Evaluation of Barley Grass as a Potential Source of Some Nutritional Substances

Ivana PAULÍČKOVÁ[1], Jaroslava EHRENBERGEROVÁ[2], Vlasta FIEDLEROVÁ[1], Dana GABROVSKÁ[1], Pavla HAVLOVÁ[3], Marie HOLASOVÁ[1], Jiří KOPÁČEK[2], Jarmila OUHRABKOVÁ[1], Jitka PINKROVÁ[1], Jana RYSOVÁ[1], Kateřina VACULOVÁ[4] and Renata WINTEROVÁ[1]

[1]Food Research Institute Prague, Prague, Czech Republic; [2]Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic; [3]Research Institute of Brewing and Malting, Brno, Czech Republic; [4]Agricultural Research Institute Kroměříž, Ltd. and Agrotest fyto, Ltd. Kroměříž, Kroměříž, Czech Republic

Abstract

Paulíčková I., Ehrenbergerová J., Fiedlerová V., Gabrovská D., Havlová P., Holasová M., Kopáček J., Ouhrabková J., Pinkrová J., Rysová J., Vaculová K., Winterová R. (2007): **Evaluation of barley grass as a potential source of some nutritional substances**. Czech J. Food Sci., **25**: 65–72.

Barley green matter was analysed for the contents of vitamin C, total polyphenols, phenolic compounds, proteins, amino acids, and saccharides; the activity of catalase was also determined. The contents of vitamin C, total polyphenols, and ferulic acid decreased with the age of barley plants. The influence of the variety has not been proved unequivocally. The contents of vitamin C between 0.107–6.357 g/kg DM, of total polyphenols between 17.167–35.559 g/kg DM, and of ferulic acid between 0–5.916 g/kg DM were found. Catalase activity amounted to 4.5–29.7 TSU. The monosaccharide profile showed high contents of glucose (15.40–88.40 g/kg DM) and fructose (37.60–81.40 g/kg DM) which decreased with the plant growth. The contents of saccharose and galactose were low, ranging between 0–7.70 g/kg DM and 3.70–5.30 g/kg DM, respectively. The relations between their contents and the growth phase were insignificant. The total amino acid content decreased with the plant age. High contents of aspartic (15.232–28.682 g/kg DM) and glutamic acids (16.694–35.526 g/kg DM), as well as minimal contents of sulphur amino acids, especially methionin (2.586–5.03 g per kg DM), could be noted. The highest catalase activity was found in the early growth phase (18.5–35.1 TSU), being higher in all samples grown at the location Kroměříž. The yield of juice pressed out from frozen green matter amounted to 68%. The pressed out juice was preserved by fluid drying, freeze drying, and freezing. In respect to folates and total polyphenols contents and the antioxidant activity, freezing appears the most suitable procedure for preserving.

Keywords: barley grass; nutrition; preservation; antioxidants; vitamins

The relation between nutrition and health has been unequivocally established. Wrong eating habits and losses of nutritional factors during technological processing, storage, and culinary treatments can lead to the diet deficient in some nutritional factors.

Supported by the Czech Science Foundation, Project No. 525/05/0781.

One of the possibilities how to improve the balance of the nutrient intake in a natural way is the diet enriched by the so-called "green foods", i.e. fresh or delicately preserved foods of plant origin.

Generally speaking, young plant parts are characterised by increased contents of some vitamins, provitamins, antioxidants, and other bioactive substances. Barley grass contains significant quantities of calcium, copper, iron, magnesium, potassium, zinc, β-carotene, folate, pantothenic acid, vitamins B_1, B_2, B_6, C, and E, superoxide dismutase, catalase, and chlorophyll. However, the nutrient contents of all barley varieties depend on where the plants are grown, the soil quality, the average rainfall, and the harvest technique (Droushiotis 1984). It is known that the highest concentrations of nutrients are present for just a few critical days.

Barley grass is promoted as a source of antioxidants, the most important being *O*-glycosyl isovitexin, superoxide dismutase (SOD), catalase (CAT), vitamin E, vitamin C, and carotenoids (Bamforth 1983; Aruoma & Halliwell 1987; Kitta *et al*. 1992; Osawa *et al*. 1992; Nakajima *et al*. 1998; Acar *et al*. 2001; Janda *et al*. 2003; Lee *et al*. 2003).

Food supplements based on green plant parts have their tradition especially in East Asian countries. In the USA, the food supplement Green Barley is on the market. This is basically pressed out and dried juice from barley grass grown under strictly controlled conditions. The researches conducted in the USA and Japan proved that an extract from young barley leaves helps to suppress a number of health disorders including obesity, diabetes, circulatory disorders, arthritis, anaemia, excessive cholesterol levels, renal difficulties, and cancer (Nishiyama *et al*. 1994; Shibamoto *et al*. 1994). An extraordinary feature of the products derived from young barley is their well-established ability to degrade organophosphate pesticides (Durham *et al*. 1999).

The aim of our work was to evaluate the contents of the selected nutritional parameters in barley grass grown under the soil and climatic conditions of the Czech Republic. The retention of nutrients was followed during three possible ways of processing – freezing, freeze drying, and fluid drying.

MATERIAL AND METHODS

Material. In the course of the year 2005, the experiments were started and conducted at Kroměříž (KR) and Žabčice (ŽB) with two malting varieties of hulled barley, i.e. Sebastian and Malz, and with a hulless variety of spring food barley KM1910, under restricted chemical inputs (basic fertilisation – autumn 2004), winter wheat having been the previous crop. Analytical determinations of the selected nutrients were carried out in three samplings of barley green matter taken in defined growth phases, as described by the decimal code (DC) scale: sampling I at growth phase DC 29, sampling II at phase DC 31, and sampling III at phase DC 32 to 33 (Zadoks 1974).

According to the character of the individual substances determined, the samples were analysed either as soon as possible after the harvest (vitamin C), or after having been frozen (e.g. saccharides, phenolic compounds, total polyphenols, amino acids).

Because of their specific structure, the samples for the analytical determinations were homogenised in two steps, starting with Ultimate Chopper (WS Teleshop International) followed with the mixer A11 (IKA). To test various ways of preserving juice from barley green matter, the variety Malz in sampling III from the location Žabčice was chosen. For practical reasons, all the products obtained by three different ways of preservation were made from previously frozen raw materials. Juice from barley green matter was made using the extractor Green Power (Woorideul Industrial Co. Ltd.) and it was further processed by the laboratory freeze dryer Lyovac GT2 (FINN-AQUA) and the laboratory fluid dryer TG1 (Retsch).

Methods. Dry matter was determined by a gravimetric method (Davídek *et al*. 1981). The protein content was determined by the Kjehldal method (Davídek *et al*. 1981). Ascorbic acid was determined by titration with 2,6-dichlorphenolindophenol (ČSN ISO 6557/2 Method A). A potentiometric indication of the equivalence point was used.

The enzyme activity of superoxid dismutase was assessed using the kit Ransod. This kit, made by the British company Randox, is intended for the analysis of SOD activity in blood samples. The necessary modifications of the respective phases of the procedure, especially the sample preparation, to suit the plant material were applied (Belcredová *et al*. 2006).

To assess the activity of catalase, a spectrophotometric method based on the measurement of the drop of absorbance at 240 nm was used (Bergmeyer 1970).

Total polyphenols were determined by a spectrophotometric method (Lachman *et al*. 1997). Phenolic compounds were quantified by a RP HPLC method (Orsák *et al*. 2000).

Total amino acids were determined after acid hydrolysis. Cysteine was oxidised to cysteic acid before hydrolysis. Ion exchange chromatography with postcolumn derivatisation using ninhydrine was applied for the sample separation and detection (Moore & Stein 1954; Spackman *et al*. 1958).

A HPLC method with a refractometric detector was used for monosaccharide analysis. Fructose, glucose, saccharose, and galactose were separated on a HPLC system under the following conditions: Hema-Bio 1000 Q (30 × 3 mm, 10 µm) and Hema-Bio 1000 SB (30 × 3 mm, 10 µm) guard columns, an Ostion LGKS 0800 Ca form (250 × 8 mm) column; column temperature 80°C; mobile phase demineralised water, flow rate of 0.3 ml/min.

Folates were assayed as 5-methyltetrahydrofolate (main representative of natural folates in plant materials). The determination of 5-methyltetrahydrofolate (5-MTHF) was performed by a RP HPLC method after thermal and enzymatic hydrolysis and purification of the samples using SPE (Holasová *et al*. 2004). The procedure cited was modified in releasing folates from food matrix. α-Amylase in addition to conjugase from hog kidney, and incubation at 37°C for 3 h was used.

The total antioxidant status was determined using the Randox kit (Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom) (Miller *et al*. 1993).

Three preservation procedures were chosen for the treatment of the pressed out barley juice, expected to keep the maximum of the original contents of the nutritionally valuable substances. The variety Malz in sampling III grown at the location Žabčice was chosen for the juice production.

One portion of barley juice was dried using the laboratory fluid dryer Retsch. Another portion was frozen at –24°C and subsequently freeze dried, and the remaining portion was frozen in 0.5 litre PET bottles at –18°C. In the case of fluid drying, guar flour was used as a vehicle. Ten parts of juice were mixed with one part of guar flour and the resulting gel was pressed through a sieve (5 mm mesh) to obtain granules. The drying was conducted in a laboratory fluid dryer under a very low temperature regime (at 30°C for 8 h), when the juice granules about 20 mm long were kept floating in an air stream. Dried granules were then ground to obtain a light green, partially water soluble powder.

RESULTS AND DISCUSSION

Young green parts of barley plants, as a potential source of nutritionally valuable substances, were analysed for the contents of vitamin C, total polyphenols, phenolic compounds, amino acids, and saccharides, and for the activity of catalase.

Vitamin C is very unstable in the non-acid barley plant environment. Due to the activity of enzymes, vitamin C was rapidly oxidised by mere plant crumpling and wilting. The results obtained (Figure 1) indicate that the content of vitamin C fluctuated

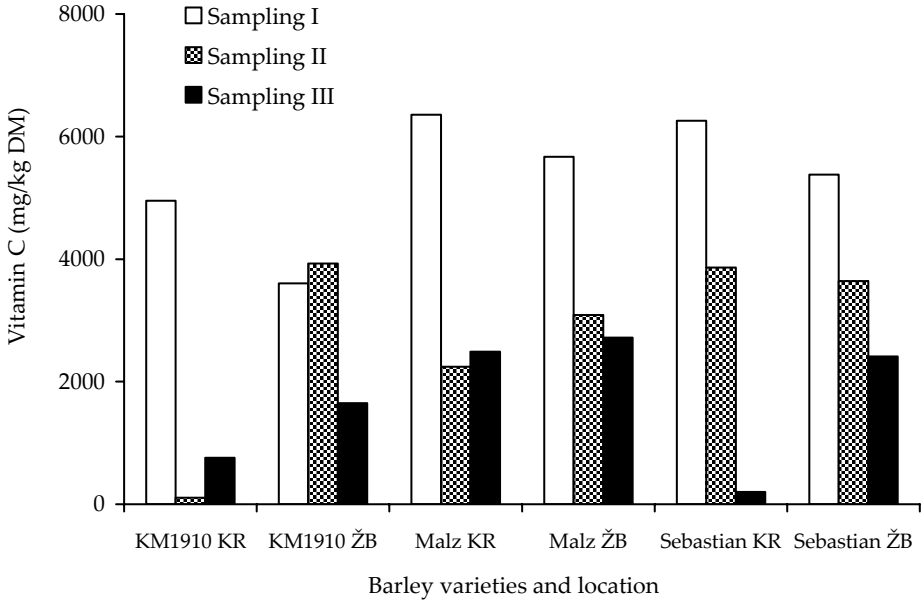

Figure 1. Total contents of vitamin C in barley varieties grown at the locations Kroměříž (KR) and Žabčice (ŽB)

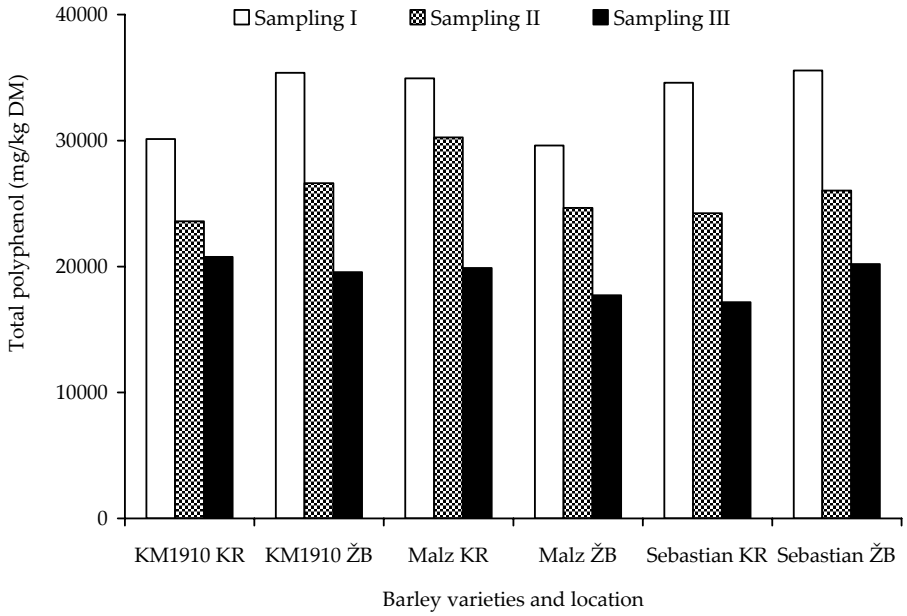

Figure 2. Total polyphenol contents in barley varieties grown at the locations Kroměříž (KR) and Žabčice (ŽB)

between 0.107 to 6.357 g/kg DM and decreased with progressing growth. Higher contents of this factor were found in the varieties Malz and Sebastian coming from both locations. No relation was found between the vitamin C content and the locality. Raw material crumpling or wilting can be a possible explanation for the dramatic drop of the vitamin C content during the growth period of barley KM1910 from Kroměříž, and in sampling III of the variety Sebastian from Kroměříž.

It is obvious from the evaluation of total polyphenols in the barley grass samples from both Kroměříž and Žabčice (Figure 2) that the total polyphenols contents decreased with the plant age. The influence of location was not proved unequivocally.

Out of the phenolic compounds analysed (catechine, epicatechine, caffeic, chlorogenic and ferulic acids) only the presence of ferulic acid was found (Figure 3). The results showed that it was most abundant in sampling I of all varieties tested, the highest values having been found in the variety KM1910 from both locations (4.962–5.916 g/kg DM). The content of ferulic acid decreased with the plant age. The influence of location was not established unequivocally. The presence of other phenolic compounds followed was not analytically proved.

The assays of simple saccharides, namely saccharose, glucose, galactose and fructose, showed low contents of saccharose and galactose (0 to 7.7 and 3.7 to 5.3 g/kg DM, respectively). In sam-

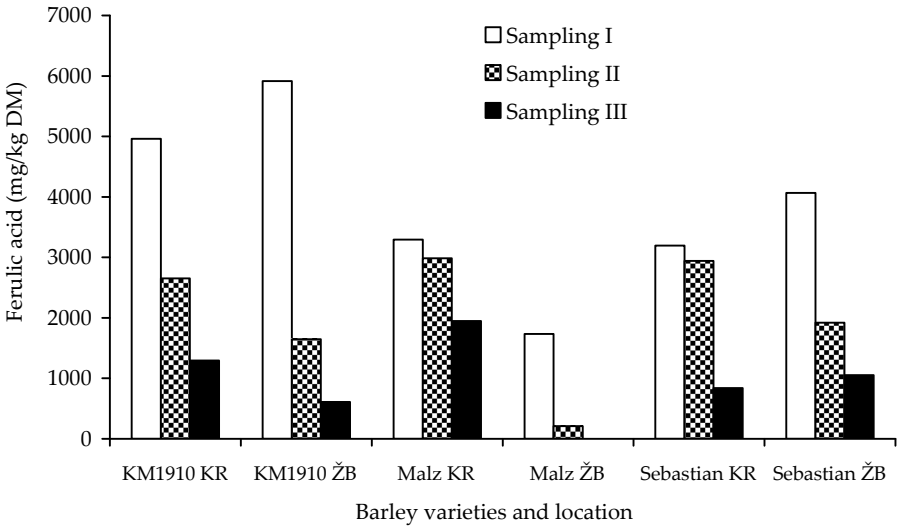

Figure 3. Ferulic acid contents in barley varieties grown at the locations Kroměříž (KR) and Žabčice (ŽB)

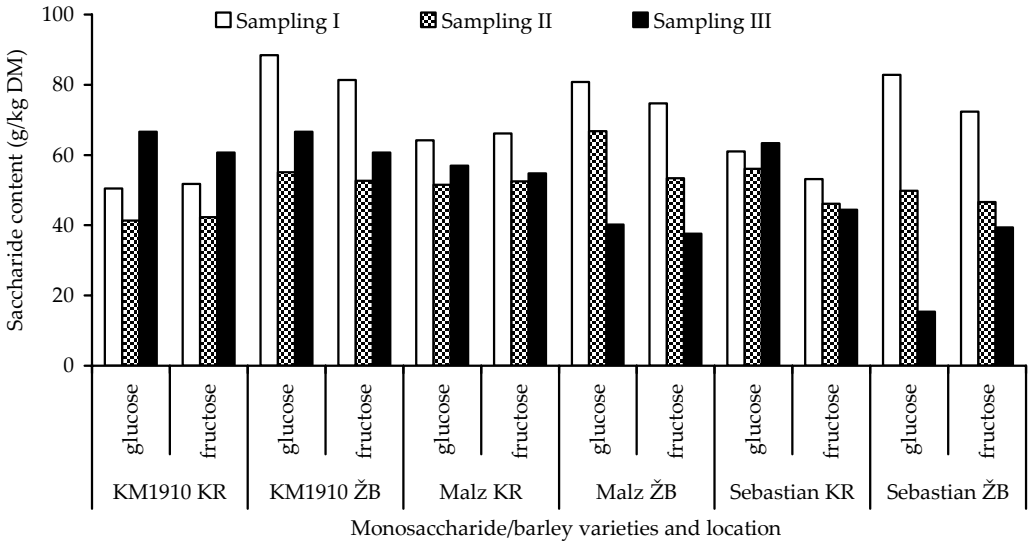

Figure 4. Monosaccharide contents in the respective growth phases of barley varieties grown at the locations Kroměříž (KR) and Žabčice (ŽB)

pling I, all varieties grown at the location Žabčice were found to have higher contents of glucose and fructose than those grown at the location Kroměříž (Figure 4). During the subsequent growth phases, both saccharides decreased markedly in the varieties from Žabčice. The varieties from the location Kroměříž did not show such changes and the contents of both saccharides fluctuated only in the range of 1 to 2% (w/w). In sampling III, the contents of glucose and fructose were higher in the samples obtained from the location Kroměříž than in those from the location Žabčice.

Total amino acids declined with the plant development; the highest content was observed in sampling I, which corresponds to the protein content in dry matter. An example of a typical amino acid spectrum is shown in Figure 5. Sampling I of the variety Malz contained 30.44 g, sampling II 23.19 g

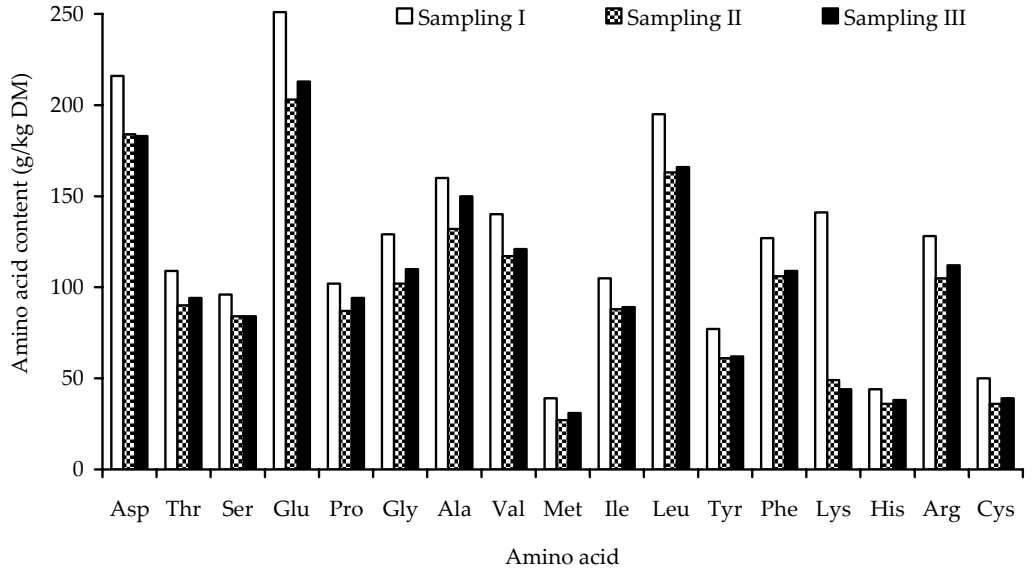

Figure 5. Total contents of amino acids in the respective growth phases of barley variety Malz grown at the location Kroměříž

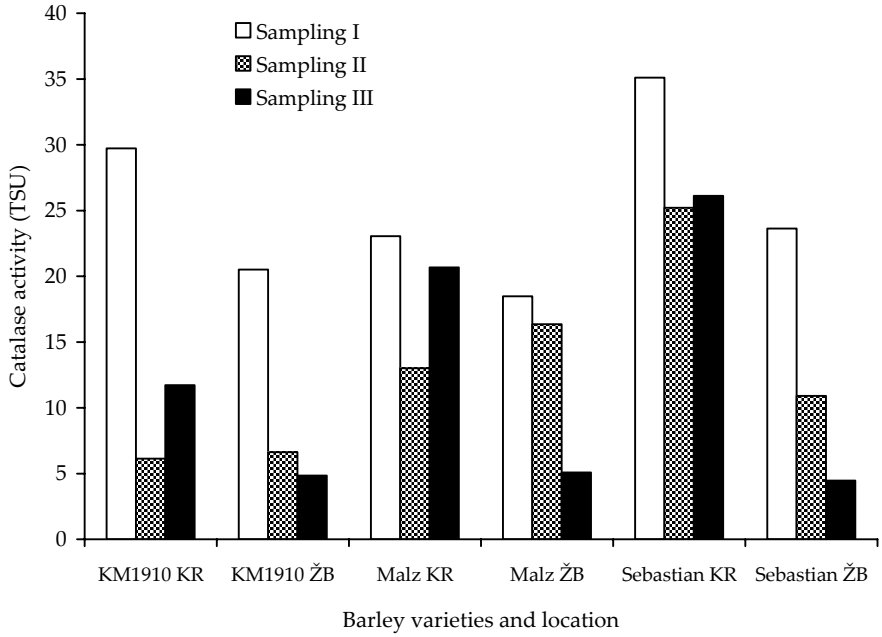

Figure 6. Catalase activity in barley varieties grown at the locations Kroměříž (KR) and Žabčice (ŽB)

and sampling III 18.81 g of protein/100 g DM, respectively. In general, high contents of aspartic and glutamic acids could be noted. Higher contents of leucine, alanine, valine, arginine and also phenylalanine were also demonstrated. The content of sulphur amino acids, notably methionine, was at a minimum. The content of histidine was also low.

The contents of the respective amino acids did not show any significant fluctuations depending on the barley variety or growth site.

The highest barley catalase activity was measured in sampling I. The location was a factor influencing significantly the catalase activity; a higher activity was found in the samples from the location Kroměříž. The samples from this location were found to have a higher catalase activity in sampling III than in sampling II, in contrast to the varieties grown at Žabčice.

For the utilisation of barley grass as a raw material for food supplements, the retention of health beneficial factors during processing is substantional. Among others, the fluid drying, freezing, and freeze drying of pressed out juice might be used. The yield of juice by pressing out was high, repeatedly amounting to 68%. The juice obtained was dark green, with a typical flavour, containing 8.2% of dry matter. Pressed out juice was preserved by fluid drying, freezing, and freeze drying.

The preserved products were then analysed for total polyphenols, ferulic acid, folates, and for the antioxidant and enzyme activities (Table 1). The lowest values of the analytes were found in the fluid dried product. The losses were much higher than related to dilution with guar flour. They might be attributed to the sample exposition to elevated temperature and oxygen for a relatively long period. Freezing appears to be the most delicate preservation procedure from the viewpoint of the antioxidant factors and the folate content. Freeze drying caused a decrease in the contents of polyphenols, folates, and antioxidant activity by approximately 30% in comparison with the freezing procedure.

Table 1. Contents of dry matter, total polyphenols, ferulic acid, and folates, and antioxidant and enzyme SOD activities

Way of preservation	Dry matter (g/100 g)	Total polyphenols (mg/kg sample)	Ferulic acid (mg/kg sample)	5-MTHF (µg/kg sample)	Antioxidant activity (mmol/kg sample)	SOD activity (U/g)
Freezing (–18°C)	8.2	2804	163	134	344	60
Fluid drying (30°C)	90.9	15 793	481	272	1677	200
Freeze drying	91.4	22 567	1717	1082	2320	800

CONCLUSION

The results of this study provide information that can help in the utilisation of barley grass as a unique and fully natural source of valuable nutritional substances. The assays of these substances characterise young barley plants grown in 2005 at two locations and harvested in three growth phases. The analysis of the contents of vitamin C, total polyphenols, ferulic acid, monosaccharides, amino acids, and the determination of the activity of catalase have yielded data indicating that this is a valuable plant material which is worth becoming an object of continued, more detailed studies. The results also indicate that the contents of nutritional substances are strongly dependent on the growth phase; the barley variety and the growth site appear to be less important.

The high yield of juice achieved by pressing out suggests that juice utilisation should be preferred to processing whole barley plants in the food supplement production. Out of the preservation procedures tested, freezing appears the most suitable for preserving selected antioxidant factors and folates.

References

Acar O., Turkan I., Ozdemir F. (2001): Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties. Acta Physiologiae Plantarum, **23**: 351–356.

Aruoma O.I., Halliwell B. (1987): Superoxide dependent and ascorbate dependent formation of hydroxy radical from hydrogen peroxide in the presence of iron. Are lactoferrin and transferrin promoters of hydroxyl-radical generation? Biochemical Journal, **241**: 273–278.

Bamforth C.W. (1983): Superoxide dismutase in barley. Journal of Institute of Brewering, **89**: 420–423.

Belcrediová N., Ehrenbergerová J., Havlová P. (2006): Enzym superoxid dismutasa v zrnu ječmene a sladu. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, LIV, No. 2: 7–14.

Bergmeyer H.V. (1970): Methoden der Enzymatischen Analyse. Verlag Chemie GmbH, Weinheim: 273–282.

Davídek J., Janíček G., Pokorný J. (1981): Laboratorní příručka analýzy potravin. SNTL-ALFA, Praha: 117–185.

Droushiotis D. (1984): The effect of variety and harvesting stage on forage production of barley in low rainfall environments. Journal of Agricultural Science, **102**: 287–289.

Durham J., Ogata J., Nakajima S., Hagiwara Y., Shibamoto T. (1999): Degradation of organophosporous pesticides in aqueous extracts of young green barley leaves (*Hordeum vulgare* L.). Journal of the Science of Food and Agriculture, **79**: 1311–1314.

Holasova M., Fiedlerova V., Roubal P., Pechacova M. (2004): Biosynthesis of folates by lactic acid bacteria and propionibacteria in fermented milk. Czech Journal of Food Sciences, **22**: 175–181.

Janda T., Szalai G., Rios-Gonzales K., Veisz O., Pldi E. (2003): Comparative study of frost tolerance and antioxidant activity in cereals. Plant Science, **164**: 301–306.

Kitta K., Hagiwara Y., Shibamoto T. (1992): Antioxidative activity of an isoflavonoid 2''-*O*-glycosylisovitexin isolated from barley leaves. Journal of Agricultural and Food Chemistry, **40**: 1843–1845.

Lachman J., Hosnedl V., Pivec V. (1997): Changes in the content of polyphenols in barley grains and pea seed after controlled accelerated ageing treatment. Scientia Agriculturae Bohemica, **28**: 17–30.

Lee S.H., Jew S.S., Chang P.S., Hong I.J., Hwang E.S., Kim K.S., Kim K.T., Sung H.L. (2003): Free radical scavenging effect and antioxidant activities of barley leaves. Food Science and Biotechnology, **12**: 268–273.

Miller N.J., Rice-Evan S.C., Davies M.J., Gopinathan V., Milner A. (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science, **84**: 407–412.

Moore S., Stein W.H. (1954): Procedures for the chromatographic determination of amino acids on four per cent cross-linked sulfonated polystyrene resins. Journal of Biological Chemistry, **211**: 893–906.

Nakajima S., Hagiwara Y., Hagiwara H., Shibamoto T. (1998): Effect of the antioxidant 2''-*O*-glycosylisovitexin from young green barley leaves on acetaldehyde formation in beer stored at 50°C for 90 days. Journal of Agricultural and Food Chemistry, **46**: 1529–1531.

Nishiyama T., Hagiwara Y., Hagiwara H., Shibamoto T. (1994): Inhibitory effect of 2''-*O*-glycosyl isovitexin and α-tocopherol on genotoxic glyoxal formation in a lipid peroxidation system. Food and Chemical Toxicology, **32**: 1047–1051.

Orsák M., Lachman J., Pivec V. (2000): Effect of UV-A and gamma-irradiation on the polyphenol levels in barley and pea seeds, seedlings and plants. Scientia Agriculturae Bohemica, **31**: 181–196.

Osawa T., Katsuzaki H., Hagiwara Y., Shibamoto T. (1992): A novel antioxidant isolated from young

green barley leaves. Journal of Agricultural and Food Chemistry, **40**: 1135–1138.

Shibamoto T., Hagiwara Y., Hagiwara H., Osawa J. (1994): A flavonoid with strong antioxidative activity isolated from young green barley leaves. American Chemical Society, Washington, ACS Symp., Ser. 547: 153–163.

Spackman D.H., Stein W.H., Moore S. (1958): Automatic recording apparatus for use in the chromatography of amino acids. Analytical Biochemistry, **30**: 1190–1206.

Zadoks J.C., Chang T.T., Konzak G.F. (1974): A decimal code for growth stages of cereals. Weed Research, **14**: 415–421.

Received for publication July 13, 2006
Accepted after corrections October 27, 2006

Corresponding author:

Ing. Ivana Paulíčková, Výzkumný ústav potravinářský Praha, Radiová 7, 102 31 Praha 10, Česká republika
tel.: + 420 296 792 368, fax: + 420 272 701 983, e-mail: i.paulickova@vupp.cz