted from the leaching of selenium from the fertilizers into the ground water. (*Mäkelä et al.*, 1995).

Lavado et al. (1999) examined the effect of agriculture and fertilization on extractable selenium content of soil. No difference was found due to fertilization and different agricultural technologies in case of soils with average selenium content of 3.33 mg Se/kg.

Martens and Suarez (1999) investigated the transformations of volatile methylated selenium in soil. Microbial volatilization of selenium as dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe) from soil is an important part of the Se cycle in nature, but little is known about the stability and transformations of these gases during residence in the soil environment before dissipation to the atmosphere. Experiments monitored by gas chromatography and atomic absorption spectroscopy were made with various clay mineral standards, charcoal, commercial humic substances and soils to determined the sorption and transformations of DMSe and DMDSe injected onto the headspace or passed through soil materials. Batch experiments conducted with 2-5 g materials placed into 40 ml Teflon centrifuge tubes equipped with Mininert gas sampling valves showed that DMSe was slowly sorbed by soil materials and most of the DMSe deficit in the headspace was recovered as selenite and selenate. In contrast, DMDSe was rapidly partitioned from the gas phase and resulted in an increased recovery of less soluble elemental and selenide forms. These results were confirmed during flow-through soil column studies with both little DMSe sorption and sorption of the majority of the DMDSe additions. Additions of selenomethionine to soil to produce DMSe and DMDSe in sealed flasks resulted in an increased partitioning of Se into inorganic when compared with a flow-through system designed to limit the contact of Se gases with soil. These results suggest that soil Se volatilization as DMSe and DMDSe results in Se loss to the atmosphere as DMSe with concomitant soil Se immobilization due to the instability of DMDSe.

Tan et al. (2002) examined the relation between soil selenium content and endemic diseases in China. In their study the geographical distribution of the total and water-soluble selenium content in topsoil (plough layer for cultivated soils, eluvial horizon for natural soils) was discussed and its relationship with some human health problems with China was evaluated. Topsoil samples, 354 in total, including 156 natural and 198 cultivated soils of 21 main soil types were collected. The total selenium concentration in soil samples was determined after derivatization with DAN (diaminonaphthalene) by spectrofluorometry. Water soluble Se concentration in soil was determined by the

same method after extraction with water in a soil to water ratio of 1:5. The geometric and arithmetic mean of total Se concentration was 0.173 mg/kg and 0.239 mg/kg, respectively, with the lowest value being 0.022 mg/kg and the highest one being 3.806 mg/kg. For the cultivated soils, the geometric mean of the total Se content was 0.188 mg/kg, and its arithmetic mean was 0.269 mg/kg, these values for the natural soils were 0.154 mg/kg and 0.206 mg/kg, respectively. Geometric mean of the water-soluble Se content of the soils was 4.0 μg/kg, the arithmetic mean was 6.4 μg/kg, the lowest value was 0.6 μg/kg and the highest one was 109.4 μg/kg. For the cultivated soils the average concentration of water-soluble Se was 4.3 μg/kg, similar to that of the natural soils (4.4 μg/kg geometric mean). Two sequences of the soil types, arranged separately in the concentration of total Se and water-soluble Se, are different and this demonstrates that the proportions of the two forms of selenium existing in various soils are different. The percentages of water-soluble Se to total Se in different types of soils varied from 1.07 to 6.69%. The laterite and other subtropic soil have relatively high water-soluble selenium contents because of their higher total selenium contents. A very significant correlation between total Se and water-soluble Se has been found in cultivated soil with a correlation coefficient of 0.58.

Dhillon et al. (2006) conducted greenhouse experiments to study the bioavailability of selenium to sorghum (Sorghum bicolor L.), maize (Zea mays L.) and berseem (Trifolium alexandrinum L.) fodders in a sandy loam soil modified with different levels os Se-rich wheat (Triticum aestivum L.) and raya (Brassica juncea L. Czern) straw containing 53.3 and 136.7 μg Se/g, respectively. Application of Se-rich straws to each crop, even at the highest rate of 1%, did not have any detrimental effect on dry-matter yield of the different crops. With an increase in the level of wheat straw from 0% to 1%, Se content in sorghum and maize plants increased to the highest level of 1.3 and 1.5 μg/g, respectively, at 0.3% of the applied straw and thereafter it decreased consistently. In case of raya sraw, the highest Se content in sorghum (2.3 μg/g) and maize (3.0 μg/g) was recorded at 0.3% and 0.4% of the applied straw, respectively. These investigations suggest that Se-rich raya and wheat straw may be disposed off safely in soils for growing fodders.

Hawkesford and Zhao (2007) analyzed various strategies for increasing the selenium content of wheat. Selenium is essential for humans and animals but has no known function in plants. Excess accumulation is toxic to both plants and animals. Dietary intake of selenium is low in a large number of people worldwide. This is due to low bioavailability of Se in some soils and consequently low concentrations of Se in plant tissues. Both selenate and selenite are

taken up by plants and subsequently translocated around the plant. Selenate is an analogue of sulphate and is transported by the sulphate transporter family. Some plants are able to accumulate high concentrations of selenium (hyperaccumulators), however, genetic variation in accumulation ability amongst non-accumulators such as cereals, is relatively small. Within plant tissues, selenium enters the pathways for sulphate assimilation and metabolism and will replace cysteine and methionine in proteins, often with detrimental effect. Alternatively, selenium may be accumulated as methylated derivatives or lost from the plant following volatilisation. Agronomic biofortification of crops with Se-containing fertilisers, which is practised in some countries, provides the best short-term solution for improving selenium content of wheat. Longer-term genetic improvement, particularly by targeting substrate discrimination of transporters between selenate and sulphate, for example, may provide a means to enhance uptake and promote accumulation.

Zhao and McGrath (2009) investigated soil selenium content and its reduction in experiments with plants. Plants and the associated rhizosphere microbes may be used to take up and/or volatilize excessive build-up of Se in contaminated soil and irrigation drainage water. Selenium, when present as selenate, is highly bioavailable to plant roots. Recent field trials have shown that transgenic Brassica Juncea (Indian mustard) overexpressing genes involved in sulphur/selenium metabolism have enhanced selenium accumulation and tolerance. The transgenic plants overexpressing adenosine triphosphate sulfurylase (APS), which catalyzes sulfate/selenate activation before they can be reduced to sulfite/selenite, accumulated 4.3-fold more Se than the wild-type plants, and extracted approximately 4% of the extractable Se from a contaminated soil. The major mechanism of Se toxicity in plants is the non-specific incorporation of selenocysteine and selenomethionine into proteins in place of Cys and Met, resulting in the alteration of protein structure. One way to enhance Se tolerance is to direct the metabolic flow of SeCys away from protein synthesis by overexpressing SeCys lyase (SL) which decomposes SeCys to elemental Se and alanine. Another way to engineer Se tolerance is to transfer the selenocysteine methyltransferase (SMT) gene from the Se hyperaccumulator Astragalus bisulcatus, which is also hypertolerant to Se, to nontolerant plants. SMT catalyzes the methylation of SeCys to methylselenocysteine, which is a non-protein amino acid non-toxic to plants. The SMT transgenic plants of B. Juncea accumulated 60% more Se from a contaminated soil than the wild-type under field conditions.

Darcheville et al. (2008) investigated the role of microorganisms in the behaviour of selenium in natural soils maintained under strictly aerobic con-

ditions. Six-day batch experiments were performed with soils constrained to different microbiological states, either by sterilization or by adding organic substrate. Selenium was added to the soil as selenite. The distribution of selenium in the gaseous, liquid and solid phases of the batch was measured. It was found that the active microorganisms played major role in the distribution of selenium within the soil. On the one hand microorganisms could promote volatilisation of selenium (in relatively small amounts), leading to the spreading of selenium compounds outside the soil. On the other hand microbial activity increased both amount of selenium retained by the soil, and the strength of its retention (less exchangeable selenium), making selenium less susceptible to remobilisation.

Selenium uptake into wheat

Increasing selenium content of wheat requires investigation of uptake mechanism catalyzed by transporters. Both selenite and selenate in the soil may be available to plants depending upon soil conditions. Several studies have demonstrated the uptake of selenite into plants (*Hopper and Parker*, 1999; *Zhang et al.*, 2003), although in some cases root to shoot translocation seemed to be more limited than for selenate (Hopper and Parker, 1999). Competition was demonstrated between selenite and phosphate in nutrient solution studies indicating a possible involvement of phosphate transporters in selenite uptake (*Hopper and Parker*, 1999), although this has not been investigated at the molecular level. It is generally accepted that selenate is taken up by plants from the soil via sulphate transporters in the roots. At the whole plant or agronomic scale the interaction between sulphate and selenate is well documented (*Barak and Goldman*, 1997; *Bell et al.*, 1992; *Broadley et al.*, 2006; *Hopper and Parker*, 1999; *Mikkelsen and Wan*, 1990; *Wu and Huang*, 1992). An implication of these interactions is that sulphate fertilization and selenium biofortification may be inextricably linked and are likely to be antagonistic to one another. The variation in the selectivity of the sulphate transporters for sulphate compared to selenate offers an opportunity for selective enrichment. Early kinetic studies of sulphate uptake into barley roots showed that selenate was a competitive inhibitor of sulphate uptake (*Legget and Epstein*, 1956). Based on this evidence antagonism for uptake is inevitable.

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Effect of various prebiotics on LA-5 and BB-12 probiotic bacteria multiplication, and on probiotic yoghurt production

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Abstract. To obtain a good quality probiotic yoghurt the quality of raw milk is a prerequisite condition. Our studies aimed the measurement of milk factors which can affect the multiplication of probiotic lactic acid bacteria (LABs) Lactobacillus acidophilus (LA-5) and Bifidobacterium (BB-12) strains from Christian Hansen company (Denmark) and importance of different added prebiotics to milk. Prebiotics are a category of functional food, defined as: Non-digestible food ingredients that beneficially affect the host by selectively stimulating the g rowth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. I studied multiplication of these two probiotic strains without prebiotics, with molasses, lactose and peptone, like prebiotic. I observed, that prebiotics have positive influence on probiotic bacteria multiplication, also their concentration is very important. Added molasses in higher concentration than 2% inhibit multiplication of LA-5 and BB-12, and have negative influence on acidification process and probiotic yoghurt production. From technological point of view, a combination of presented two probiotic strains present many advantages in taste, structure, and acidification process of probiotic yoghurt.

Key words and phrases: probiotic yoghurt, probiotics, prebiotics, survival of probiotic bacteria

1 Introduction

Yoghurt is a long time known and appreciated dairy product, obtained traditionally by the spontaneous or induced lactic fermentation of milk. The microbiology of lactic-producing bacteria and the fermentation biochemistry and technology of yoghurt is well documented (*Apostu*, *Barzoi*, 2002; *Banu*, 2002; *Banu*, *Moraru*, 1972; *Costin*, 2005; *Socaciu*, 2001).

The term "probiotic" is known since 1903 when the benefic actions of Lactobacillus acidophilus strains were observed in human intestine, and the term of "prebiotic" is known since 1961, and define the substances, generally natural ingredients or microorganisms which improve the intestinal equilibrium and defense against pathological bacteria (*Brengmark*, *Martindale*, 2006; *Costin*, *Segal*, 2001; *Macovei*, *Costin*, 2006; *Tomasik*, *Tomasik*, 2006)

Prebiotics are a category of functional food, defined as: Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health.

This was updated by Roberfroid: "A prebiotic is 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.' Today, only 2 dietary nondigestible oligosaccharides fulfill all the criteria for prebiotic classification." Those 2 are fructo-oligosaccharides and galacto-oligosaccharides. Use of the term other than in that manner is incorrect, since all oligosaccharides do not fit this definition, i.e. mannanoligosaccharides (MOS). They may confer other positive benefits, but are minimally utilized by the comensural bacteria. Typically, prebiotics are carbohydrates (such as oligosaccharides), but the definition does not preclude non-carbohydrates. The most prevalent forms of prebiotics are nutritionally classed as soluble fibre. To some extent, many forms of dietary fibre exhibit some level of prebiotic effect

Traditional dietary sources of prebiotics include soybeans, inulin sources (such as Jerusalem artichoke, jicama, and chicory root), raw oats, unrefined wheat, unrefined barley and yacon. Some of the oligosaccharides that naturally occur in breast milk are believed to play an important role in the development of a healthy immune system in infants, but these are not considered prebiotics, as they do not act through the intestinal microflora. Prebiotic oligosaccharides are increasingly added to foods for their health benefits. Some oligosaccharides that are used in this manner are fructooligosaccharides (FOS), xylooligosaccharides (XOS), polydextrose and galactooligosaccharides (GOS).

Some monosaccharides such as tagatose are also used sometimes as prebiotics. (Food-Info.net Wageningen University). Studies have demonstrated positive effects on calcium and other mineral absorption, immune system effectiveness, bowel pH, and intestinal regularity. Correlations have also been made with other positive health factors, but more research is required. The immediate addition of substantial quantities of prebiotics to the diet may result in a temporary increase in gas, bloating or bowel movement. It has been argued that chronically low consumption of prebiotic-containing foods in the typical Western diet may exaggerate this effect.

Objectives:

a) Preparing of milk samples with prebiotics: molasses, added peptone, lactose,
b) Study of molasses concentrations effect on probiotic bacteria multiplication.
c) Influence of added peptone, and combination of peptone and molasses in different concentration on LA-5 and BB-12 bacteria multiplication.
d) Multiplication of probiotics in molasses and peptone solution directly.
e) Multiplication of different probiotic bacteria in molasses added sterilized milk.
f) Obtaining of probiotic yoghurt by using molasses like prebiotic; LA5 and BB12 like probiotic strains (determination of product's shell-life).

2 Material and methods

2.1 Preparation of probiotic yoghurt with prebiotics

Technology of probiotic yoghurt processing is influenced by many factors, as presented in previous chapters.

The chemical composition of raw milk, the total bacteria number, somatic cell numbers, inhibitors in raw milk; all these factors influence the quality of the final product.

Because it is a probiotic dairy product, not only the sensorial, chemical and biological properties of product are important, but the number of probiotic bacteria after the end of product's shell-life.

How I presented in first chapters the quality of raw milk in Romania is a continuing problem yet. Specially the NTG (number of total bacteria/germ) causes problems at yoghurt production.

For probiotic yoghurt production we need raw milk with good quality parameters:

- acidity: max. 18 T°
- NTG :< 100000/ml
- NCS : < 400000/ml

Because these parameters are not realized every day in dairy factories, in my thesis I tried to find a solution for these situations.

By adding prebiotics to raw milk, specially oligosaccharides, the production time of yoghurt may be shortened, and these prebiotics are nutrients for probiotic bacteria.

I tested inulins and honey, like prebiotic and beet molasses from a sugar producing company.

Specially I tested the molasses effect on probiotics multiplication. Molasses was tested like functional food in different industries, but I wanted to demonstrate, that it may be used not only like feed for animals, but like prebiotic for special probiotic yoghurt. It is a by-product at sugar production, with high sugar content. The extraction process of sugars from beet molasses, after elimination on sucrose is really expensive; that is why it is not applied on industrial level.

Molasses I used for my trials contains 46.5% total sugars, 44.55% sucrose, 0.75% reducing sugars and 1.14% of raffinose. Its protein content was 12.4%. The detailed composition is presented in *Table 1.*

Table 1: The chemical composition of molasses used for trials

	Beet molassescomposition , %
Total Sugars	46.5
– Sucrose	44.55
– Reducing Sugars	0.72
– Raffinose	1.14
Non-Sugar Organic Matter	16
– Nitrogen components as protein (6.25 * N)	12.4
Betaine	4
Glutamic Acid	3.64
– Non-nitrogen bodies	6.92
Soluble gums/other carbohydrates	3.6
Organic acids	3
- Crude Ash	9
Sodium (as Na)	0.32
Potassium (as K)	5
Calcium (as Ca)	0.34

Molasses I used for my experiments is from a sugar producing company.

2.1.1 Production of probiotic yoghurt with molasses solution (different concentrations)

Preparing of molasses solution with different concentrations:

For these trials we used molasses solutions with different concentration: 1%; 1.5%; 2%; 3%; 5%.

Molasses solutions were prepared from molasses. To prepare solution with 1% concentration: in an Erlenmeyer 150 ml we measured (with analytical balance) 1 g of molasses and 99 ml of distilled water. After an energic shaking this solution must be sterilized in heating chamber at 105°C 30 minutes. Before starting the sterilization process the Erlenmeyer must be covered. In Gordon's lab I used Memmert heater (etuva).

After sterilizing with IBCm Bactocount is verified the number of total bacteria from solution. If it is higher than 50 CFU/ml the sterilizing process must be repeated. Do not increase the sterilizing temperature! It is recommended a longer time of sterilization for a better result (molasses contains sugars, what may be affected by a higher temperature, and the concentration may be modified, also).

The number of total bacteria may be determined by inoculation on MRS agar or nutritive agar, also, but this process takes a very long time (72 hours), and in this time the NTG of solution may be modified.

For inoculation of molasses solution we used LA-5 and BB12 strains prepared from freeze dried cultures, offered by Christian Hansen company.

Preparing method of culture is presented at chapter: *Influence of NTG on probiotics multiplication.*

3.33 units of freeze-dried culture has to be used to 19.98 liter of milk or molasses solution (in this situation). By inoculation we add the culture to sterilized molasses solution. After 4 hours of incubation on 38°C we tested the modified NTG (Lactobacillus acidophylus number).

Bacteria number was determined after 1,2,3,4 hours of incubation.

The same steps were followed with the other molasses solutions with: 1% 1.5%; 2%; 3%; and 5% concentrations.

The preparing method of molasses solutions:

- 1% concentration: 1 g molasses + 99 ml distilled water
- 1.5% concentration: 1.5 g molasses + 98.5 ml distilled water
- 2% concentration: 2 g molasses + 98 ml distilled water
- 3% concentration: 3 g molasses + 97 ml distilled water
- 5% concentration: 5 g molasses + 95 ml distilled water

2.1.2 Multiplication of LA-5 and BB-12 probiotic strains in molasses solution with added peptone

The object of trial is to demonstrate if the added peptone may accelerate the multiplication of probiotic bacteria and to study the adequate concentration for this additive to obtain the best results.

The molasses solution preparing method has been presented. Because the 1.5% solution was the most adequate for probiotic bacteria multiplication I used this concentration for next testing.

On same way like molasses solutions we obtained the peptone solutions from special peptone powder (from Sanimed company): to 0.1 g of peptone by adding 99.9 ml of molasses solution to 1% concentration of peptone solution. The molasses solution (1.5% concentration) with added peptone solution in different concentration has to be inoculated with LA-5 strains, and testing the number of total bacteria after 1,2,3,4 hours.

2.1.3 Adding molasses solutions directly to pasteurized milk and its inoculation with probiotics (LA-5 and BB-12)

The aim of experiments was to test if probiotics can multiplicate faster if they are inoculated directly into milk with added prebiotics (molasses) and to verify if molasses in milk may accelerate the multiplication of probiotics.

For testing molasses effect on probiotics (LA-5 and BB-12) we have to choose a milk with good chemical parameters, total bacteria number and somatic cells number.

Milk we used to this trial was tested by IBCm Bactocount before pasteurizing (determination of total bacteria number); by Somatos analyzer (somatic cells number determination) and by Lactostar (chemical and physical parameters determination).

Fat content of milk was standardized to 2.8%. Its protein content was: 3.38%; somatic cells number: 164000 CFU/ml; total bacteria number before pasteurization: 78000 CFU/ml, after pasteurization on 95°C, 4200 CFU/ml (determined by inoculation).

Samples were obtained by adding molasses solution to milk, incubated at 36°C, respecting the steps of yoghurt processing technology, presented in previous chapter.

Parameters of milk we use for trials:

- Fat content: 3.85%
- Protein content: 3.47%

- Dry substance: 8.55%
- Freezing point: – 0.534°C

Preparing of molasses solution to samples:

To 5 grams of molasses we added 95 ml of distilled water, obtained a molasses solution with 5% concentration. Molasses solution was sterilized in an Erlenmeyer at 105°C, one hour. To 450 ml of pasteurized milk at 95°C we added 150 ml; 5% molasses solution, obtained milk with 1.25% concentration.

This preparing will be repeated one more time.

The samples (600 ml) will be divided in six, and numbered: sample 1 → sample 6.

Samples prepared in second step will be divided in six and numbered: sample 1'→ sample 6'.

Both samples will be inoculated with 0.02 units of culture=0.006g culture/100 ml.

Samples 1 → sample 6 were inoculated with LA-5 culture, sample 1'→ sample 6' with BB-12.

To obtain a more homogeneous solution it is recommended the inoculation before dividing, but in this case must be very careful with hygiene.

All samples were introduced to incubator (36°C). After 1; 2; 3; 4; 5; 6 hours we measured the pH, and in every hour one sample from each group were eliminated.

By introducing the eliminated sample to cooler (2-4°C) the activity of microorganism was stopped.

2.1.4 Preparing of probiotic yoghurt with molasses

Preparation process of probiotic yogurt is presented in chapter VIII. (flow chart). Together with milk protein and milk powder has to be added the molasses solution. It is very important to be added before pasteurizing process.

After preparing molasses solution was sterilized and its NTG (number of total bacteria) determined by presented methods.

Pasteurized milk with added molasses solutions in different concentrations (one sample with molasses 1.5% concentration and 0.1% of peptone) will be cooled till 38°C and inoculated with LA-5 strain (Lactobacillus acidophilus).

All prepared sample must be divided in two parts (min. 50 ml of sample). One of the samples will be inoculated with LA-5, the other one with BB-12. After inoculation we determine the NTG of inoculated samples by inoculation on Petri plates.

Prepared milk with molasses solutions (different concentrations) after pasteurization on 95°C will be inoculated with LA-5 and BB-12 cultures. From every sample (molasses with 1%; 1.5%; 2%; 3%; 5% concentration) one cup will be inoculated with LA-5 and one cup with BB-12. Molasses solution before adding was sterilized, and it was added before pasteurization.

We used milk with 84000CFU/ml, NTG before pasteurization, NTG after pasteurization was 3600 CFU/ml. Its fat content was 3.8%, protein content 3.35%.

After an incubation of samples on 38°C the number of total bacteria has to be determined.

2.1.5 Influence of added lactose content on probiotic bacteria multiplication

Beneficious effect of lactose on acidification process is demonstrated, already. The objective of my trial was to determine the lactose content influence on probiotic bacteria multiplication, and what is the most recommended dosage for probiotic yogurt production. To increase lactose content I used skimmed milk powder from Bucovina company, with the parameters presented in *Table 2*.

Table 2: Parameters of skimmed milk powder added to milk sample

Milk powder "Bucovina"	Protein content %	Lactose content, %	Fat content, %	NTG, CFU/g	Coliform bacteria CFU/g	Salmonella	E.coli Staph. aureus CFU/g
	34	48	1.5	Max.50000	< 10	Abs/25g	Abs.

To do not modify fat content of milk we used for probiotic yoghurt production it was added skimmed milk powder. Milk we used for testing had 4.52% lactose content, and 3.87% fat content. Its NTG = 74000CFU/ml, NCS = 264000 CFU/ml. Milk powder has to be added to milk before pasteurization process, in this way it is possible destroying of microorganisms from raw milk and reconstructed milk.

2.1.6 Testing survival of probiotic bacteria during the shelf-life of product

I compared probiotic bacteria activity in milk without prebiotics, milk with molasses (added molasses to milk directly) and milk with probiotic strains multiplied on molasses substrates).

The aim of trial was to compare the survival of probiotic bacteria in shelf-life of product, to determine the right shelf-life, and to test if addition of prebiotics has influence on survival of bacteria LA-5. NTG was determined by Bactocount IBCm.

3 Results and discussions

3.1 Properties of probiotic yoghurt using molasses as prebiotic

The influence of molasses concentration on LA-5 multiplication is presented in *Table 3*.

Table 3: Influence of molasses concentration on LA-5 multiplication

Molasses solutions concentrations %	NTG of solutions before inoculation CFU/ml	Number of total bacteria after			
		1 hours; CFU/ml	2 hours; CFU/ml	3 hours; CFU/ml	4 hours; CFU/ml
0	18	13000	1620000	2570000	2650000
1	18	13760	1642000	2580000	2670000
1.5	16	13840	1840000	2199000	2479000
2	12	14100	1214000	2178000	2182000
3	17	11200	884000	1460000	1570000
5	19	10750	812000	1260000	1294000

Molasses solutions concentrations %	pH of solution before inoculation	pH after			
		1 hours;	2 hours;	3 hours;	4 hours;
0	7.20	5.82	4.90	4.40	4.40
1	7.20	5.86	4.92	4.44	4.42
1.5	7.21	5.84	4.98	4.51	4.45
2	7.19	6.07	5.17	4.76	4.55
3	7.17	6.34	5.84	5.14	4.82
5	7.16	6.75	6.05	5.62	5.02

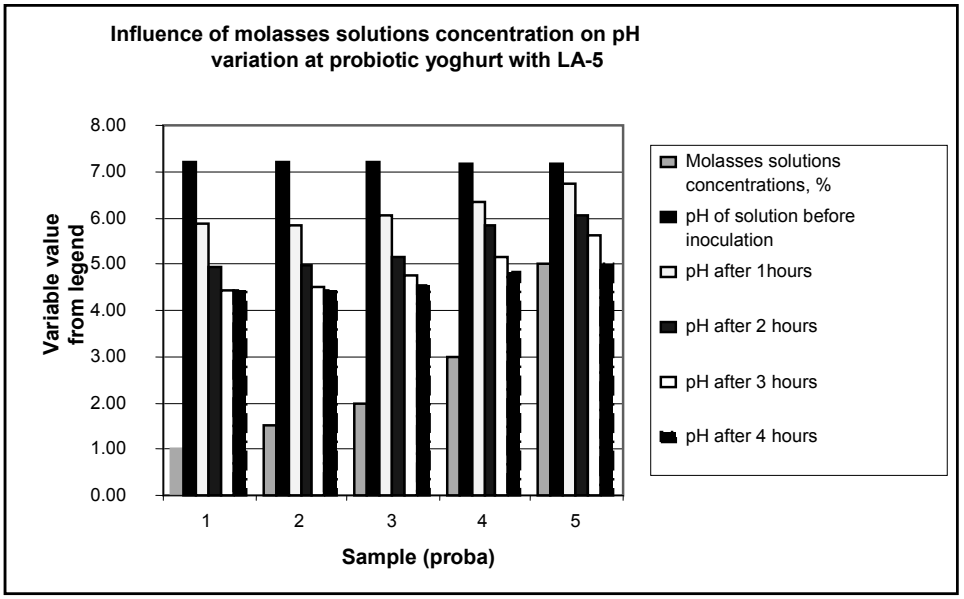

Figure 1: Influence of molasses concentration on LA-5 multiplication

We observe that molasses in 1-1.5% concentration helps the multiplication of LA-5 bacteria. In about three hours the number of total bacteria gets up to maximum level. This is the right time for inoculation of milk with this molasses solution. If we keep bacteria on incubation temperature for a longer time there is a self-inhibition of bacteria. A part of bacteria will be destroyed. If the inoculation of milk is not possible immediately, the inoculated molasses solution must be cooled till 4-6°C. This temperature inhibits the multiplication of bacteria and the acidification of solution.

Higher concentration of molasses solution inhibits the multiplication of probiotic bacteria. So to use molasses as a prebiotic, a 1-1.5% concentration of molasses solution is recommended.

3.1.1 Multiplication of LA-5 and BB-12 probiotic strains in molasses solution with added peptone

Multiplication of LA-5 and BB-12 probiotic strains in molasses solution with added peptone is presented in *Table 4*.

Table 4: Influence of peptone concentration on LA-5 multiplication (original)

Concentration of peptone solution %	Nr. of total bacteria of peptone + molasses solution	N1	N2	N3	N4
0	13570	1960000	2468000	2812000	2822000
0.1	13570	1970000	2476000	2822400	2822600
0.15	13820	1955000	2514000	2842000	2843000
0.2	13100	1953000	2486000	2796000	2798000
0.3	13600	1964000	2416000	2804000	2804000
0.5	13750	1965000	2482000	2614000	2615000

The results of trials are presented in the same *Table 4*. Number of total bacteria is measured in CFU/ml, for inoculation we used LA-5 strain. Concentration of molasses solution was 1.5 %.

N1- Number of total bacteria of peptone + molasses solution after 1 hour of incubation (inoculation with LA-5)

N2- Number of total bacteria of peptone + molasses solution after 2 hours of incubation (inoculation with LA-5)

N3- Number of total bacteria of peptone + molasses solution after 3 hours of incubation (inoculation with LA-5)

N4- Number of total bacteria of peptone + molasses solution after 4 hours of incubation (inoculation with LA-5)

B1- Number of total bacteria of peptone + molasses solution after 1 hour of incubation (inoculation with BB-12)

B2- Number of total bacteria of peptone + molasses solution after 2 hours of incubation (inoculation with BB-12)

B3- Number of total bacteria of peptone + molasses solution after 3 hours of incubation (inoculation with BB-12)

B4- Number of total bacteria of peptone + molasses solution after 4 hours of incubation (inoculation with BB-12)

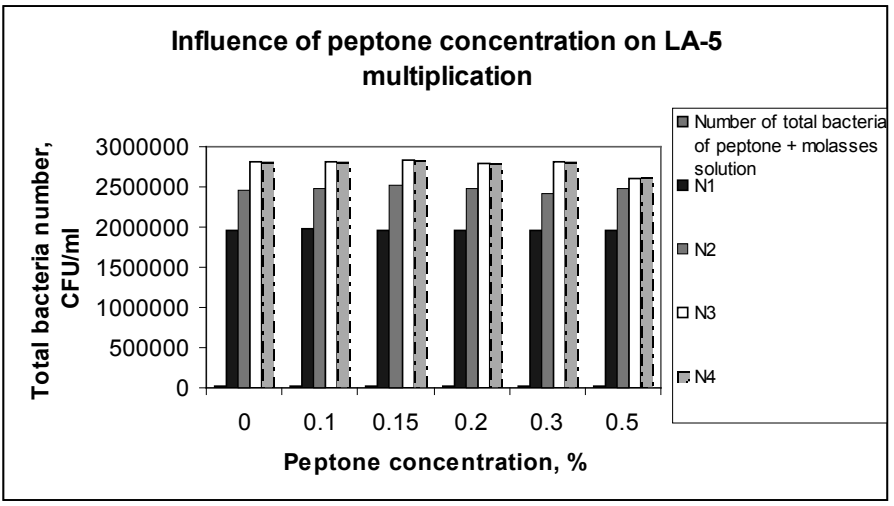

Figure 2: Influence of peptone concentration on LA-5 multiplication

For inoculations we used LA-5 strains in dosage presented in previous trial. The test was repeated with BB-12 probiotic strain. BB-12 strains preparing method was the same like we used to LA-5.

The number of total bacteria of 1 ml of prepared culture is: 13720 CFU /ml. The results of trials are presented in *Table 5.* Number of total bacteria is measured in CFU/ml, for inoculation we used BB-12 strain. Optimum incubation time is 3-4 hours.

Table 5: Influence of peptone concentration on BB-12 multiplication

Concentration of peptone solution %	Nr. of total bacteria of peptone + molasses solution	B1	B2	B3	B4
0	12470	1640000	2173000	2322000	2320000
0.1	12470	1650000	2175000	2322400	2322000
0.15	12810	1645000	2114000	2340000	2340000
0.2	12100	1613000	2085000	2292000	2292500
0.3	12700	1644000	2012000	2200000	2200000
0.5	12450	1647000	2125000	2314000	2312000

Probiotic bacteria are multiplying with different speed. *Bifidobacterium's* BB-12 multiplication speed is lower than LA-5's. The acidification process, measured in pH, is more intensive at LA-5, also. But as common properties we can observe:

Both culture's multiplication were accelerated by molasses solution 1.5%, and by peptone, also. 0.1% of peptone solution helps the multiplication of probiotic bacteria, but the same results were obtained with 0.2, 0.3, 0.5 % added peptone. So added peptone in 0.1% helps the multiplication of bacteria and the acidification process, but a higher concentration of peptone it is not necessary, it would not be useful, only uneconomical.

3.1.2 The influence of molasses on probiotic yoghurt obtained from pasteurized milk inoculated with LA-5 and BB-12

The influence of molasses on probiotic yogurt obtained from pasteurized milk inoculated with LA-5 and BB-12 is presented in *Table 6.* First six saples were inoculated with LA-5, samples 1'-6' with BB-12 strain.

Table 6: Molasses influence on acidification process

Sample/hours of incubation	pH	Lactic acid content%
1	6.69	0.20
2	6.63	0.22
3	5.59	0.21
4	4.70	0.71
5	4.51	0.84
6	4.46	0.92
1'	6.73	0.21
2'	6.08	0.34
3'	5.69	0.43
4'	5.17	0.50
5'	4.99	0.64
6'	4.42	0.94

After 4 hours of incubation from each sample we determined the pH, lactic acid content by photometer, and product's sensorial properties. This concentration of molasses solution was used, because at previous trials we observed that bacteria multiplication was at maxim level if molasses solution's concentration was between 1-2%.

I tried a higher concentration for molasses solution in milk (5%), but as I observed at previous experiments, also, this concentration inhibited the mul-

tiplication of bacteria and another problem was the lactic acid content's determination. At this concentration it was impossible todetermethe lactic acid content of yoghurt, because the sample's color became darker.

Not only colour of product was affected, but its taste, also. So a concentration between 1-2% of molasses has stimulating effect on probiotic bacteria multiplication and doesn't modify appreciably the final product's sensorial properties. The next parameters were determined after 1; 2; 3; 4; hours of incubation: acidity of product; NTG; lactic acid content; pH; taste; sensorial properties. We used the same raw milk for all trials. Dates of trials are presented in table 7.

Table 7: Production of yogurt with molasses-inoculation with LA-5

Molasses solutions concentrations %	NTG of milk before inoculation CFU/ml	Number of total bacteria after			
		1 hours; CFU/ml	2 hours; CFU/ml	3 hours; CFU/ml	4 hours; CFU/ml
0	3600	18400	1860000	2620000	2615000
1	3600	19760	1963100	2780000	2680000
1.5	3600	19840	1960000	2799000	2779000
2	3600	14600	1414000	2478000	2382000
3	3600	14200	874000	2160000	2160000
5	3600	11850	841000	2044000	2033000

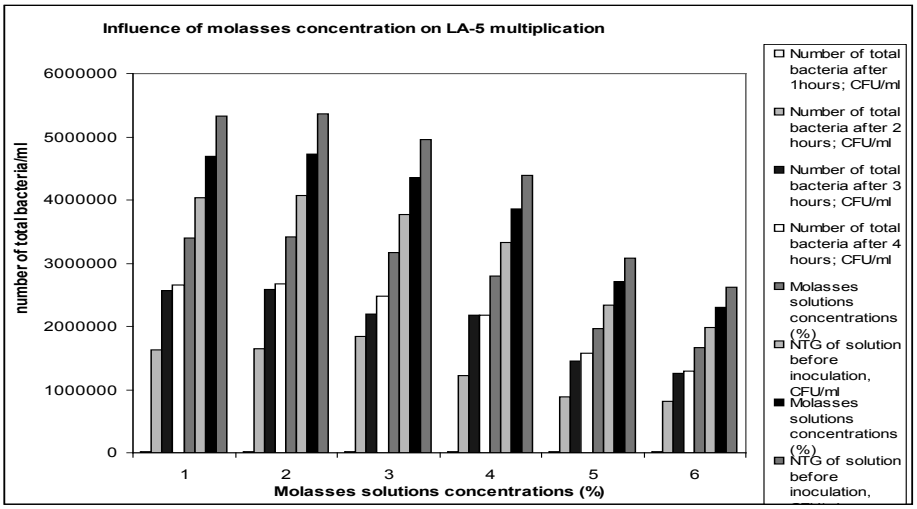

Figure 3: Production of yogurt with different concentration of molasses-inoculation with LA-5

Table 8: Production of yogurt with molasses-inoculation with BB-12

Molasses solutions concen-trations %	NTG of milk before inoculation CFU/ml	Number of total bacteria after			
		1 hours; CFU/ml	2 hours; CFU/ml	3 hours; CFU/ml	4 hours; CFU/ml
0	3600	18400	1860000	2620000	2615000
1%	3600	15460	1663100	2380000	2384000
1.5%	3600	15840	1660000	2394000	2396000
2%	3600	13500	1463000	2175000	2173000
3%	3600	12200	679000	2060000	2110000
5%	3600	11650	646000	2034000	2133000

By keeping the yoghurt in small sterilized cups it's possible to deduce product's shelf-life. For the determination of product shelf-life we have to analyze some parameters: sensorial properties; biological properties of product and chemical modifications (specially the acidity of yoghurt). Yoghurt samples were tested after 5 days; 10 days; 15 days; 16 days; 17 days; 18 days; 19 days; 20 days. Results are presented in table 9.

Table 9: Modification of LA-5 a number in product shelf-life

Yoghurt/ molasses	Acidity, T°	pH	NTG after (× 1000)						
			days: 5	10	15	16	17	18	20
0%	84	4.42	2670	2670	2670	2660	2660	2630	2580
1%	86	4.44	2680	2680	2680	2570	2560	2470	2300
1.5%	83	4.51	2779	2779	2779	2450	2450	2380	2260
2%	77	4.76	2382	2382	2382	2360	2360	2280	1982
3%	62	5.14	2160	2160	2160	2000	2000	1950	1096
5%	49	5.52	2033	2033	2033	1960	1950	1860	1080

Table 10: Modification of BB-12 a number in product shelf-life

Yoghurt/ molasses	Acidity, T°	pH	NTG after (× 1000)				
			days: 5	10	15	16	20
0%	81	4.50	2370	2370	2340	2340	2110
1%	82	4.52	2380	2380	2350	2340	2130
1.5%	79	4.57	2394	2394	2320	2310	2010
2%	74	4.62	2175	2175	2085	2085	1980
3%	62	5.18	2060	2060	1960	1960	1650

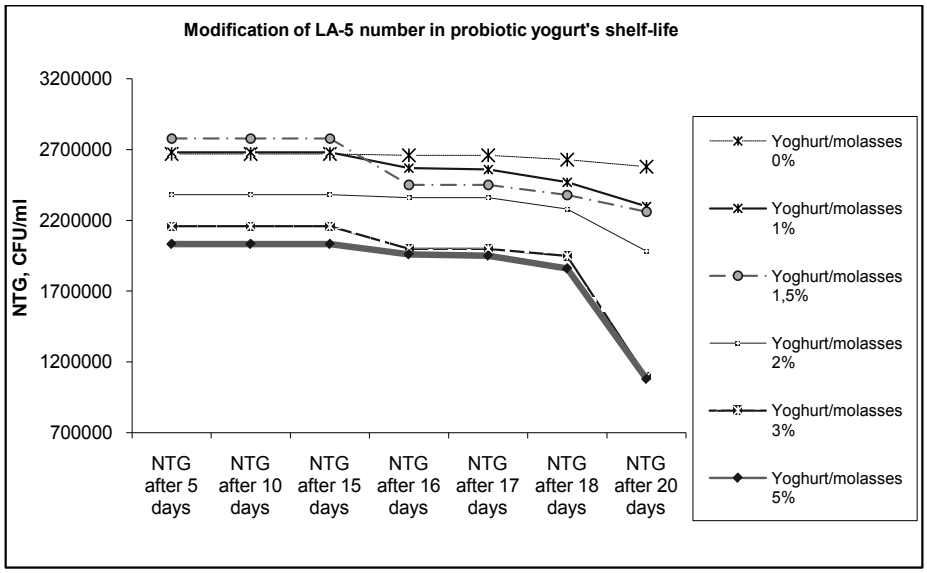

Figure 4: Modification of LA-5 a number in product shelf-life

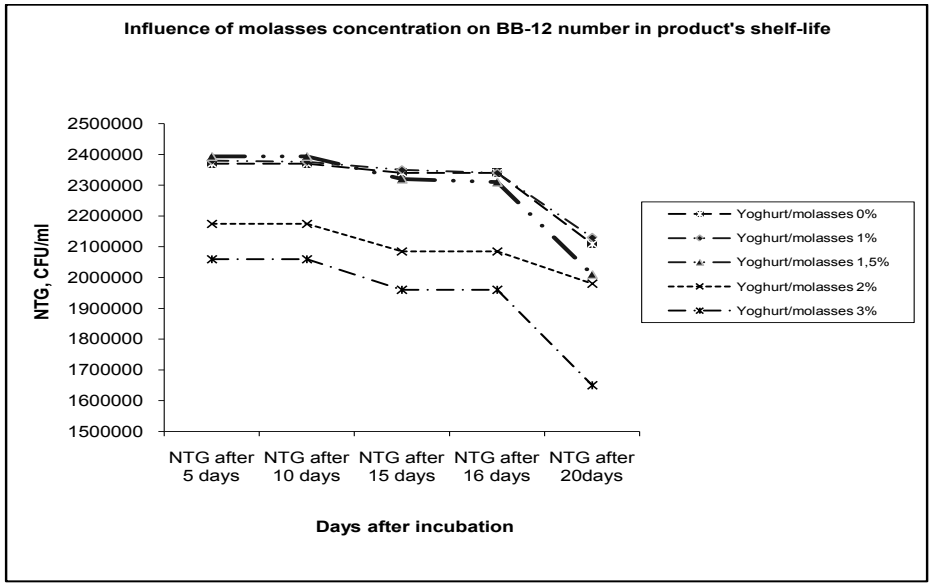

Figure 5: Modification of BB-12 a number in product shelf-life

Added molasses is influencing the multiplication of probiotic bacteria, the acidification process, pH modification and lactic acid content, also. How it was proved by previous trials it is recommended a molasses concentration between 1-2%. By this experiment we tested the molasses concentration effect on post-acidification process. Number of bacteria may not decrease significantly in product's shelf-life, because probiotic bacteria number at the end of product's shelf-life has to be higher than 108 CFU/175g of yoghurt (legislation). After 18 days of storage the number of LA-5 and BB-12 d ecreased considerably. A high concentration of added molasses influenced negative survival of probiotic bacteria. High molasses concentration results in a higher acid content of product, and this acidity destroyed a part of probiotic bacteria. Taste of yogurt with 5% molasses concentration caused a bitter taste, and the color of product became darker.

A commercial and technological advance may be obtained by producing probiotic fruit yoghurt. In this case the taste of molasses in not palpable. Fruits have to be sterilized before using and they have to be added to yoghurt after yoghurt producing, before dosage.

Probiotic yoghurts we obtained may have a shelf-life of 14-18 days. Shelf-life is depending on storage conditions, temperature, and manipulation. Probiotic yoghurt has to be stored at 2-6°C, in clear and clean deposits.

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Evaluation of biological value of sprouts I. Fat content, fatty acid composition

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Abstract. During our research work fatty acid content of the most important sprouts, wheat, lentil, alfalfa, radish and sunflower seed was investigated during the germination. It was found that both the saturated and unsaturated fatty acids hardly changed during the germination. The most important saturated fatty acid of the sprouts investigated by us is palmitic acid, amount of which hardly changed or even increased in the case of alfalfa sprout during germination. Oleic acid and linoleic acid were present in the highest concentration among the unsaturated fatty acids in the sprouts investigated. The concentration of oleic acid remained unchanged during the germination period, and the same applies, except lentil, to linoleic acid in case of every sprouts examined. In the case of lentil sprout the concentration of oleic acid decreased while linoleic acid content increased significantly. Based on our investigation it can be stated that most of the fatty acids hardly changed during the germination, and there was no verifiable tendency.

Key words and phrases: sprouts, chemical changes during germination, fat content, fatty acid composition

1 Introduction

In the recent decades more and more attention is paid to the healthy nutrition, to its role in health-maintaining and in the prevention of certain diseases. Changes in the nutritional habits, in foods consumed and in the food preparation methods have contributed to the decrease of the nutritional value. Various pathological researches came to the conclusion that consumption of higher amount of foods of vegetable origin could be effective in the prevention against some chronic diseases. These beneficial effects have been attributed partly to the high antioxidant activity of the vegetables. Most important antioxidants found in the plants are vitamin C, carotenoids and phenolic derivatives especially flavonoids.

Sprouting is a natural biological process that every higher plants exhibit, during which the seed at rest starts to grow under favourable environmental conditions (appropriate moisture content, temperature, oxygene) and a new plant develops. 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 in breaking down the high-molecular complex materials into their building blocks. 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. (*Sangronis and Machado*, 2007). 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).

The sprouts fulfill the requirements of the modern nutritional science for whole-food. Compared to seeds, the sprouts have a higher nutritional value: higher quality of protein, more favourable amino acid composition, higher polyunsaturated fatty acid content, better bioavailability of trace elements and essential minerals and higher vitamin content. During sprouting the amount of such antinutritive materials as haemagglutinins, trypsin inhibitor activity, tannins, pentosans, phytic acid, decreases. Researches found that the sprouts are a good source of ascorbic acid, riboflavin, choline, thiamin, tocopherol and pantothenic acid (*Lintschinger et al.*, 1997).

Urbano et al. (2005) examined the protein digestibility of various sprouts and bioavailability of the minerals, *Gill et al.* (2004) the relationship between the consumption of vegetables and the prevention of cancer, and *Clarke et al.* (2008) the effeciency of the sulforaphane content of different sprouts in cancer prevention. *Kim et al.* (2004) examined the change in fatty acid composition due to sprouting. It was established that in most of the sprouts among the fatty acids linolenic acid was present in the highest concentration, its concentration increased during seven days up to 52.1% and the total amount of the fatty acids was higher than 83%, that is, the unsaturated ones dominated over the saturated ones. The amount of oleic acid was 36.8%, that of linoleic acid was 38.1%, and that of linolenic acid was 2.7% in the original seed. During sprouting the concentration of the saturated fatty acids rapidly decreased, and myristic acid and stearic acid disappeared from the sample during one day of germination. 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 since linoleic acid, linolenic acid and arachidonic acid are essential for the human organism. Linoleic acid is capable of transporting bioactive compounds and can transform into arachidonic acid from which hormone-like compounds are forming. Summarized, it was established that majority of fatty acids of buckwheat is unsaturated ones, out of which linoleic acid can be found in the highest amount.

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

Studying the literature, we found no more data on the change in fat content and fatty acid composition during sprouting. Furthermore, we find some of the findings of the above two cited articles available to be hard to imagine, the mechanisms transforming the saturated fatty acids into unsaturated ones, and the monounsaturated oleic acid into polyunsaturated fatty acids, are unknown. Because of the above we started our investigation relating to fatty acid composition of nutritional sprouts and the changes in the fatty acid composition due to germination. During our work we determined the fatty acid composition of wheat, lentil, sunflower, alfalfa and radish seed sprouts and its change in the function of germination time. In this paper we wish to report our results.

2 Materials and methods

The examined samples, germination

Commercially obtainable organic wheat, lentil, sunflower, alfalfa and radish seeds were obtained. The seeds were washed in 0.1% H_2O_2 for 1 min then soaked in distilled water for 24 hrs. After the 24 hrs elapsed, the seeds were placed into germination bowls, and germinated at 20 °C in a Memmert 200 incubator. They were rinsed twice a day with distilled water and samples were taken in every 24 hrs. According to the domestic practice and international recommendations wheat and lentil were germinated for 3 days, radish for 7 days, alfalfa for 8 days, sunflower for 5 days. After germination the sprouts were washed with distilled water, dried at 60 °C, then stored frozen at -10 °C until the analyses. Crude fat content of the sprouts were determined in a Soxhlet extractor after extraction with diethylether according to the Hungarian Standard.

Crude fat content was given with 0.1% accuracy as the mean value of two repetitions. The allowed maximal difference between the two repetitions was 0.3%.

During the sample preparation for the fatty acid analysis a sample quantity containing 1 g fat was destructed with 8-20 cm^3 of concentrated hydrochloric acid (37%) for 1 hour on hot water bath. After having cooled down, 7 cm^3 of ethanol was added. Lipids were extracted with 15 cm^3 diethylether then with 15 cm^3 benzine (b.p.<60 °C), and the organic layers were combined. The solvents were removed under reduced pressure. To the residue 4 cm^3 of 0.5 M sodium hydroxide methanol solution was added and under a reflux cooler it was boiled until all the fat drops disappeared (approx. 5 min), then 4 cm^3 of 14% boron trifluoride methanol solution was added, boiled for 3 min, finally 4 cm^3 of hexane dried on water-free sodium sulphate was added and boiled for 1 min, and the mixture was allowed to cool down. The reflux cooler was removed and saturated aqueous sodium chloride solution was added and after having separated the organic layer was collected into a 4 cm^3 vial containing water-free sodium sulphate and was directly examined by gas chromatography.

Determination of the fatty acid composition was performed using a Varian 3800 gas chromatograph. The chromatographic column was a fused silica capillary column (100 m,·0.25 mm id) with a CP-Sil 88 (FAME) stationary phase (film thickness: 0.2 μm). FID detector was used. Detector gas flow rates were as follows: hydrogen 30 ml/min, air 200 ml/min, make up gas 30 ml/min. Detector temperature was 270 °C. The carrier gas was high-purity

hydrogen, column head pressure was 235 kPa. Temperature program: 140 °C for 10 min; at 5 °C/min up to 235 °C; isotherm for 30 min. Injected volume was 1 µl. The fatty acid methyl esters were identified using the "37 component FAME Mix" standard from Supelco. Results were given as fatty acid methyl ester relative weight%.

3 Results and discussion

Table 1 shows crude fat content of the starting seeds and the sprouts. Results were given in weight% on air dry matter basis.

Table 1: Crude fat content of the seeds and sprouts

Number	Description	Crude fat content (%) (on air dry-matter basis)
1.	Wheat seed	1.7
2.	Wheat sprout, day 3	1.7
3.	Lentil seed	1.4
4.	Lentil sprout, day 3	1.4
5.	Alfalfa seed	10.3
6.	Alfalfa sprout, day 3	9.8
7.	Alfalfa sprout, day 7	4.5
8.	Radish seed	39.0
9.	Radish sprout, day 2	39.2
10.	Radish sprout, day 6	20.2
11.	Sunflower seed	60.3
12.	Sunflower sprout, day 3	57.7
13.	Sunflower sprout, day 5	43.4

In case of wheat and lentil sprouts no change was experienced, in case of the alfalfa sprout the fat content decreased around to its half value, and the same applies for the six-day radish sprout. In case of sunflower sprout the decrease is around 30%.

Table 2 shows fatty acid composition of wheat seed and wheat sprout.

In the wheat sprout the fatty acids being present in the highest concentration are palmitic acid, linoleic acid and oleic acid. Out of the saturated fatty acids palmitic acid is present in 33.5% in the wheat sprout which value is higher than that for the wheat seed (31.2%), that is, due to the germination the

concentration of palmitic acid increased.

Table 2: Fatty acid composition of wheat seed and wheat sprout

Fatty acid		Wheat seed	Wheat sprout Day 3
		Fatty acid methyl ester %	
Undecanoic acid	11:0	1.7	1.7
Lauric acid	12:0	0.1	0.1
Tridecanoic acid	13:0	0.7	0.7
Myristic acid	14:0	0.8	0.5
Pentadecanoic acid	15:0	0.3	0.3
Palmitic acid	16:0	31.2	33.5
Stearic acid	18:0	1.9	1.2
Oleic acid	18:1	10.7	7.8
Linoleic acid	18:2	25.6	27.3
Arachidic acid	20:0	0.3	0.2
Eicosenoic acid	20:1	0.9	0.4
α-Linolenic acid	18:3n3	2.0	2.5
Behenic acid	22:0	1.2	1.2
Eicosatrienoic acid	20:3n6	1.4	1.5
Eicosatrienoic acid	20:3n3	2.3	0.2

The value for stearic acid decreased from 1.9% in the seed to 1.2% in the wheat sprout. Out of the saturated fatty acids beyond the mentioned ones also undecanoic acid (1.7% in the wheat seed and also in the sprout), lauric acid (0.1% in both of the samples), tridecanoic acid (0.7% in the seed and the sprout), myristic acid (0.8% in the wheat seed and 0.5% in the wheat sprout), pentadecanoic acid (0.3% in both samples), arachidic acid (0.3% in the seed and 0.2% in the sprout) and behenic acid (1.2% in both samples) could be detected in the samples.

Out of the monounsaturated fatty acids oleic acid is present in the highest concentration, its value decreased from 10.7% in the wheat seed to 7.8% due to germination. In the samples also eicosenoic acid could be detected: 0.9% in the wheat seed and 0.4% in the wheat sprout.

Out of the polyunsaturated fatty acids linoleic acid was present in the highest concentration, with 25.6% in the starting wheat seed and increasing to 27.3% in the sprout. In the samples also α-linolenic acid (2.0% in the wheat seed and 2.5% in the sprout) and eicosatrienoic acid (C20:3n6): (1.4% in the

wheat seed and 1.5% in the wheat sprout), (20:3n3): (2.3% in the wheat seed, 0.2% in the sprout) were detectable.

Table 3 contains fatty acid composition of lentil and lentil sprout.

Table 3: Fatty acid composition of lentil seed and lentil sprout

Fatty acid		**Lentil seed**	**Lentil sprout, Day 3**
		Fatty acid methyl ester %	
Undecanoic acid	11:0	0.4	0.6
Lauric acid	12:0	0.2	0.2
Tridecanoic acid	13:0	0.4	0.4
Myristic acid	14:0	1.1	1.1
Pentadecanoic acid	15:0	0.4	0.7
Palmitic acid	16:0	26.2	27.0
Stearic acid	18:0	1.6	2.2
Oleic acid	18:1	14.0	9.3
Linoleic acid	18:2	19.4	27.4
Arachidic acid	20:0	0.3	0.5
Eicosenoic acid	20:1	0.3	0.4
α-Linolenic acid	18:3n3	3.3	4.7
Behenic acid	22:0	1.2	1.6
Eicosatrienoic acid	20:3n6	1.4	1.4
Eicosatrienoic acid	20:3n3	0.1	0.1

In the lentil sprouts palmitic acid, linoleic acid and oleic acid can be found in the highest concentration. Out of the saturated fatty acids palmitic acid can be found in the lentil seed in 26.2%, this value is higher in the sprout (27.0%). Out of the saturated fatty acids also undecanoic acid (0.4% in the lentil seed, 0.6% in the lentil sprout), lauric acid (0.2% in both samples), tridecanoic acid (0.4% in both samples), myristic acid (1.1% in both samples), pentadecanoic acid (0.4% in the lentil seed, 0.7% in the sprout), stearic acid (the value of 1.6% in the starting seed increased to 2.2% in the sprout), arachidic acid (0.3% in the lentil seed, 0.5% in the sprout) and behenic acid (1.2% in the lentil seed, 1.6% in the sprout) could be detected.

Out of the unsaturated fatty acids linoleic acid and oleic acid can be found in the highest amount. Concentration of linoleic acid in the lentil seed was 19.4%; due to the germination this value increased up to 27.4% in the sprout. Oleic acid is present in the seed in 14.0% which decreases to 9.3% in the sprout.

Out of the unsaturated fatty acids also eicosenoic acid was detectable (0.3% in the lentil seed and 0.4% in the sprout). Out of the polyunsaturated fatty acids concentration of α-linolenic acid increased from 3.3% in the starting lentil seed up to 4.7% in the sprout due to the germination, concentration of eicosatrienoic acid hardly changed, however (20:3n6: 1.4% in the seed and 1.5% in the sprout, 20:3n3: 0,1% in both samples).

Table 4 contains fatty acid composition of alfalfa seed and alfalfa sprout.

Table 4: Fatty acid composition of alfalfa seed and alfalfa sprout

Fatty acid		Alfalfa seed	Alfalfa sprout, Day 3	Alfalfa sprout, Day 7
		Fatty acid methyl ester %		
Lauric acid	12:0	0.1	0.2	0.1
Myristic acid	14:0	0.5	0.4	0.6
Pentadecanoic acid	15:0	0.2	0.3	0.6
Palmitic acid	16:0	15.9	15.9	22.4
Palmitoleic acid	16:1	0.1	0.1	0.3
Margaric acid	17:0	0.1	0.2	0.3
Stearic acid	18:0	3.2	2.9	4.4
Oleic acid	18:1	10.4	9.1	9.8
Linoleic acid	18:2	34.3	34.7	29.1
Arachidic acid	20:0	0.7	0.8	1.0
γ-Linolenic acid	18:3n6	0.2	0.2	0.2
Eicosenoic acid	20:1	0.3	0.2	0.3
α-Linolenic acid	18:3n3	24.9	24.9	15.8
Eicosadienoic acid	20:2	0.1	< 0.1	0.1
Behenic acid	22:0	0.8	1.0	1.7
Eicosatrienoic acid	20:3n6	0.4	0.7	1.3
Eicosatrienoic acid	20:3n3	0.4	0.2	0.3
Arachidonic acid	20:4n6	0.1	0.1	0.7
Docosapentaenoic acid	22:5n3	0.9	1.2	1.6

In the sprout the fatty acids being present in the highest concentration are linoleic acid, α-linolenic acid, palmitic acid, and oleic acid. Ouf of the saturated fatty acids in the highest concentration palmitic acid could be detected. Its concentration increased due to the germination: 15.9% in the seed, the same value in the three days old sprout, and 22.4% in the seven days old sprout. Concentration of stearic acid increased during the germination (from 3.2% to 4.4%). Out of the saturated fatty acids under the given chromatographic conditions lauric acid, myristic acid, pentadecanoic acid, margaric

acid, arachidic acid and behenic acid were detectable, their amount remained below 1%, however.

Out of the unsaturated fatty acids linoleic acid, α-linolenic acid and oleic acid were present in the highest concentration. Concentration of linoleic acid in the original seed was 34.3%, which decreased during the germination to 29.1%. Concentration of α-linolenic acid also decreased during the germination: from 24.9% in the starting alfalfa seed to 15.8% in the sprout. The amount of oleic acid also decreased during the germination: from 10.4% in the seed to 9.4% in the sprout. Out of the unsaturated fatty acids also palmitoleic acid, α-linolenic acid, eicosenoic acid, eicosadienoic acid, eicosatrienoic acid, arachidonic acid and docosapentaenoic acid could be detected, their amount remained below 1%, however.

Table 5 contains fatty acid composition of radish seed and radish sprout.

Table 5: Fatty acid composition of radish seed and radish sprout

Fatty acid		Radish seed	Radish sprout, Day 2	Radish sprout, Day 6
		Fatty acid methyl ester %		
Lauric acid	12:0	<0.1	<0.1	<0.1
Myristic acid	14:0	0.1	0.1	0.1
Pentadecanoic acid	15:0	<0.1	<0.1	<0.1
Palmitic acid	16:0	8.2	6.5	8.0
Palmitoleic acid	16:1	0.2	0.2	0.2
Stearic acid	18:0	3.2	2.7	3.0
Oleic acid	18:1	35.1	27.4	34.6
Linoleic acid	18:2	15.5	12.6	15.9
Arachidic acid	20:0	2.0	1.6	2.0
α-Linolenic acid	18:3n6	0.1	0.1	0.1
Eicosenoic acid	20:1	14.8	11.5	15.1
α-Linolenic acid	18:3n3	13.6	11.3	14.0
Eicosadienoic acid	20:2	0.6	0.5	0.7
Behenic acid	22:0	1.9	1.4	1.9
Arachidonic acid	20:4n6	0.1	<0.1	0.1
Docosadienoic acid	22:2	0.4	0.3	0.4
Lignoceric acid	24:0	1.2	0.9	1.2

In the radish sprout in the highest concentration oleic acid, linoleic acid, eicosenoic acid, α-linolenic acid and palmitic acid were present. Out of the saturated fatty acids concentration of palmitic acid in the radish seed was 8.2%, during the germination this reduced to 6.5% in the two days old radish

sprout, whereas in the six days old sprout it increased to 8.0%. Concentration of stearic acid decreased from 3.2% in the starting radish seed to 2.7 and 3.0% in the sprout. Out of the saturated fatty acids also lauric acid, myristic acid, pentadecanoic acid, arachidic acid, behenic acid and lignoceric acid were detectable, their concentration was below 2%, however.

Out of the unsaturated fatty acids oleic acid was present in 35.1% in the radish seed, in 27.4% in the two days old sprout, in 34.6% in the six days old sprout. Concentration of linoleic acid increased due to the germination from the starting 15.5% to 15.9% in the six days old sprouts. Concentration of eicosenoic acid also increased due to the germination from the starting 14.8% to 15.1%, whereas that of α-linolenic acid from 13.6% to 14.0%. Under the given chromatographic conditions also palmitoleic acid, α-linolenic acid, eicosadienoic acid, arachidonic acid, docosadienoic acid, however, their concentration was less than 0.5%.

Table 6 contains fatty acid composition of sunflower seed and sunflower sprout.

Table 6: Fatty acid composition of sunflower seed and sunflower sprout

Fatty acid		Sunflower seed	Sunflower sprout, day 3	Sunflower sprout, day 5
		Fatty acid methyl ester %		
Myristic acid	14:0	0.1	0.1	0.2
Pentadecanoic acid	15:0	<0.1	<0.1	<0.1
Palmitic acid	16:0	5.8	5.7	5.8
Palmitoleic acid	16:1	0.1	0.1	¡0.1
Margaric acid	17:0	0.1	0.1	0.1
Stearic acid	18:0	5.4	5.4	5.6
Oleic acid	18:1	21.7	21.0	20.9
Linoleic acid	18:2	65.0	65.6	64.8
Arachidic acid	20:0	0.4	0.4	0.4
Eicosenoic acid	20:1	0.2	0.1	0.2
α-Linolenic acid	18:3n3	0.1	0.4	1.0
Behenic acid	22:0	0.9	0.9	1.0
Arachidonic acid	20:4n6	0.2	<0.1	<0.1
Lignoceric acid	24:0	0.2	0.3	0.3

In the sunflower sprout out of the saturated fatty acids palmitic acid and stearic acid are present in the highest concentration. Concentration of palmitic acid hardly changes due to the germination: it is 5.8% in the seed, in the three

days old sprout is 5.7% , and in the five days old sprout 5.8%. Also the value of stearic acid changes to a very little extent during the germination, the starting value of 5.4% in the seed remains unchanged in the three days old sprout, it increases in the five days old sprout to 5.6%, however. Out of the saturated fatty acids also myristic acid, pentadecanoic acid, margaric acid, arachidic acid, behenic acid and lignoceric acid were detectable, their concentration was negligible, however.

In the sunflower sprout linoleic acid is the highest concentration unsaturated fatty acid which increased from the starting 65.0% by day 3 to 65.6%, then reduced by day 5 to 64.8%. Oleic acid is present similarly in a high concentration, the starting value of 21.7% reduced to 21.0% in the three days old sprout and to 20.9% in the five days old sprout. Out of the unsaturated fatty acids also palmitoleic acid, eicosenoic acid, α-linolenic acid, arachidonic acid could be detected, their concentration was below 1%, however.

By the analysis of the fatty acid composition of wheat, lentil, sunflower, alfalfa and radish seed sprouts we established that by far no such radical changes occurred during the germination as reported by *Kim et al.* (2004), and *Tokiko and Koji* (2006). Calculating on dry-matter basis the crude fat content of the sprouted plant either did not change or decreased during the sprouting.

Regarding the fatty acid composition, concentration of palmitic acid, the saturated fatty acid being present in the highest concentration, increased in case of wheat, lentil and alfalfa sprout, in the case of radish sprout decreased somewhat, and in case of sunflower sprout practically did not change during the germination. Similar change can be reported in case of stearic acid, and we cannot give a definite answer to how the stearic acid content changed during the germination. It is very probable that either the stearic acid content or palmitic acid content does not suffer a substantial change due to the germination.

We can formulate almost similar tendencies in case of unsaturated fatty acids. In case of wheat, lentil and alfalfa sprout the amount of oleic acid decreased somewhat, in case of radish and sunflower sprout the change is minimal. The increase of linoleic acid is considerable only in case of the lentil sprout, whereas for all the other sprouts its amount remains practically unchanged in the germination period. The other polyunsaturated fatty acids occur in such a small concentration, that even the tendency of the changes is difficult to follow.

Summarized, it can be established that some of the saturated fatty acids decrease minimally, others remain unchange. Out of the unsaturated fatty

acids oleic acid practically hardly changes, and also the amount of linoleic acid shows a considerable increase only in case of the lentil sprout. From hardly affected the fatty acid composition of the fat of the sprouting plant, consequently the biological value of the fat. We cannot confirm the results found in the literature reporting that due to the germination the amount of the saturated fatty acids considerably decreases, and the amount of the polyunsaturated essential fatty acids considerably increases.

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The influence of disposal technology obtained with alkaline treatments on D-amino acid content of slaughterhouse waste

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Abstract. One possibility of disposing of animal proteins is alkaline hydrolysis that can be applied even for waste materials infected with *transmissive encephalopathy* since the alkaline hydrolysis decomposes the prions therefore it proved to be especially effective for the treatment of materials of animal origin of the highest risk. In our experiment the change in D-amino acid content of slaughterhouse waste due to the treatments was examined. The treatments were done with sodium and potassium hydroxide solution, respectively, for 2, 3 and 6 hours at 135, 150 and 153 °C. D-Asp, D-Glu,and D-Trp content was determined using a *Hitachi Merck LaChrom HPLC*, and D-allo-Ile content using an *INGOS* amino acid analyzer. In case of tryptophan the hydrolysis was carried out with 3 M p-toluenesulfonic acid in the presence of 3-indolylpropionic acid, in other cases the 6 M hydrochloric acid hydrolysis was applied. Hydrolysis temperature was in both cases 110±2 °C. Summarized, it can be said that due to the heat and alkali combinations we used aspartic acid, glutamic acid, tryptophan and isoleucine transform in 40-50% into the D-isomer. Even though the hydrolysed product obtained this way met in other parameters the requirements of the modern feeding, one should be expect that most of the amino acids undergo full racemization during this process.

1 Introduction

Waste materials are necessary by-products of the modern civilization and everyday's life. By producing more and more waste materials humans significantly disturb the natural balance. Therefore one of the most important tasks of environmental protection is solving the problem of waste materials. According to the recent standpoints such strategies should be elaborated whose aim is to keep the sources of the nature and protect the environment. As basic target was defined the reduction of the amount of pollutive materials by recycling them with new, modern technologies.

Animal by-products include mostly slaughterhouse waste of ungulated farm animals and poultries since consumption of meat of these animals continuously increased during the last 20 years. Animal by products were processed earlier into meat-flour and industrial fat. Meat- and bone-flour were potential sources of animal protein in feeding. Animal proteins have high biological value, their composition is much more favourable than that of yeast, soybean or sunflower. But after the appearance of *bovine spongiform encephalopathy* (BSE) the use of animal proteins was banned in animal feeding. Vegetable proteins can be of high biological value in animal feeding if well combined with each other.

Recently meat- and bone flour are used for fertilizing, animal proteins are

used for energy production in increasing amounts. Energy is very important nowadays in both economic and social respect. Today's technologies are not suitable yet so that the polluting materials can be used as source of energy without dangering the environment. In order to achieve this, further researches are necessary, among which developments associated with animal proteins are of special importance. Alkaline hydrolysis is a possibility for the treatment of by-products of animal origin which can provide a solution in case of proteins.

In its entirety, waste of animal origin does not represent a significant problem in waste material processing, however, the contamination of soil, water and air that can be caused by improper treatment of animal waste, can be dangerous to our environment (*Vučemilo et al.*, 2003).

According to the data of FAO the world's meat production reached 250 million tons per year in 2004 which is continuously growing. In the Republic of Croatia the number of slaughtered animals hardly changed during the passed 10 years, around 37 million chickens, 100 thousand cattles, 900 thousand pigs per year (*Vrabec*, 2004). In Hungary in 2006 the quantity of slaughtered animals was as follows: beef-cattles 84 thousand tons, hogs 613 thousand tons, poultries 632 thousand tons (*KSH*, 2007). If we take into consideration that around 50-58% of these quantities can be utilized in the human nutrition, it can be established that around 25% of the body mass of slaughtered animals is a slaughterhouse waste.

With the appearance of BSE Europe faced a huge problem, as processing of waste material that can be brought into connection with BSE is strictly forbidden. By the introduction of the new EU regulations (*EU Directive* 1774/2002) slaughterhouse waste has been categorized into three categories (high, medium and low risk) and conditions of waste treatment were defined. As a result of the restrictions the protein demand of animal breeding considerably increased and also the demand for elaborating new methods for the treatment of waste of animal origin. Such a possiblity is the alkaline hydrolysis of animal proteins which can be applied even in case of waste infected by transmissive encephalopathy. The procedure has been applied in the United States at the Albany Veterninary School since 1993 (*Kaye et al.*, 1998). There are several results showing that alkaline hydrolysis destructs the prions (*Ernst*, 1993; *Tagochi et al.*, 1991; *Taylor et al.*, 1999; *Taylor*, 2000), therefore it proved to be especially effective in the treatment of materials of animal origin of the highest risk.

The alkaline treatment is a procedure during which due the alkaline medium the high molecular proteins disintegrate into smaller peptides, free amino acids. This is a very important step e.g. in the disintegration of prion pro-

teins causing BSE. The alkaline hydrolysis is carried out with NaOH or KOH. The process can be accelerated by heating, where 150°C is considered to be the optimal temperature. The reaction takes place in a steel reactor under continuous stirring at 4 bar pressure in 3-6 hours (*92*/2005/EEZ). During the alkaline hydrolysis the peptide bonds are cleaved and depending on the alkali used peptides with low molecular weight and potassium or sodium salts of free amino acids are forming (*Neyens et al.*, 2003).

It was obtained in earlier investigations that every impact involving high temperature alkaline treatment results in racemization of most of the amino acids (*Imai et al.*, 1996; *Man and Bada*, 1987; *Friedman*, 1991). When treating the proteins with alkali at low temperature or the hydrolysis is carried out at high temperature in neutral or acidic medium, this can also cause racemization, but the combination of alkaline treatment and high temperature results in racemization almost surely. In the course of our research the racemization of amino acids including epimerization of isoleucine was examined in the products of an experiment targeting to render harmless slaughterhouse waste.

2 Materials and methods

Treatment of the samples

In the experiments ox brain samples obtained from Croatia were used. As no cases of BSE were recorded in Croatia and the samples were taken from cattles younger than 30 months old, no prion analysis was conducted.

After sampling the samples were stored at −20°C, then after defrosting they were homogenized and divided into 400 g parts. During treatments to 400 g of sample 600 cm^3 of distilled water and 44 cm^3 of 45% KOH or 30 cm^3 of 45% NaOH solution was added. Accordingly, to the control sample 644 and 630 cm^3 of distilled water was added. The alkaline mixtures were heated at different temperatures and under different pressures (135°C, 2.75 bar; 150°C, 4.78 bar and 153°C, 5.17 bar) and after 2, 3 and 6 hrs sample was taken from the reactor. At the bottom of the reactor a magnetic stirrer ensured the homogenity of the solution. After the treatment the samples were centrifuged at 5,000 rpm for 2 min., the sediment of approx. 0.5% was removed, and the liquid phase was stored in a deep-freeze. Out of each treatments the measurements were carried out in 3 repetitions. Along with the control altogether 19 treatments were carried out. Parameters of the treatments are summarized in *Table 1.*

Table 1: Alkaline treatments applied

Control samples			C
Alkali used	Treatment temperature, °C	Treatment time, hours	Marking
NaOH	135	2	N1
		3	N2
		6	N3
	150	2	N4
		3	N5
		6	N6
	153	2	N7
		3	N8
		6	N9
KOH	135	2	K1
		3	K2
		6	K3
	150	2	K4
		3	K5
		6	K6
	153	2	K7
		3	K8
		6	K9

Hydrolysis conditions

In order to release the amino acids the hydrolysis was carried out using 6 M hydrochloric acid. As tryptophan decomposes under acidic conditions and alkaline conditions lead to racemization, we applied a less rigorous acidic medium during the determination of the Trp enantiomers. The hydrolysis mixture was a 3 M p-toluenesulfonic acid solution containing 3-indolylpropionic acid. The protein/3-indolylpropionic acid ratio was set to the value of 1:1 (*Liu and Chang*, 1971; *Gruen and Nichols*, 1972).

In both cases the hydrolyses were carried out in closed ampoules under nitrogen atmosphere at 110°C for 24 hrs. Th pH of the hydrolysates was set with 4 M NaOH solution.

Derivatization and analysis

Before the analysis the samples were filtrated on a 0.45 μm membrane filter, then from the amino acid enantiomers during precolumn derivatization

with OPA (o-phtaldialdehyde) and TATG (1-thio-β-D-glucose-tetraacetate) diastereomers were formed (*Einarsson et al.*, 1987; *Csapó et al.* 1995).

The derivatization and analysis were carried out using a MERCK-Hitachi HPLC apparatus consisting of the following units: L-7250 programmable autosampler, L-7100 pump, L-7350 column thermostat, L-7480 fluorescence detector, AIA data processing unit for D-7000 HPLC system manager.

Diastereomer derivatives of Asp and Glu were separated on a 125mm×4 mm i.d. Superspher 60 RP-8e column. Composition of the mobile phase was at start 28% (v/v) methanol, 72% (v/v) phosphate buffer (50 mM, pH=7). After 10 min the proportion of acetonitrile was increased from 0% to 17% and at the same time that of the phosphate buffer was decreased to 55%. From the 40. min the proportion of acetonitrile was increased to 40%, whereas that of the phosphate buffer decreased to 36%, and that of methanol to 24%.

For the analysis of the Trp enantiomers a 125 mm×4 mm i.d. Purospher RP-18e column was used. Composition of the mobile phase was as follows: at the start of the measurement 20% (v/v) methanol (remained unchanged during the analysis), 64% (v/v) phosphate buffer (50 mM, pH=7), and 16% (v/v) acetonitrile. After 38 min isocratic elution the proportion of phosphate buffer was decreased to 53% and that of acetonitrile was increased to 27%. From the 50. min the proportion of the phosphate buffer decreased further to 45% and at the same time that of acetonitrile increased to 35%. From the 65. min the starting composition was used again.

For both HPLC separations the columns were thermostated at 40 °C. Flow rates were 1 cm^3/min. The derivatives were detected by fluorescence detector (ex.: 325 nm, em.: 420 nm).

D-allo-isoleucine content was determined using an *INGOS* AAA 400 amino acid analyzer. Separation took place on a 350×3.7 mm, OSTION Lg ANB cation-exchange column. In the amino acid analyzer working on the principle of the ion-exchange chromatography D-allo-isoleucine eluted between methionine and isoleucine and was detected via postcolumn derivatization with ninhydrin.

3 Results

As a result of the treatments the concentration of the individual amino acids in the samples increased compared to the starting materials. The reason for this was that during the treatments the samples lost some amount of solvent and by this they become more concentrated.

Racemization of aspartic acid and glutamic acid

It was established that even during the hydrolysis carried out at the lowest temperature and for the shortest time (135 °C, 2 hrs) more than 40% of both amino acids racemized (Fig. 1. and 2.). Proportion of the D-amino acids is expressed by the formula of D/(D+L)×100 (Table 2. and 3.). After analysis of the control, not heat-treated sample for D-aspartic acid 3.9% and for D-glutamic acid 1.6% was obtained which could be attributed to the intervention prior to the heat treatment and to the racemization occurred during sample preparation and protein hydrolysis, respectively.

It was observed that at the two higher temperatures, during longer treatment the concentration of the amino acids decreased despite the solutions becoming more concentrated the concentration of the amino acids decreased, reason for which was presumably the decomposition of Asp and Glu.

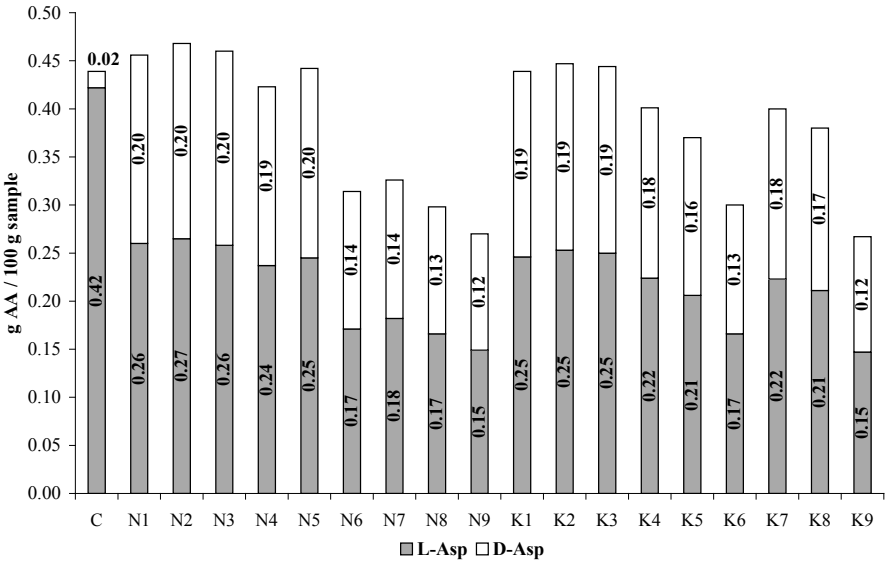

Figure 1: Change in the amount of aspartic acid enantiomers due to alkaline treatment

Table 2: Extent of racemization of aspartic acid due to alkaline treatments

Marking	N1	N2	N3	N4	N5	N6	N7	N8	N9
$\frac{D}{D+L} \times 100$	43.0%	43.4%	43.9%	44.0%	44.6%	45.5%	44.2%	44.3%	44.8%
Marking	K1	K2	K3	K4	K5	K6	K7	K8	K9
$\frac{D}{D+L} \times 100$	43.2%	43.4%	43.7%	44.1%	44.3%	44.7%	44.3%	44.5%	44.9%

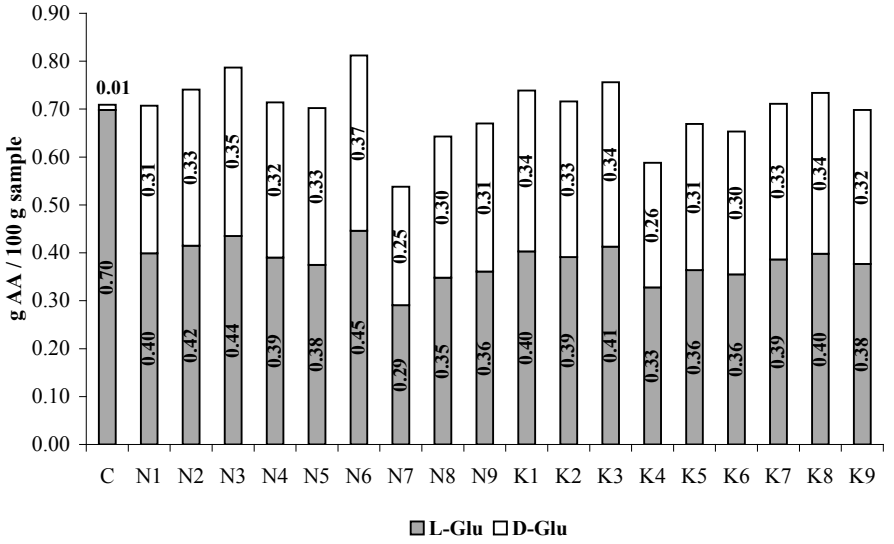

Figure 2: Change in the amount of glutamic acid enantiomers due to alkaline treatment

Table 3: Extent of racemization of glutamic acid due to alkaline treatments

Marking	N1	N2	N3	N4	N5	N6	N7	N8	N9
$\frac{D}{D+L} \times 100$	43.6%	44.0%	44.7%	45.4%	46.6%	45.1%	45.9%	45.9%	46.1%
Marking	K1	K2	K3	K4	K5	K6	K7	K8	K9
$\frac{D}{D+L} \times 100$	45.5%	45.4%	45.4%	44.2%	45.6%	45.6%	45.7%	45.8%	46.0%

Racemization of tryptophan

In case of tryptophan it was necessary to employ another hydrolysis method because tryptophan completely decomposes during the 6 M hydrochloric acidic hydrolysis for 24 hrs due to cleavage of the indole group. During the acidic hydrolysis we applied, using the protecting agent more than 80% of tryptophan

could be recovered from the protein.

Change in the Trp enantiomer content of the samples is shown in Fig. 3, the extent of racemization is given in Table 4. It was found that 39-45% of tryptophan racemized during the heat treatment. Like in case of aspartic acid and glutamic acid, treatment at higher temperature and longer treatment time, respectively, led to decomposition of tryptophan.

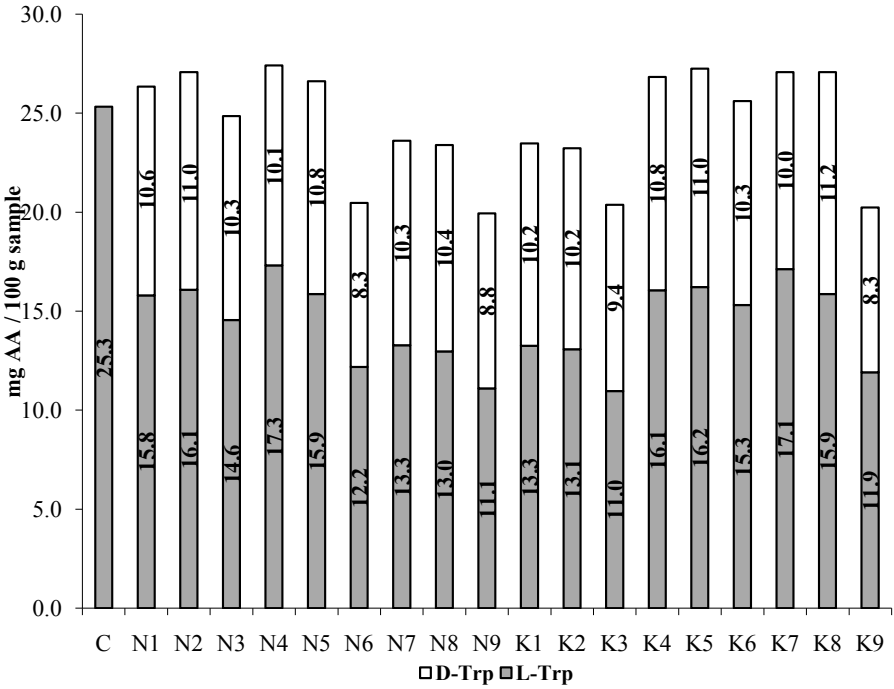

Figure 3: Change in the amount of tryptophan enantiomers due to alkaline treatment

Table 4: Extent of racemization of tryptophan due to alkaline treatments

Marking	N1	N2	N3	N4	N5	N6	N7	N8	N9
$\frac{D}{D+L} \times 100$	40.0%	40.6%	41.5%	36.8%	40.4%	40.4%	43.8%	44.6%	44.3%
Marking	K1	K2	K3	K4	K5	K6	K7	K8	K9
$\frac{D}{D+L} \times 100$	43.5%	43.7%	46.2%	40.2%	40.5%	40.2%	36.8%	41.4%	41.2%

Epimerization of isoleucine

In the last phase of our experiments the epimerization of isoleucine was examined. It was found that the control sample did not contain D-allo-isoleucine even in traces. The results of the treatments are shown in Fig. 4, the extent of the racemization is shown in Table 5. Based on the results it can be said that in the treated samples the total amount of the isomers practically does not change. In contrast with the other three examined amino acids, in case of isoleucine we do not have to reckon with the decomposition of the amino acid.

Examining the extent of the racemization it was established that carrying out the treatment at 135°C with NaOH, the extent of the epimerization of Ile is less than in the other treatments, although it is above 40% also in this case. In the other treatments the racemization can be considered as complete.

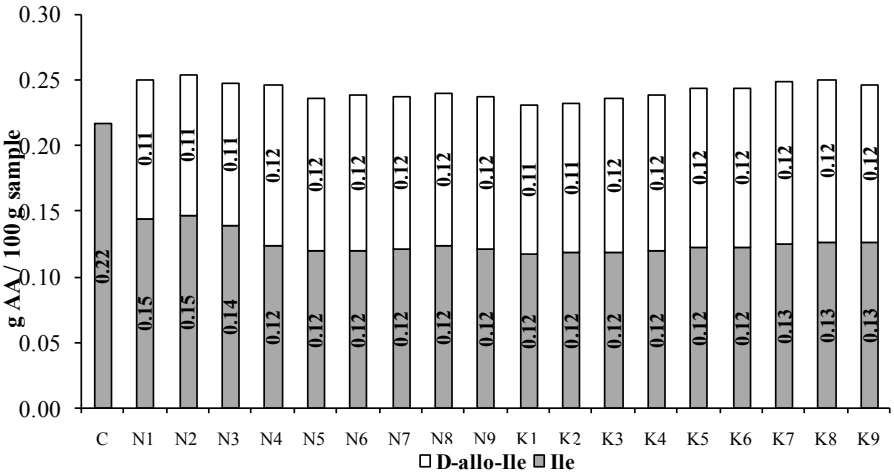

Figure 4: The amount of the isoleucine enantiomers due to alkaline treatment

Table 5: Extent of epimerization of isoleucine due to alkaline treatments

Marking	N1	N2	N3	N4	N5	N6	N7	N8	N9
$\frac{D}{D+L} \times 100$	42.2%	42.4%	43.5%	49.8%	49.4%	49.8%	48.7%	48.3%	48.7%
Marking	K1	K2	K3	K4	K5	K6	K7	K8	K9
$\frac{D}{D+L} \times 100$	48.9%	48.9%	49.6%	49.8%	49.6%	49.6%	49.4%	49.4%	48.6%

4 Conclusions

During our research ox brain samples obtained from Croatia and treated with NaOH and KOH, respectively, at different temperatures for different durations, were analyzed. Aspartic acid, glutamic acid, isoleucine and tryptophan enantiomers of the hydrolysate were determined.

Aspartic acid and glutamic acid were chosen because proteins used in animal feeding contain relatively much of these two amino acids, in some cases their total amount can reach even 30-40% of the crude protein content. Furthermore, these two amino acids were chosen as according to our previous experiences they belong to amino acids racemizing relatively easily, hence they can be used as markers for the estimating the racemization of the other proteinous amino acids. Third reason why we chose these two amino acids was that during the high-performance liquid chromatographic analysis we applied these two amino acids appear at the very beginning of the chromatogram therefore they provide quick information as to whether it is worth to determine the D- and L- enantiomers of the other amino acids as well. If no D-aspartic acid or D-glutamic acid can be detected in the sample, it is not worth continuing with the chromatographic analysis as it is almost for sure that the analyzed material does not contain the D-enantiomers of the other proteinous amino acids, either.

Isoleucine was chosen because the determination of D-allo-isoleucine formed due to the alkaline treatment does not require special analytical procedure, it can be easily analyzed by an automatic amino acid analyzer working on the principle of ion-exchange column chromatography, that can be found in most of the food and feedstuff laboratories, its peak between methionine and isoleucine can be well separated from that of the other proteinous amino acids. On the other hand, this amino acid was chosen because isoleucine belongs – along with leucine and valine – to the amino acids racemizing the hardest, that is, if we can detect D-allo-isoleucine in the sample even in traces, that means that also the other proteinous amino acids underwent racemization with high probability.

Tryptophan was chosen because it is an essential amino acid on the one hand, and the alkaline hydrolysis conditions are favourable for avoiding the decomposition of tryptophan, since the indole group of tryptophan completely decomposes under acidic circumstances. Based on the literature, we can be sure that during the alkaline treatment we applied a considerable amount of tryptophan remains unchanged, it is highly probable, however, that its considerable part racemizes during the alkaline treatment.

Based on the analyses it appears that on the racemization in the temperature range of 135-153 °C as well as between the treatment times of 2-6 hrs neither the temperature nor the treatment time have any considerable effect. This is shown by the fact that between the sample treated with NaOH for 2 hrs and the one treated for 6 hrs at 153 °C only a difference of 1.8% in the ratio of D-aspartic acid was obtained which is a negligible difference compared to the average racemization of 43-45%. The same tendency appears in case of the hydrolysis with KOH where between the hydrolysis carried out at the lowest temperature for the shortest time and the hydrolysis at the highest temperature for the longest time a difference of 1.7% was obtained which is negligible compared to the total racemization of 43-45%. In case of aspartic acid, comparing the treatments with NaOH and those with KOH, no significant difference could be found, that is, in the respect of racemization the two alkaline hydrolyses can be considered as equal. It appears that the hydrolysis carried out at 153 °C for 6 hrs in case of both KOH and NaOH leads to a considerable decomposition of aspartic acid. In case of glutamic acid almost fully similar tendency was obtained as described for aspartic acid. This is not surprising because racemization of these two acidic amino acids - based on our earlier experiences - occurs in a similar way and ratio due to the different technological interventions. In case of the treatment with NaOH including the total temperature and time combinations, the proportion of D-glutamic acid varied between 43.6 and 46.1%, while in case of the treatment with KOH between 44.2 and 46.0%. No difference could be found between the two kinds of alkaline treatment regarding racemization of glutamic acid. Although the difference is not so definite than in case of aspartic acid, it can be established that the hydrolysis carried out at higher temperature for a longer time results not only in racemization but also decomposition of glutamic acid.

In case of tryptophan the situation was more complicated as not the usual protein hydrolysis was applied but the protein treated with alkali was hydrolyzed with 3 M p-toluenesulfonic acid in the presence of protecting agent (3-indolylpropionic acid). Regarding the racemization of tryptophan practically the same can be said as already discussed in case of aspartic acid and glutamic acid. Comparing both hydrolyzing media and all the three time and temperature combinations it was found that the extent of racemization of tryptophan varied between 43.6 and 46.1%. Considerable loss of tryptophan was experienced only in case of the six-hour hydrolysis. Due to the alkaline treatment the amount of tryptophan practically did no change in comparison to the control sample.

Evaluating the results for D-allo-isoleucine it was found that the alkaline treatment carried out at 135°C with NaOH resulted in around by 5-6% less racemization than at 150 and 153°C, respectively. At 135°C the extent of the racemization varied between 42.2 and 43.3%, while at the two higher temperatures between 48.3 and 49.8%. In case of treatment with KOH no such a difference was experienced, the racemization ranged between 48.6 and 49.8%.

As it was expected, isoleucine did not decompose practically in any of the treatments since it is known that the aliphatic side chain amino acids resist almost every technological interventions.

Evaluating the results for the four amino acids it was established that during the alkaline treatment each amino acid racemized in 42-46%, hence in practical respect the obtained material can be considered as racemic mixture of the amino acids. The amino acid content of the samples decreased only in the treatment carried out at the highest temperature for the longest time. As the racemization can be considered as complete even in the treatment carried out at the lowest temperature and for the shortest time and since racemization is a result of the roughest technological interventions, we suppose that a treatment at 135°C for 2 hrs with NaOH or KOH is sufficient for the entire destruction of the protein structure.

Based on the above the almost complete racemization makes the obtained material unfit for being used as animal feedstuff since the higher animals – with the exceptions of ruminants – can utilize only L-amino acids, D-amino acids act as growth inhibitor. Nothing seems to be against, however, that the product of the hydrolysis with KOH, after neutralization, is used as nitrogen fertilizer in the soil.

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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 isonflavons 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 Brassicae 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 mirosinase 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-Payrastre*, 2006).

The primary *in vivo* methabolic 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 chemotherapic 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-Payrastre*, 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 (≈660 μmol/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-picril-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 therapic 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 microrganisms 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 citotoxicity 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 citotoxicity 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 a*l. (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 B_2 content increased significantly, at the same time there was no significant change in the vitamin B_1 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 lenght 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 peripheric 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. epigallokatechin-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, epigallokatechin-3-gallate, epigallokatechin, epikatechin and katechin. 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-dihydroxyphenylanaline 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-dihydroxyphenylanaline 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 B_{12} content of the Japanese radish looked for the answer to how the plant can absorb it from a vitamin B_{12}-containing solution and incorporate into their cells. It was established that the B_{12} 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 B_{12} content was used during the germination. Vitamin B_{12} 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, kumoestrol, formononetin and biokanin) 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 phytooestrogens 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 Se-methionine 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 that 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 mammilary cancer induced by 7,12-dimethyl-benz(a)antracene 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 ibarine 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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A study of 34 cultivars of basil (*Ocimum* L.) and their morphological, economic and biochemical characteristics, using standardized descriptors

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Abstract. Basil is an important medicinal and aromatic plant that is widely used in many fields. The aim of this study was to evaluate the oil content and ornamental value of a wide range of species and cultivars of *Ocimum* L. from various different sources, using standardized descriptors. 34 cultivars of basil (*Ocimum* L.) were grown in two different years at The Faculty of Horticulture in Lednice, Czech Republic. The cultivars were divided into groups according to leaf colour and leaf size. The cultivars displayed a wide diversity of morphological, biological and economic characteristics. The groups of green small-leafed and purple-leafed varieties were judged to be the most decorative. To improve the ornamental value of the *Ocimum* species the uniformity, colour stability and earliness of flowering should be examined. The essential oil was extracted using hydro-distillation methods. Most of the samples had high essential oil levels (1.8 – 14.3 ml.kg^{-1}), which were influenced by cultivar, environmental conditions and storage length. The major

Key words and phrases: *Ocimum* (Lamiaceae), morphological characteristics, biochemical characteristics, descriptor, essential oil

volatile oil constituents were determined by gas chromatography. Levels of linalool, 1,8-cineol and eugenol were determined in all samples. The small-leaved cultivars, *O. basilicum* (pravá trpasličí) and *O. b. minimum* 'Spicy Bush', were richest in essential oil content, followed by cv. 'Citriodora' and the traditional Czech cultivars 'Ohře' and 'Litra'. The results suggest that further research to improve the quality of the essential oil content is desirable for essential oil production.

1 Introduction

Basil has been used as a medicinal and aromatic plant for centuries. Nowadays it is used in many fields of human activity, especially in the food industry and in gastronomy. Basil is also important for the pharmaceutical industry and is still used in traditional medicines in many parts of the world. It is appreciated for its essential oil, which gives it a unique flavour.

The genus *Ocimum* is characterized by great variability among its constituent species, including morphology, growth habit, the colour of flowers, leaves and stems, and chemical composition. The ease of cross-pollination has given rise to a large number of species, subspecies, varieties and forms [28, 30]. However, [7] have found that their chemical constituents are not necessarily correlated with their taxonomy. Different authors have divided basil into groups based on various points of view, ranging from their chemical composition to morphology [1, 5, 7, 10, 11, 20, 27, 33]. Some systems of descriptors combining morphological, economical and biochemical characteristics of *Ocimum* species have also been proposed [8, 18].

The levels of essential oil and other compounds vary between the different basil species and cultivars, and are also influenced by growing conditions [7, 18, 34, 35]. According to [12], the variation in essential oil content and composition of *Ocimum basilicum* coming from different countries might be attributed to different environmental conditions, as well as genetic factors, different chemotypes and differences in the nutritional status of the plants. Most of the essential oil is concentrated in the leaves, and the amounts obtained from branches and stems is not economically significant [3, 17, 23].

In the last few decades the importance of basil as an ornamental plant has grown and the number of cultivars on sale has increased significantly, it is now available in a wide range of habits, colours and flavors. Apart from their ornamental value, these cultivars may also be rich in essential oils and could offer a new source of oil for industrial exploitation [34]. However, according to [30], for example, only a few of the North-American lemon-scented cultivars

could compete successfully as industrial sources of citral. On the other hand, they suggest that the cultivar 'Camphor' could be a potential industrial source of camphor and the cultivars 'East Indian' and 'Tree' could be a useful source of eugenol.

This is the first study of a large number of basil cultivars using a large number of standardized descriptors and with an emphasis on detailed morphological characteristics. The aim was to evaluate basil cultivars according to their essential oil content and composition as well as their ornamental value. Domestic and foreign cultivars of basil were collected to form a field trial. The morphological development of cultivars was observed to assess the best time of harvest for aromatic and medicinal purposes. The essential oil content was examined qualitatively and quantitatively to assess the potential of cultivars for industrial use.

2 Material and methods

Plant material

34 basil cultivars were purchased from commercial growers in different countries (Tab. 1).

Field trial

The plants were cultivated in a sandy-loam soil in the experimental grounds of Mendel University of Agriculture and Forestry (Brno), at the Faculty of Horticulture in Lednice, Czech Republic. Average temperature from April to August was 18.5°C with 199 mm of rainfall in 2003, and 16.8°C with 364 mm of rainfall in 2005. A drip-irrigation system was installed to provide water when necessary.

34 basil cultivars were sown in containers in a greenhouse in the middle of April. Newly germinated plants were pricked out into pots. The plants were planted out in the field from 40 to 45 days after germination. Each cultivar was represented by 20 plants in 2003 and by 15 plants in 2005. The distance between the plants was 0.4 m in the rows and 0.6 m between the rows.

Plants were harvested at the beginning of flowering from the middle of June to the end of July in 2003 and from the end of June to the middle of August in 2005. The fresh biomass was weighed immediately after harvest, then dried at room temperature and weighed again.

Table 1: Origin of the plant material

Semo Smržice, Czech Republic:
Ocimum basilicum L. 'Purple Opaal'
Ocimum basilicum L. 'Cinamonette'
Ocimum basilicum L. 'Lime'
Ocimum basilicum L. 'Compakt'
Ocimum basilicum L. 'Blue Spice'
Ocimum basilicum L. 'Dark Green'
Ocimum basilicum L. 'Lettuce Leaf'
SEVA FLORA, Valtice, Czech Republic:
Ocimum basilicum L. 'Ohře'
Ocimum basilicum L. 'Litra'
NOHEL GARDEN, Dobříš, Czech Republic:
Ocimum basilicum L. (pravá trpasličí)
Ocimum basilicum L. (pravá zelená)
Ocimum basilicum L. (pravá červená)
Botanical garden of medicinal plants,
Hortus Plantarum Medicinarum Academii Mediceae, Wroclav, Polland:
Ocimum basilicum L. 'Kardinal'
Ocimum basilicum L. 'Cinnamon'
Ocimum tenuiflorum L.
IHP ŽALEC, Slovenia:
Ocimum basilicum L. 'Genovese'
Ocimum basilicum L. 'Citriodora'
Ocimum basilicum L. 'Grant Vert'
Ocimum basilicum L. 'Sweet Dani'
JELITTO, Schwarmstedt, Germany:
Ocimum basilicum L.
Ocimum basilicum L. 'Großblättrig'
Ocimum basilicum L. 'Purple Ruffles'
Johny's Selected Seeds, Maine, USA:
Ocimum basilicum L. 'Italian Large Leaf'
Ocimum basilicum L. 'Nufar'
Ocimum basilicum L. 'Magical Michael'
Ocimum basilicum L. 'Genovese Compact Improved'
Ocimum basilicum L. 'Red Rubin'
Ocimum basilicum L. 'Osmin Purple'
Ocimum basilicum L. 'Cinnamon'
Ocimum basilicum L. 'Fino Verde'
Ocimum basilicum citriodora 'Mrs. Burns' Lemon'
Ocimum basilicum minimum 'Spicy Bush'
Ocimum americanum 'Lime'
Tsukuba Medicinal Plant Research Station,
National Institute of Health Sciences, Japan:
Ocimum basilicum L.

Descriptors for the genus *Ocimum* L. [8] was used to evaluate morphological, biological and economic characters. An evaluation of plant width, stem branching, inflorescence length and density of flowers were added to the list of morphological characters to be assessed. The number of days of flowering was added to the list of biological characters. Fresh and dry yields of biomass

per plant and vigour rating were added to the list of economic characters.

Analysis of essential oil content

The analysis were made in the laboratories of Mendel University of Agriculture and Forestry (Brno), at the Faculty of Horticulture in Lednice, Czech Republic. The essential oil content was measured by hydrodistillation. 20 g of the dry biomass with 400 ml of distilled water was boiled in the distillation apparatus. After three hours volume of distilled essential oils was noted.

Gas chromatography (GC) analysis of essential oils

The analysis were made by the Prague-Ruzyne Crop Research Institute at their Gene Bank Department in Olomouc, Czech Republic. The essential oil was analyzed on a Hewlett-Packard gas chromatograph model 5890A SERIES II equipped with flame ionization detector (FID) and HP-INNOVAX capillary column (60 m – 0.53 mm). Injector and detector temperatures were set at 220°C. The column temperature programme was: 100°C (3 min, 3°C/min), 150°C (2°C/min) and 200°C (15 min). Nitrogen was used as carrier gas at 39 kPa and at a flow rate of 50 ml/min. A sample of 1.0 µl was injected, using split mode (split ratio, 1:20). The relative retention times of the compounds were compared with those of standards. The content of 1,8-cineole, linalool and eugenol was determined for each sample. Other essential oil compounds were identified if having a significant percentual content in comparison with the other compounds.

Statistical analysis

Statistical analysis of the data was performed by analysis of variance (ANOVA) using Unistat 4.53 software.

3 Results

Morphological characteristics

The plant heights varied from 143 to 570 mm. The smallest was *Ocimum basilicum minimum* 'Spicy Bush', the tallest were *Ocimum basilicum* from Germany (Jelitto) and *Ocimum americanum* 'Sweet Dani'. The plant spread was from 213 mm in *O. basilicu*m minimum 'Spicy Bush' to 610 mm in *O. basilicum* 'Ohře'.

Most plants had a semi-erect habit, semi-dense branching and sparse stem pubescence. The prevalent leaf-shape was lanceolate. In green-leafed basils with large leaves, and purple-leafed basils, a serrate leaf margin was prevalent. In the green small-leafed basils the leaf margin was entire.

Most cultivars had glabrous leaves apart from the cultivars 'Blue Spice', 'Piperitum' and *O. tenuiflorum*, whose leaves are sparsely pubescent. Plants had prevalently plain leaves in the medium and small green-leafed and purple-leafed cultivars. In the large green-leaved cultivars most leaves were undulate with small or sparse blisters. The prevalent leaf colour varied from green to dark green according to the RHS colour chart.

Flower size was intermediate. The green-leafed cultivars predominantly had a green calyx and a white corolla. In the purple-leafed cultivars the calyx and corolla were various shades of purple, and in most cultivars the calyx was pubescent. The length of the flowering spike varied from 29 to 178 mm. Sparse and semi-sparse inflorescence density was prevalent.

Biological characteristics

Most cultivars do not show stand uniformity, although the following cultivars do: *O. basilicum* 'Litra', 'Blue Spice', 'Cinnamon', 'Cinamonette', 'Citriodora', 'Kardinal', 'Lime', 'Piperitum', *O. americanum* 'Lime', *O. tenuiflorum* and *O. basilicum minimum* 'Spicy Bush'.

The beginning of flowering varied from 19 to 64 days after planting. The cultivars which were the latest to initiate flowering were *O. basilicum* (Tsukuba, Japan), 'Fino Verde', 'Magical Michael' and 'Purple Ruffles'. The earliest flowering cultivars were 'Blue Spice', 'Sweet Dani' and *O. tenuiflorum*.

Economic characteristics

Fresh weight yields varied from 14 to 713 g per plant. The lowest fresh weight yield was given by *O. americanum* 'Lime', the highest by *O. basililcum* 'Fino Verde'. Dry weight yields varied from 25 to 499 g per plant. The lowest dry weight yield was given by the cultivar 'Spicy Bush', the highest by 'Fino Verde'. Vigour ratings varied from 4:1 to 9:1.

Biochemical characteristics

Essential oil contents varied from 1.8 to 14.3 $ml.kg^{-1}$ (0.18 – 1.43%) in the dry herbage. According to the descriptors [8] the oil content was high in most cultivars (over 4 $ml.kg^{-1}$ of dry biomass). In 94.1% of the cultivars the

essential oil content was more than 0.3% of dry weight. The lowest oil content was found in the cultivar 'Piperitum', the highest oil content in dwarf basil *O. basilicum* (pravá trpasličí) and *O. basilicum* 'Lime'.

Linalool content varied from 3.9 to 19.9%. The highest linalool content was found in the cultivar 'Red Rubin' and the lowest in 'Blue Spice'. The content of 1,8-cineole varied from 0.9 to 18.5%. The highest levels were found in the cultivar 'Piperitum', the lowest in *O. americanum* 'Lime'. The eugenol content varied from 0.3 to 13.3%, with the highest levels in the cultivar 'Magical Michael' and the lowest in *O. americanum* 'Lime'. Some cultivars also contained significant levels of one or more of the following compounds: methylchavicol, camphor, citral, limonene, methylcinnamate, caryophyllen-β, anethol, terpinen-4-ol, myrcene, thymol, ocimene and cinnamaldehyde. (Tab. 2 - 5)

Statistical results

Statistically highly significant differences were observed between the two years (due to environmental factors) regarding plant height, essential oil content and eugenol content, and also in the fresh and dry weight yields per plant and in the vigour rating. Statistically highly significant differences were observed between cultivars regarding plant height, leaf-length, flower-size, flowering time, essential oil content, anethol and terpinen-4-ol content, and also in the fresh weight yield per plant and levels of linalool, 1,8-cineole, eugenol, methylchavicol, citral and β-caryophyllene. There were differences seen between the first and the second harvest in yield, content of 1,8-cineole and eugenol, but not in essential oil content and linalool content.

4 Discussion

Only a few articles about basil have focussed on essential oil levels and its composition, and also on morphological characteristics as well e.g. [7, 16, 18, 19, 22, 30]. The present study gives a detailed overview of the morphological, biological, economic and biochemical characteristics of 34 basil cultivars evaluated using Dušková's minimal set of descriptors [8].

According to [2] plant height varies with the cultivar and the number of harvests in the season, as is confirmed in this research too. [16] found that harvesting only the secondary branches of *O. tenuiflorum* led to maximum plant height and number of secondary branches per plant. On the other hand, secondary branches give the least biomass yield.

Tabel 2: Biochemical characteristics - green leafed cultivars with large leaves (> 60 mm)

Green leafed cultivars with large leaves (> 60 mm)	Essential oil content [ml.kg^{-1}]			Linalool content [%]			1,8-cineol content [%]			Eugenol content [%]			Content of other essential oil compounds		
	2003	2005		2003	2005		2003	2005		2003	2005		2003	2005	
	1st Harvest	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harvest	1st Harvest	2nd Harvest
Ocimum basilicum **L. ´Dark Green´**	3.25	3.88	4.63	18.00	14.25	15.60	14.40	13.50	14.90	9.65	9.20	**11.10**	-	-	methylcinnamate (11,3 %)
Ocimum basilicum **L. ´Genovese´**	**1.88**	6.00	-	14.40	16.50	-	11.75	13.50	-	11.85	8.50	-	methylchavicol (14,35 %)	-	-
Ocimum basilicum **L. ´Genovese Compact Improved´**	**4.25**	4.00	-	14.40	**18.10**	-	14.40	**15.80**	-	10.95	9.90	-	-	-	-
Ocimum basilicum **L. ´Grant Vert´**	3.75	4.75	5.50	13.15	14.65	16.25	13.15	12.75	**16.25**	10.85	8.75	7.75	-	-	-
Ocimum basilicum **L. ´Großblättrig´**	3.88	4.25	4.88	14.35	17.35	**15.10**	11.90	**6.60**	15.05	11.15	9.40	8.20	-	myrcene (12,75 %), limonene (5,35 %)	-
Ocimum basilicum **L.´Italian Large Leaf´**	-	4.50	-	-	-	-	-	-	-	-	-	-	-	-	-
Ocimum basilicum **L. (Jelitto)**	1.90	**2.65**	**3.88**	14.80	11.25	**19.65**	13.80	8.80	14.95	**12.45**	-	9.50	-	thymol (9,1 %)	-
Ocimum basilicum **L. ´Lettuce Leaf´**	4.00	4.50	4.25	13.70	**14.20**	17.10	**4.00**	12.00	**13.45**	**7.00**	9.10	6.70	camphor (10 %)	-	-
Ocimum basilicum **L. ´Litra´**	6.00	**7.05**	**8.00**	**15.90**	16.85	16.50	8.80	9.15	16.05	9.90	7.50	**6.40**	anethol (15,8 %)	anethol (15,7 %), camphor (5,25 %)	anethol (16,5 %), camphor (1,4 %)
Ocimum basilicum **L. ´Nufar´**	-	6.00	-	14.90	15.80	-	-	-	-	-	-	-	-	-	-
Ocimum basilicum **L. (pravá zelená)**	3.75	4.00	-	**12.30**	14.40	-	**14.95**	14.40	-	11.90	**10.20**	-	-	-	-
Ocimum basilicum **L. (Tsukuba)**	8.00	5.00	-	-	-	-	12.30	14.40	-	9.65	**8.10**	-	-	-	

Tabel 3: Biochemical characteristics - green leafed cultivars with middle size leaves (30-60 mm)

Green leafed cultivars with middle size leaves (30-60 mm)	Essential oil content [ml.kg^{-1}]			Linalool content [%]			1,8-cineol content [%]			Eugenol content [%]			Content of other essential oil compounds		
	2003	2005		2003	2005		2003	2005		2003	2005		2003	2005	
	1st Harvest	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harvest	1st Harvest	2nd Harvest
Ocimum americanum **L. ´Lime´**	3.00	5.25	5.50	12.80	16.05	**15.70**	**0.90**	**0.65**	1.60	-	0.65	**0.90**	citral (12,8%)	citral (17,1 %), β-caryophyllene (6,2 %)	citral (15,8 %), β-caryophyllene (5,9 %)
Ocimum basilicum **L. ´Blue Spice´**	3.63	**4.75**	4.63	**3.95**	**4.80**	**10.00**	12.65	**16.20**	10.00	8.80	4.05	-	methylchavicol (11 %)	methylchavicol (11,2 %)	methylchavicol (10,6 %)
Ocimum basilicum **L. ´Cinnamon´**	6.38	9.50	7.50	14.10	14.55	13.80	4.80	2.05	6.30	10.20	7.60	9.40	methylcinnamate (14,1 %)	myrcene (6 %), methylcinnamate (11,8 %)	myrcene (0,5 %), methylcinnamate (13,9 %)
Ocimum basilicum **L. ´Cinamonette´**	7.75	6.50	6.75	14.75	**16.45**	11.70	8.85	2.95	5.50	8.00	**10.55**	9.55	methylcinnamate (14,8 %), methylchavicol (10,2 %)	methylcinnamate (16,4 %), methylchavicol (4,8 %)	methylcinnamate (11,7 %), methylchavicol (6,2 %)
Ocimum basilicum **L. ´Citriodora´**	-	11.50	-	13.65	10.65	-	-	-	-	-	-	-	-	-	-
Ocimum basilicum **L. ´Compakt´**	4.88	6.38	-	12.90	11.95	-	6.30	9.20	-	10.00	8.90	-	terpinen-4-ol (4,8 %)	terpinen-4-ol (5,1 %)	-
Ocimum basilicum **L. ´Kardinal´**	2.88	6.25	-	12.90	15.10	15.60	8.50	2.80	-	8.65	4.65	-	methylchavicol (12,8 %), anethol (4,7 %)	myrcene (12 %), limonene (4,9 %), methylchavicol (6,3 %), anethol (8,7 %), methylcinnamate (6,7 %)	-
Ocimum basilicum **L. ´Lime´**	2.25	8.00	6.25	**15.35**	17.30	-	2.00	1.00	4.65	**1.65**	**1.30**	2.25	citral (11,5 %), β-caryophyllen (4,5 %)	β-caryophyllen (9,3 %), citral (14,3 %)	citral (15,6 %), β-caryophyllene (4,2 %)
Ocimum basilicum **L. ´Magical Michael´**	6.01	6.75	-	13.10	11.40	14.70	4.85	2.70	-	**13.25**	10.50	-	-	terpinen-4-ol (7,3 %)	-
Ocimum basilicum **citriodora L. ´Mrs.**	**7.25**	**9.63**	**8.50**	14.40	13.10	-	3.60	4.30	**2.25**	-	0.75	**1.60**	citral (11,7 %), terpinen-4-ol (8,9 %),	citral (11,4 %), terpinen-4-ol (7,6 %),	citral (14,7 %), terpinen-4-ol (7,1 %),

Burns` Lemon´													β-caryophyllene (5,3 %)	β-caryophyllene (7,5 %)	β-caryophyllene (7,5 %)
Ocimum basilicum **L. ´Ohře´**	6.50	7.38	-	14.65	3.65	10.60	10.00	13.10	-	8.80	8.05	-	anethol (15,1 %)	anethol (13,1 %)	-
Ocimum basilicum **L. ´Piperitum´**	**1.75**	5.50	**4.38**	8.60	13.60	-	**13.75**	11.10	**18.15**	5.85	5.20	5.80	methylchavicol (9,5 %)	methylchavicol (11,1 %)	methylchavicol (10,7 %)
Ocimum basilicum **L. ´Sweet Dani´**	1.88	7.38	-	6.40	6.50	-	1.20	11.05	-	1.80	4.20	-	citral (12,1 %), β-caryophyllen (4,8 %), cinnamaldehyd (3,9 %), methylchavicol (2,4%)	methylchavicol (13,6 %) citral (7,65 %), β-caryophyllen (3,7 %)	-
Ocimum tenuiflorum **L.**	3.75	5.60	-	-	-	-	11.00	12.35	-	10.00	7.20	-	-	-	-

Tabel 4: Biochemical characteristics - green leafed cultivars with small leaves (< 30 mm)

Green leafed cultivars with small leaves (< 30 mm)	**Essential oil content [ml.kg^{-1}]**			**Linalool content [%]**			**1,8-cineol content [%]**			**Eugenol content [%]**			**Content of other essential oil compounds**		
	2003	**2005**		**2003**	**2005**		**2003**	**2005**		**2003**	**2005**		**2003**	**2005**	
	1st Harvest	**1st Harv.**	**2nd Harv.**	**1st Harv.**	**1st Harv.**	**2nd Harv.**	**1st Harv.**	**1st Harv.**	**2nd Harv.**	**1st Harv.**	**1st Harv.**	**2nd Harv.**	**1st Harvest**	**1st Harvest**	**2nd Harvest**
Ocimum basilicum **L. (pravá trpasličí)**	**11.38**	10.25	-	**11.90**	**11.75**	-	**11.95**	**11.75**	-	**11.90**	**11.75**	-	-	ocimene (3,75 %), terpinen-4-ol (4,5 %)	-
Ocimum basilicum **L. ´Fino Verde´**	-	**2.50**	-	**-**	**-**	-	**-**	**-**	-	**-**	**-**	-	-	-	-
Ocimum basilicum minimum ´**Spicy Bush**´	**11.15**	**11.25**	-	**11.50**	**12.90**	-	**11.55**	**13.00**	-	**11.55**	**12.05**	-	-	thymol (9,3 %)	-

Tabel 5: Biochemical characteristics - purple leafed cultivars (> 60 mm)

Purple leafed cultivars (> 60 mm)	Essential oil content [ml.kg^{-1}]			Linalool content [%]			1,8-cineol content [%]			Eugenol content [%]			Content of other essential oil compounds		
	2003	2005		2003	2005		2003	2005		2003	2005		2003	2005	
	1st Harvest	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harv.	1st Harv.	2nd Harv.	1st Harvest	1st Harvest	2nd Harvest
Ocimum basilicum **L. ´Osmin Purple´**	-	**4.50**	-	-	-	-	-	-	-	-	-	-	-	-	-
Ocimum basilicum **L. (pravá červená)**	**3.63**	-	-	17.50	-	-	**14.40**	-	-	7.75	-	-	-	-	-
Ocimum basilicum **L. ´Purple Opaal´**	-	4.25	-	-	-	-	-	-	-	-	-	-	-	-	-
Ocimum basilicum **L. ´Purple Ruffles´**	**2.63**	-	-	12.50	-	-	12.50	-	-	**6.30**	-	-	methylchavicol (8,5 %)	-	-
Ocimum basilicum **L. ´Red Rubin´**	3.00	**3.25**	-	**19.9**	**14.55**	-	**10.35**	**10.30**	-	**7.85**	**7.05**	-	-	methylchavicol (8,53 %)	-

Also, in comparing various methods of harvesting, the biomass of secondary branches gave higher essential oil yields when cut 30 cm above ground. The present study also confirms that the method of harvest and the height of the cut can influence the number of lateral branches and therefore the yield of the next harvest too. However, [23] found that the essential oil yield was not affected by the number of cuts.

[8] describes stand uniformity as being uniformity in the time of flowering. However, uniformity in the sense of the homogenous appearance of plants of each cultivar should be added to the set of descriptors as another significant parameter for both ornamental and industrial uses of basil. Moreover, very low uniformity was observed in some cultivars and [30] have also rated most basil plants as being semi-uniform. In addition, [28] warn of low uniformity in plants grown from seeds.

Basil was harvested at identical or comparable developmental stages [7, 18]. The differences found in chemical composition during the different growth stages would suggest that different harvest times must be adopted according to the intended use of the oil [14, 22]. [17] found the highest essential oil yields at the pre-flowering stage compared to all others. This study also emphasizes the need to harvest basil at an identical growth stage – at the beginning of flowering. Therefore it is important to consider the differences in earliness of flowering between the cultivars. There was no statistical difference between the flowering times in the two years, but there were highly significant differences in the flowering times between the cultivars. This demonstrates the greater influence of the typical character of each cultivar than of the growing conditions.

The cultivars differed in morphological characteristics of the leaves and stems, and in the flowers regarding various colours, shapes and sizes. In addition, leaf size has been found to be an important character for distinguishing basil cultivars, because it is correlated with height and it is the most distinctive leaf trait [7, 30, 35]. This has lead to the grouping of cultivars according to leaf colour and size [1, 5], an approach supported by the present study.

Present study has confirmed, as proved by [29] too, that the problem with weeds lasts until the first harvest and until there is a complete covering of the surface by crop plants. The optimal plant densities with respect to the habits and growth rates of each cultivar have to be established. The basil was grown at a plant density of 4.5 plants per m^2. However, [29] suggest a much higher plant density (15 - 17 plants per m^2) in field conditions, which would be insufficient for some of the cultivars with a prostrate habit examined in the present study. Densities smaller than 0.4 × 0.6 m would be suitable mainly

for dwarf basils like 'Spicy Bush'.

There was a significant difference in fresh and dry yields per plant between the two years. These results confirm the significance of environmental factors [7, 18, 33]. Moreover, in 2005 the yield of some cultivars was reduced by sunburn. The purple-leafed cultivars were the most sensitive, especially 'Purple Ruffles', which contains a relatively high level of the red pigment anthocyanin [30].

There were highly significant differences in essential oil content between the two years. The essential oil content in 2003 was measured after 18 months of storage, in 2005 it was measured after some 3 weeks to 5 months in storage. As the essential oil content was higher in 2005, it is to be supposed that the length of storage had some influence perhaps on the observed essential oil levels [6, 29]. [4] found that higher temperatures before harvest lead to higher essential oil levels. However, even if in 2003 the temperatures were higher, the observed essential oil content was lower because of the longer storage period.

The yield and physical properties of the *O. basilicum* essential oils are affected by seasonal changes and the content of essential oils is distributed unevenly among the seasons of the year [12]. However, [6] found no seasonal variation in the composition and content of essential oil major components. However, the content of essential oil was influenced by the time of harvest. According to [29] the essential oil of each harvest has its own qualitative parameters. This was statistically proved with regard to the 1,8-cineole and eugenol content but not in the case of linalool.

The levels of essential oils observed in the basil cultivars in this study are comparable to those reported by [21, 26, 34]. The major constituents of essential oils of *Ocimum basilicum* L. were linalool, eugenol and 1,8-cineole [9, 13, 15, 18, 21, 23, 26]. However, [3] found low levels of 1,8-cineole and linalool. Minor compounds were camphor, limonene, methyl-chavicol [18], caryophyllene and methyl-cinnamate [13]. In all lemon-scented basils, such as *O. basilicum* 'Lime', 'Mrs. Burns' Lemon', 'Sweet Dani' and *O. americanum* 'Lime', citral was found, as also reported by [30].

The eugenol content varied considerably between the two years of cultivation but there were no differences in the 1,8-cineole and linalool contents. According to [6] the eugenol and linalool content increased during storage. This was might be confirmed by the present study in the case of eugenol, where the content was generally higher after longer storage in most cultivars. These results also demonstrate the influence of growing conditions on the eugenol content but not on the content of linalool and 1,8-cineole.

O. americanum 'Lime' was the basil with the most significantly different essential oil composition in comparison with other cultivars. In addition, a relatively high content of β-caryophyllene was observed in *O. americanum* 'Lime', which is in agreement with the study of [32], who found that β-caryophyllene is a major compound in O. americanum 'Clove'. A much lower content of 1,8-cineole and eugenol was observed in *O. americannum* 'Lime' and in *O. tenuiflorum* than in cultivars of *Ocimum basilicum*, which confirms that they belongs to different chemotypes. In contrast, [24] found eugenol to be a major component of the essential oil of *O. sanctum* L. (syn. *O. tenuiflorum* L.). A lower content of eugenol was recorded in *O. tenuiflorum* compared to the results of [31]. In addition, 1,8-cineole was found to be a prevalent component in *O. tenuiflorum* and the presence of linalool was also observed.

According to [11], methylchavicol is one of the main essential oil components in basil, but this compound was found at levels greater than 5% only in the following cultivars: 'Genovese', 'Blue Spice', 'Cinamonnette', 'Kardinal', 'Piperitum' and 'Sweet Dani'.

Linalool was the prevalent component of the essential oils found in *O. basilicum* cultivars by [22], and in only one cultivar, 'Blue Spice', was the linalool content significantly lower than in the others.

The eugenol content was also evaluated for its role in the metabolism of toxic methyleugenol, mentioned also by [25]. Higher levels of methyleugenol were reported in *O. tenuiflorum* by [31]. In studying essential oil compounds it is highly recommended that methyleugenol levels are also observed.

One interesting observation on a basil with japanese origins, *O. basilicum* (Tsukuba), showed that the levels of linalool, 1,8-cineole and eugenol were equal, which has not been mentioned in any previous study to our knowledge.

In conclusion, the main contribution of the present study is the complete evaluation of the essential oil content and its composition and at the same time the detailed evaluation of their morphological, biological and economic characteristics. In this way it is possible to identify the cultivars with high values in all parameters. Moreover, an evaluation of the genus *Ocimum* L. according to the descriptors gives an unambiguous characterisation of the cultivars, which should be provided for all species. Such a characterization is essential not only for describing the accessions in the gene banks, which is what it was originally designed for, but it is also helpful in practice for growers who wish to choose a cultivar with specific characteristics. However, the quality of the essential oils in the ornamental cultivars of *Ocimum* L. should be improved to become commercially usable for essential oil production. To use basil as an ornamental plant, research should be focused mainly on the uniformity,

colour composition and earliness of flowering, which is also important for the industrial cultivation of basil.

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Supporting information

Morphological and biological characteristics of basil cultivars are available as supporting information.

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