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INFLUENCE OF ABIOTIC STRESS AND TECHNOLOGICAL TREATMENT ON CHEMICAL COMPOSITION OF CEREAL GRAINS AND LEGUME SEEDS

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Contents of total and damaged starch were observed, together with lipid and protein contents, during the evaluation of abiotic stresses influence (dry conditions, high temperature, low pH value) on the yield and seed of wheat. Contents of total and damaged starch were not changed. Seed growing in filial generation (2nd year) from stressed seeds becomes evident decrease of lipids (0.71 times) and increase of damaged starch 1.31 times. Changes of the total starch and proteins contents were minor. The starch assay procedures were used for the evaluation of the influence of microwave treatment on chemical and physicochemical characteristics of rice. Microwave treatment did not affect the total content of starch in rice, the but the content of damaged starch increased with absorbed microwave energy and temperature of treatment, chiefly for the moisture of 30% and a temperature of 100°C. Changes of damaged starch were in accordance with amylographic characteristics of rice flour. Changes in the contents of total starch, proteins and α-galactooligosaccharides and trypsin inhibitors activity were also monitored during microwave treatment of germinated pea seeds. The contents of total starch, lipids and proteins were without significant changes, whereas the content of α-galactooligosaccharides decreased up to 42% of the value before treatment and trypsin inhibitor activity decreased up to 56-60%. These changes are favourable from the nutritional point of view.

INTRODUCTION

Changes in the chemical composition of cereals and grain legume seeds are important either for the evaluation of seeds quality from agronomical and breeding points of view and for the evaluation of seeds and cereal or legume products for food and feed applications as well. Starch and soluble carbohydrates are apart from proteins, lipids and minerals the main subject of interest. Enzyme kits Megazyme (Megazyme International Ireland Ltd.) for determination of total starch, starch damage and α-galactooligosaccharides represent very suitable way for this purpose. Total Starch Assay and Starch Damage Assay Kits can serve in bread production, because the knowledge of both the starch content and the water absorption of the flour are essential. The major variable affecting water absorption is the level of damaged starch granules which are produced during the milling of the grain. Properties of starch are also important in determining the end-use application. Raffinose/ /Stachyose/Verbascose Assay Kit was developed for simple recognition of non-digestible α-galactooligosaccharides (raffinose family oligosaccharides, RFO) which are the major anti-nutritional factors in legume seeds [Megazyme, 1999].

The use of these kits for the observation of the starch and α-galactooligosaccharides contents in cereals and grain legume seeds brings out the examples of three following investigations: (1) Abiotic stresses influence on the chemical composition of wheat seeds. (2) Influence of microwave (MW) treatment on the chemical and physicochemical characteristics of rice. (3) Changes in the contents of starch and α-galactooligosaccharides during germination and microwave treatment of pea seeds.

Stress abiotic factors (drought, high temperature, low level of nutrients, low pH, and others) affect the seed quality, seed morphological, physiological and biochemical traits, activity of enzymes, performance of progeny generations, *i.e.* water uptake, plant development, yield formation and especially basic root traits of sprouting plants: length, surface, weight, nutrient uptake, number of root tips, number of and lateral roots and root density. From the agronomic point of view, the influence of seed provenance has an impact on the seed traits, chemical composition of seeds, traits of sprouting plants and development of plant in next generation. There is with large probability a significant influence of gibberelin and abscisic acid content on the development of sprouting plants.

By using microwave treatment of rice, it is possible to gain the following effects: to improve physical and chemical characteristics; to optimize conditions of cooking (save energy and cleanup time); to keep the nutritional and sensory properties; to substitute steaming and conventional drying of rice during parboiling processing by microwave drying; to optimize processing of puffed rice products. The general attractions of microwave energy include: high rates of heating, absence of surface changes to the food and to finish partly dried foods or low-moisture foods. Microwave ovens are widely used for cooking and food processing. In some applications, the duration of MW exposure has no pronounced influence on the sensory evaluation [Wadsworth & Koltun, 1986; Doos *et al*., 1993]. Many studies showed equal or better retention of some vitamins (B_1, B_2, B_6, C and folic acid) after MW heating compared with conventional heating [Cross & Fung, 1982; Hoffman & Zabik, 1985]. However, conversion of vitamin

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B_{12} to inactive vitamin B_{12} degradation products occurs in foods during MW heating [Watanabe *et al*., 1998]. On the other hand, uneven product heating and local temperature differences in heated material are the main disadvantage of microwave application.

α-Galactooligosaccharides – raffinose, verbascose, stachyose (RFO), are the major components in many grain legumes, and their antinutritional activity is frequently associated with the presence of these oligosaccharides. RFO are not hydrolyzed in the upper gut due to the absence of α-galactosidase. In the lower intestine they are metabolized by bacterial action, producing methane, hydrogen and carbon dioxide, which leads to flatulence and diarrhea. RFO are thus a factor limiting the use of grain legumes in monogastric diets. There are many ways how to decrease the content of RFO in grain legumes. One of the most effective processes for the reduction of RFO in grain legumes is germination whereby RFO can be converted to digestible sugars by α-galactosidase treatment.

MATERIALS AND METHODS

Plant material. The 4 measured winter wheat cultivars (Samanta, Estica, Ebi, Šárka) from three different localities (different soil and climatic conditions) were used. The influence of abiotic stresses in pot greenhouse experiments (15 seeds per pot, ten pots for every cultivar, five repetition) using a mixture of soil (50% of soil, 50% of sand): temperature/night 20°C, temperature/day 35°C, water/soil capacity 40%, pH 4.5 were compared with standard pot and field experimental conditions.

Samples of rice (*Oryza sativa* var. *indica*), white, and short-grains were used.

Samples of pea (*Pisum sativum* ssp. *sativum* L.) - cultivars Komet, Lantra, year of harvest 2000, were germinated in aerated water media. Seeds were incubated in 9 aeration bottles, 80 g of seeds and 200 mL of water in each bottle, water was changed after 24 h, temperature was 20°C and time of germination reached 72 h.

Total starch determination. The content of total starch was determined by means of Megazyme assays procedure [Megazyme, 1999]. Total starch (TS) analysis procedure proceeds in two phases. In phase I, starch is partially hydrolyzed and totally solubilized by cooking the sample in the presence of thermostable α-amylase. In phase II, the starch dextrins are quantitatively hydrolyzed to glucose by amyloglucosidase [AOAC Method 996.11, AACC Method 76.11]. For most samples (*e.g.* wheat flour), complete solubilisation of starch can be achieved by cooking the sample in the presence of thermostable α-amylase. However, for samples containing high levels of resistant starch (*e.g.* high amylose maize starch), complete solubilisation and dextrinisation requires pre-treatment with dimethyl sulphoxide at 100°C. The samples containing high levels of glucose and maltodextrins are washed with aqueous ethanol before analysis. An analysis of a single sample can be performed within 70 min. Twenty samples can be analysed within 2 h.

Damaged starch determination. The content of damaged starch was determined by means of Megazyme assays procedure [Megazyme, 1999]. Damaged starch granules (DS) are hydrated and hydrolyzed to maltosaccharides plus α-limit dextrins by carefully controlled treatment with purified fungal α-amylase. The fungal α-amylase treatment is designed to give near complete solubilization of damaged granules with minimum breakdown of undamaged granules. This reaction is terminated on addition of dilute sulphuric acid, and aliquots are treated with excess levels of purified amyloglucosidase to give complete degradation of starch-derived dextrins to glucose. The glucose is specifically measured with a high purity glucose oxidase/peroxidase reagent mixture. The values determined are presented as damaged starch as a percentage of flour weight on an "as is" basis [ICC Method 164].

α-Galactooligosaccharides (RFO) determination. The content of α-galactooligosaccharides (for short RFO, according Raffinose Family Oligosaccharides) was determined by means of Megazyme assays procedure [Megazyme, 1999]. α-Galactooligosaccharides are hydrolyzed to galactose, glucose and fructose using α-galactosidase and invertase. The glucose is then determined using glucose oxidase/peroxidase reagent. The method does not distinguish between raffinose, stachyose and verbascose, but rather measures these as a group. Since one mole of each of the RFO contains one mole of glucose, the concentrations are presented on a molar basis. Free glucose and sucrose in sample extracts are determined concurrently.

Statistical evaluation of all enzyme methods (TS, DS, RFO) was carried out for 6 repeated analyses of the same sample of pea seeds (Table 1).

TABLE 1. Statistical evaluation of enzymatic methods – analysis of pea seeds.

	RFO	TS	DS
Mean value (% d.s.)	1.149	43.86	1.00
Standard deviation	0.048121	0.30042	0.20343
Variation	0.002316	0.091832	0.000414

Trypsin inhibitor activity (TIA) determination. Glycine buffer pH 11 containing urea and EDTA was used for the extraction of proteins. The sample was extracted, stirred for 2 h, and then centrifuged. Synthetic substrate BAPNA (N-α-benzoyl-DL-arginin-p-nitroanilide) was hydrolysed by trypsin and the absorbance of dislodged yellow p-nitroanilide was measured at 410 nm [Gatta *et al.,* 1988].

Lipids and proteins determination. The content of lipids was determined by Soxhlet method on a Soxtherm Apparatus (Gerhardt). The content of proteins was calculated from the nitrogen content determined by the Kjeldahl's method on a Kjeltec Apparatus (Tecator).

Parameters of microwave oven. A domestic MW oven Whirlpool MT 243/UKM 347 (Norrköpping, Sweden), with a frequency of 2450 MHz, pulsed variable MW rated power output from 90 to 1000 W by a timer, inner cavity volume of 25.4 L, without sample rotation during measurement, was used in the study. The MW oven was preheated before each measurement by heating 2 L of water for 5 min. The absorbed power according to BSEN 60705 test [International Standard BSEN 60705, 1995] was determined every day as well. Load of water for this test was 350 +/- 5 g and initial water temperature reached 10 +/- 2°C. Mean value of absorbed power (n = 22) corresponding to the rated power output 350 W was 298.42 W; standard deviation was 8.5 and variation coefficient (relative standard deviation) accounted for 2.85% [Skulinová *et al*., 2002].

Microwave treatment of rice. Power output was changed from 90 to 160, 350 and 500 W; final temperature of heated rice was 60, 80, 100°C; and initial moisture content of rice was 12, 23 and 30%. The following tests were used to evaluate the influence of MW treatment on the chemical and physicochemical characteristics of rice: damaged and total starch content, water absorption at 70°C [Hogan & Planck, 1958] and amylographic gelatinization and pasting characteristics [Anon., 1982].

Microwave treatment of germinated pea seeds. Germinated pea seeds were treated in microwave oven at power output 350 W up to the final temperature of 80°C. Besides of total starch, damage starch and RFO contents, the contents of lipids, proteins and trypsin inhibitors activity (TIA) were determined [Urbánková, 2003].

RESULTS AND DISCUSSION

Abiotic stresses influence on the chemical composition of wheat seed

The average values of chemical composition of 4 measured winter wheat cultivars (Samanta, Estica, Ebi, Šárka) are presented in Table 2. The changes in the chemical composition of wheat seeds under stress conditions were not large in starch content but in the content of lipids (increase 1.27 times) and protein (decrease 0.83 times). In seed growing in filial generation (2nd year) from stressed seeds, a decrease in lipid content (0.71 times) and an increase in DS content (1.31 times) became evident. Changes in the contents of TS and proteins were minor [Bláha *et al.,* 2002]. Short germination had no influence on the ratio of starch, damage starch, protein and lipid content. However a decrease in weight of seeds could be observed as a result of respiration.

On the basis of an analysis of energy accumulation, it can be stated that the combination of abiotic stresses leads to a conclusive decrease in energy accumulation into the generative organ of wheat, which results in worse germination of seed stock (namely, a decrease of vigour) and decreased viability of sprouting plants. The great influence of localities on the technological quality of seeds also exists.

TABLE 2. Changes in the chemical composition of wheat seeds.

Provenance	TS (% d.s.)	DS (% d.s.)	Proteins (% d.s.)	Lipids (% d.s.)
Field test	59.14	1.52	14.80	1.08
Germinated	60.05	7.29	15.23	1.00
Stress conditions	59.39	1.54	12.23	1.37
2nd year	61.83	2.02	12.40	0.98

The influence of MW treatment on chemical characteristics of rice

MW treatment did not affect the total content of starch in rice (Figure 1), but the content of damaged starch increased with absorbed microwave energy and temperature of treatment, chiefly for the moisture of 30% and a temperature of 100°C (Figure 2). Amylographic characteristics and water absorption indicated minimal changes resulting from MW treatment of rice for moisture lower than 23%. The rise of water absorption is connected with the degree of starch gelatinization. The obvious decrease in amylograph height was determined with increasing temperature of MW treatment and increasing power output for the moisture of 30%. A lower decrease was observed for the moisture of 23% and minimal changes for dry samples with the moisture of 11%. These results are in agreement with the study of Halick and Kelly [1959], where the amylographic viscosity curves were essentially identical for the control and the MW-treated dry samples of rice and MW-treatments did not materially affect neither the cooking nor processing quality of the milled rice. The variabilities in water absorption among rice lots with similar cooking and processing

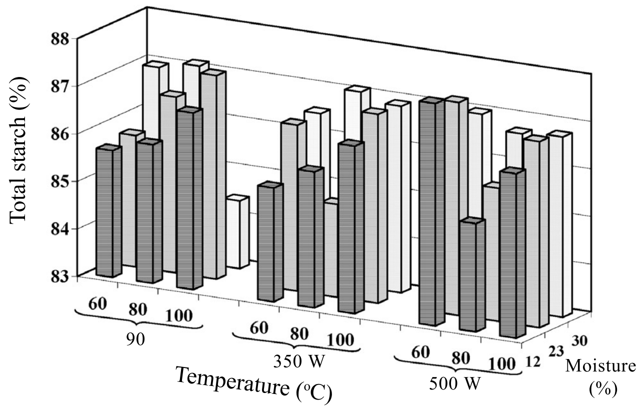

FIGURE 1. Changes in the total starch content during microwave treatment of rice.

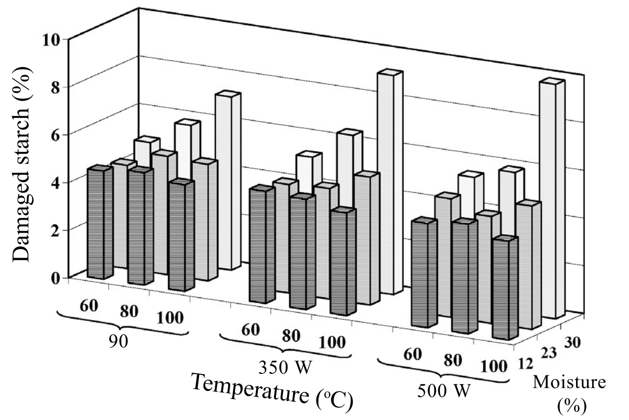

FIGURE 2. Changes in the damaged starch content during microwave treatment of rice.

TABLE 3. Changes in the chemical composition of germinated pea seeds during microwave treatment.

Sample	Lipids (% d.s.)	Proteins (% d.s.)	TS (% d.s.)	DS (% d.s.)	RFO (mmol/100g)	TIA (mg)
Komet Germinated	1.58	23.46	45.00	0.87	6.71	4.38
MW-treated	1.82	25.86	43.05	4.51	2.82	2.45
Lantra Germinated	1.49	24.28	44.23	0.94	7.26	5.62
MW-treated	1.90	27.04	43.67	6.02	3.13	3.39

characteristics were much greater than the observed changes caused by the MW-treatment. In addition, according to sensory evaluation it is not possible to consistently detect a difference in flavour or texture resulting from MW drying [Kaasová *et al*., 2000].

Changes during microwave treatment of germinated pea seeds

The average values of chemical composition of 2 measured pea cultivars are in Table 3. The row in the table with the name of cultivar (Komet, Lantra) indicates the value of seeds before treatment and the second row for each cultivar indicates values after germination and MW-treatment. Relative changes in total starch were minimal, the contents of proteins and lipids were higher by about 10–28% for treated seeds. The highest changes were reported for damaged starch, the increase was 5.2–6.4-fold for treated seeds as a consequence of microwave treatment. The positive influence of germination and MW-treatment from the nutritional point of view was observed on the changes in RFO content and TIA. The RFO content decreased up to 42% of the value before treatment, the value of trypsin inhibitor activity decreased up to 56-60%. These results confirm the hypothesis of RFO decline during germination of legumes and they are in accordance with the results of studies by Górecki *et al*. [1997] and Muzquiz *et al*. [1992], and also with our previous paper [Kadlec *et al.,* 2000]. As to the minimal changes in proteins, these are in agreement with Alonso *et al*. [1998], who reported a 3.5% increase in proteins after 48 h of pea germination.

CONCLUSIONS

1. The changes in the chemical composition of wheat seeds under stress conditions were not large in total starch content but were considerbale in the contents of lipids (a 1.27--fold increase) and protein (a 0.83-fold decrease). In seed growing in filial generation (2nd year) from stressed seeds, a decrease in lipids (0.71 times) and an increase in damaged starch (1.31 times) became evident.
2. MW treatment did not affect the total content of starch in rice, but the content of damaged starch increased with absorbed microwave energy and temperature of treatment, chiefly for the moisture of 30% and a temperature of 100°C. The results of damage starch determination are in accordance with amylographic readings of changes in the maximum viscosity. Amylographic characteristics indicates minimal changes resulting from MW-treatment of rice for the moisture lower than 23%.
3. The similar changes were observed during microwave treatment of germinated pea seeds: minimal changes in total starch, lipids and proteins contents, but relatively high increase (5.2-6.4 times) of damage starch. The positive influence of germination and MW treatment from the nutritional point of view was observed in changes of the RFO content and TIA. The RFO content decreased up to 42% of the value before treatment, and the value of trypsin inhibitor activity decreased up to 56-60%.

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