Formation of Acrylamide During Baking of Shortcrust Cookies Derived from Various Flours

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Abstract

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Asparagine and reducing sugars are the principal acrylamide precursors in foods. Their main sources in pastries are flour and hen egg yolks. The concentrations of asparagine and carbohydrates vary with flour and depend on multiple factors like environmental conditions during the cultivation of cereals and post-harvest processing methods. An objective of this study was finding the interplay between amino acid and carbohydrate profiles of the selected flours and their blends and acrylamide concentrations in the cookies derived from them. Shortcrust cookies were prepared from five different flours such as wheat Poznań flour and flours from spelt-wheat, rice, chickpea, and *Amaranth* seeds. The rice, chickpea, and amaranth flours were mixed with the wheat Poznań flour in the proportions of 1:1 (w/w), 1:1 (w/w), and 1:3 (w/w), respectively. The cookies were baked at a temperature of 180°C for 10 minutes. It was found that the cookies obtained from the blend of wheat and chickpea flours (1:1, w/w) contained much less acrylamide (5.7 μg/kg) compared to those derived from the wheat Poznań flour only (41.9 μg/kg). The concentrations of reducing sugars and sucrose in the mixture of wheat and chickpea flours were relatively low compared to wheat flour alone. Consequently, the decrease in the concentrations of carbohydrates, which are acrylamide precursors, was the smallest.

Keywords: acrylamide; asparagine; reducing sugars; shortcrust cookies; flours

Pastries, including cookies, are willingly consumed foods because of their smashing taste and attractive appearance. Baking, which determines the specific flavour and taste of pastries, is one of the principal technological operations during their manufacture. Pastries are baked at temperatures above 120°C. This factor and chemical composition of the cake batter ingredients are responsible for the formation of anti-nutritive compounds like acrylamide and its derivatives during the baking processes.

A growing concern about acrylamide was triggered by a publication of Swedish researchers who reported on high concentrations of this compound in numerous foods containing amino acids and reducing sugars, and its apparent negative impact on human health (Mustafa *et al*. 2008). Thermal processing of foods gives rise to a plethora of transformations generating high molecular weight polymers and copolymers known as melanoidins as well as low molecular weight compounds like furfural, acrolein and acrylamide. Acrylamide is formed through Maillard reactions which generate substances responsible for the taste and aroma of browned products (Chico & galdo 2006; Szczerbina *et al*. 2008).

Acrylamide is toxic to humans and its intake can contribute to the development of many diseases. This compound was classified in 1994 by the International Agency for Research on Cancer (IARC) as

"potentially carcinogenic to humans". Acrylamide displays neurotoxic and genotoxic (contributes to cell damage at genetic level) activities, negatively affects fertility, and induces cancers in animals (Atay *et al*. 2005).

Humans and animals absorb acrylamide through skin, lungs, and gastrointestinal tract. Only the monomer is toxic while polyacrylamide (polymerisation product) is not harmful to humans and animals. Investigations on model animals revealed that acrylamide added to food was virtually completely absorbed from the gastrointestinal tract while only 25% of the dose was absorbed through the skin. The conversion of the absorbed acrylamide to its more reactive epoxy derivative (glycidamide) is catalysed by cytochrome P450. The resulting metabolites are partially secreted with urine (Claunig 2008, EFSA'11 Scientific Colloquium 2008).

The mechanisms of acrylamide action in animals have not been explained in detail. However, acrylamide and glycidamide are believed to form adducts with hemoglobin and DNA, thereby leading to the synthesis of truncated proteins and cell function disorder. Besides, they can cause gene mutations and damage to chromosomes (Lopachin 2004).

The results of epidemiology studies have not provided unambiguous evidence that the intake of acrylamide with diet increases the risk of cancer development in humans. Toxicology investigations on model animals revealed that its single dose resulting in death of 50% tested animals (rats, rabbits, Guinea pigs) ranged between 150 mg/kg and 180 mg/kg. When the animals were administered with smaller acrylamide doses (2–3 mg/kg body weight per day), the results of its toxic impact were not observed for a few months. This study demonstrated that the presence of acrylamide in a diet of rats considerably increased the frequency of cancers of central nerve system, thyroid gland, nipple, and scrotum.

The neurotoxic effect of acrylamide on humans has also been proven. A prolonged exposition to acrylamide was found to cause damage to central and circumferential nerve systems in humans and animals (Taeymans *et al*. 2004).

Because of the aforementioned reasons, acrylamide contents in foods have to be minimised. This goal can be achieved through decreasing pH of batters with organic acids (mainly citric acid), supplementing them with calcium or magnesium ions, lowering the temperature of baking (Biedermann & Grob 2003; Surdyk *et al*. 2003; Graf *et al*. 2006), or treating dough with the enzyme asparaginase (Vass *et al*. 2004). However, this technological intervention should be preceded by a careful selection of the batter ingredients.

Shortcrust cookies are made from flour, sucrose, hen egg yolks, and fat. Cereal flours are rich in free amino acids including asparagine contributing along with reducing sugars to acrylamide formation. The amounts of free amino acids depend on the type of flour. According to Fredriksson *et al*. (2004), asparagine concentrations in wholegrain wheat and rye flours are 0.5 g/kg and 1.1 g/kg, respectively.

In potatoes, the ratio of reducing sugars to free amino acids contents is relatively low compared to cereal products, and therefore the formation of acrylamide in potato-based products is thought to be limited by the sugars (Zyzak *et al*. 2003). The concentration of free asparagine in wheat flour varies between 74 mg/kg and 664 mg/kg (Hamlet *et al*. 2008) while in rye flour it ranges between 319 mg/kg and 791 mg/kg (Springer *et al*. 2003; Noti *et al*. 2003).

Many factors like the botanical origin, environmental conditions during cultivation, year of harvest, fertilisers, and conditions of grain storage and processing affect the levels of free amino acids in flours. Claus *et al*. (2006) who investigated the effects of climatic conditions during wheat and rye cultivation on asparagine contents in grains harvested in 2003 and 2004, observed that their amounts were much lower in flours from the grains harvested in 2004 compared to 2003 harvests. The discrepancy in asparagine and protein contents between these flours was ascribed to different climatic conditions. According to the authors, in 2003 the insolation level was higher and precipitations were fewer than in 2004.

Fertilisers rate among the key factors affecting the quantity and quality of harvests. According to Claus *et al*. (2006) and Muttucumaru *et al*. (2006), the levels of free amino acids and reducing sugars in cereal grains and flours are also affected by fertilisers. Nitrogen fertilisers increased the amounts of free asparagine in wheat grains but had no impact on reducing sugar content (Lerner *et al*. 2006). Also Zahedi *et al*. (2004) reported that the concentrations of reducing sugars in wheat grains were not affected by nitrogen fertilisers. Sulphur fertilisers decreased the level of asparagine in wheat grains.

Apart from the environmental conditions, also grain processing methods affect the level of free amino acids in flours (Claus *et al*. 2006). According to Surdyk *et al*. (2004), the concentration of asparagine in flour depends on the extraction rate which also affects the ash content.

The objective of this study was finding the interplay between amino acid and carbohydrate profiles in the selected flours and their blends, and acrylamide concentrations in the cookies obtained from these materials.

The flours which were used in this study had different chemical compositions and physical properties. Flours from amaranth seeds, chickpea and rice are deprived of gluten and can be used to produce foods for people with coeliac disease.

The proteins from amaranth seeds are characterized by an attractive amino acid composition because they are among others rich in lysine (its content is double that in wheat flour) which is essential for children and teenagers. Also, sulphur-containing amino acids (methionine, cysteine) are abundant in this flour. Only the level of leucine is lower in amaranth flour than in the flours from wheat, barley, and maize. Because of the high content of iron, the flour from amaranth seeds can be an excellent component of foods for people suffering from anemia (Majewska *et al*. 2007). Also the lipids contained in amaranth seeds possess a high nutritive value. One of the valuable lipid substances which are dissolved in amaranth oil is squalene (concentration in the oil of 6–6.3%) which is a triterpene hydrocarbon with chemical formula $C_{30}H_{50}$. Its basic element is isoprene unit. Squalene is a strong antioxidant because it contains 6 double bonds with *trans* configuration. It stimulates human immune system through a positive impact on lymph glands, adrenal glands, and bone marrow. It supports the function of lymphocytes and macrophages. Squalene protects skin from harmful effects of acids and bases and promotes excretion of all types of non-polar xenobiotics (they dissolve in squalene and are excreted together) (Kłoczko 2008).

The seeds of spelt-wheat contain more proteins, lipids, unsaturated fatty acids, and dietary fiber than wheat grains. They are also slightly less caloric than the latter. The biological value and digestibility of spelt-wheat proteins, which contain 20–60% more essential amino acids, e.g. lysine, threonine, leucine, and isoleucine, are superior to wheat proteins. Spelt-wheat flour contains high-quality gluten.

Spelt-wheat lipids are rich in unsaturated fatty acids including linoleic acid with two double bonds and monounsaturated oleic acid which are beneficial for the heart and circulatory system. Spelt-wheat flour contains more macro- and microelements (magnesium, calcium, potassium, phosphorus, iron, zinc, copper, manganese) and lipid-soluble vitamins (A, D, E) than wheat flour (Haase *et al*. 2003; Czerwińska 2009).

The advantages of rice flour, which has been increasingly used in Polish cuisine, are low contents of sodium and potassium, good thickening properties, and high stability during freezing/thawing processes (Nikolić *et al*. 2008). Rice flour can replace wheat flour in the diet of people who suffer from gluten intolerance (Rutkowski 2008).

Chickpea has gained increasing acceptance among vegetarians. Chickpea flour obtained through grinding dried chickpea (Italian pea) was found to be a rich source of protein, mineral salts (calcium, magnesium, iron, zinc, copper, potassium), thiamine, niacin, folic acid, riboflavin, and vitamin C. Although the dough produced from this flour does not grow, the baked products have an excellent taste (Kaur *et al*. 2005).

MATERIAL AND METHODS

Chemicals. The standards of amino acids (Asn – aspartic acid, Thr – threonine, Ser – serine, Glu – glutamic acid, Pro – proline, Gly – glycine, Ala – alanine, Cys – cysteine, Val – valine, Met – methionine, Ile – isoleucine, Leu – leucine, Tyr – tyrosine, Phe – phenylalanine, His – histidine, Lys – lysine, Arg – arginine) were purchased from Sigma-Aldrich (St. Louis, USA). The standards of sugars (glucose, fructose, maltose, ribose, sucrose) were supplied by Fluka (St. Louis, USA). Acrylamide (99%) was procured from Merck (Darmstadt, Germany), and 2,3,3-d_3-acrylamide (> 98%) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, USA). Acetonitrile (99.9%) was purchased from Sigma-Aldrich (St. Louis, USA). All other reagents were of analytical grade and were supplied by POCH (Gliwice, Poland).

Ingredients of shortcrust cookies. Five flour types were used in the experiments: wheat Poznań flour type 500, spelt-wheat flour type 630, rice flour chickpea flour, and flour from *Amaranth* seeds.

Flours from rice, chickpea, and *Amaranth* seeds were blended with wheat Poznań flour type 500

in the proportions of: 1:1, 1:1, and 1:3 (w/w), respectively.

The other batter ingredients were: fat (margarine Kasia), class A fresh hen egg yolks (from the local market), white refined sucrose, and baking powder containing disodium pyrophosphate, sodium hydrocarbonate, and wheat flour.

Batter formulation and baking conditions. The cake batter formulation was as follows: flour (149 g), margarine (99 g), sugar (99 g), hen egg yolks (49 g), and baking powder (2 g). The cookies were baked in Ariston C 3 VP6 electric oven (Ariston, Fabriano, Italy) at 180°C for 10 minutes.

Analyses of flours, doughs, and baked cookies. *Ash content* was quantified in flours according to PN-ISO 2171:1994 (ICC- Standard No. 104/1, 1990). *Solid substance content* in flours, doughs and baked cookies was determined according to PN-91/A-74010. *Acidity* was determined according to the standard PN-ISO 7305:2001. The acidity of doughs and baked cookies was estimated by titration of their water extracts with NaOH solution using phenolphtalein as pH marker (Krełowska-Kułas 1993). *Wet gluten content* was estimated according to PN-EN ISO 21415:2007. *Gluten number* (GN) was calculated using the equation:

$$LG = a \times (2 - 0.065 \times R)$$

where:
a – wet gluten content in flour (%)
R – flowness of gluten (mm)

Falling number of the tested flours was determined by Hagberg-Perten method according to PN-EN ISO 3093:2010. *pH* of doughs and baked cookies was measured by using pH-meter ULAB 2002. The samples (10 g) of doughs and ground baked cookies were suspended in 100 ml of distilled water, filtered, and pH was measured in the resulting filtrates. *Fat content* in flours and their blends was assayed by Soxhlet method according to Krełowska-Kułas (1993). *Protein content* in flours was determined by Kjeldahl method according to the standard PN-75/A-04018. Nitrogen content was multiplied by the coefficient of 5.7.

Free amino acids and the sum of aspartic acid and asparagine contents

Sample preparation. Ground and de-fatted 50 g samples were extracted 3 times for 5 min with boiling 80% ethanol using a reflux condenser (for the first time with 200 ml and then twice with 100 ml). The resulting extracts were pooled and centrifuged at 8000 rpm for 15 minutes. The precipitate was discarded and the supernatant was kept overnight in a refrigerator and then filtered through blotting paper. The extracted amino acids were purified by ion-exchange chromatography on a strong cationite (Dowex 50) column (16 ml) previously regenerated with 2N HCl. The amino acid solution (100 ml) was applied onto the column at a flow rate of approximately 5 ml/minutes. Plant pigments, sugars, and organic and inorganic cations were eluted with 300 ml of distilled water. Amino acids were eluted with 100 ml of 2N NH_4OH followed by 100 ml of 4N NH_4OH at a flow rate of 5 ml/min. The eluate was evaporated to dryness under vacuum at 40°C and the amino acids were dissolved in 10% isopropanol.

Quantification of amino acids. The separation and quantification of amino acids was carried out using AAA-400 amino acid analyser (INGOS, Prague, Czech Republic). The separated amino acids were identified based on their retention times and their comparison with those of amino acid standards. The amounts of amino acids in the tested samples were determined based on the surface areas under their peaks and relevant standard curves.

Carbohydrate contents. Carbohydrates contained in the tested flours, their blends and cookies were quantified according to Claus *et al*. (2006).

Sample preparation. The ground and de-fatted samples (15 g of cookies, 10 g of flour) were mixed in 250 ml Erlenmayer flasks with 100 ml of 60% boiling ethanol, sonicated for 20 min at 70°C (in a water bath) and then centrifuged for 20 min at 10°C and 8000 rpm (to separate the precipitated proteins and starch). The resulting supernatants were kept overnight in a refrigerator, filtered through filter paper, and evaporated under vacuum. The remaining solid residue was dissolved in 5 ml of 70% acetonitrile, centrifuged, and analysed by HPLC.

HPLC analysis. Sugars were quantified by using Dionex (Chelmsford, USA) HPLC system equipped with a pump (P680), an autosampler with 50 µl loop (ASI-100), Corona CAD detector (Esa Inc., Chelmsford, USA), and data recording program (Chromeleon version 6.70). Sugars were separated at 35°C on amino column – SUPELCOSIL LC-NH_2 (250 mm × 4 mm) equipped with a precolumn

SUPELCOSIL LC-NH_2 SUPELGUARD KIT (20 mm × 4 mm). The injected sample volume was 20 µl. Sugars were eluted with a gradient of acetonitrile (B) in distilled water (A) according to the following program: –2 min to 0 min, flow rate: 1.0 ml/min, 85% B (column reconditioning); 0–2 min, flow rate: 1.0 ml/min, 85% B; 2.1–20.1 min, flow rate: 0.8 ml/min, 80% B; 20.1–25 min, flow rate: 1.0 ml/min, 50% B.

Starch content. Starch content in flours, their blends, and baked cookies was determined by polarimetric method of Ewers according to the standard PN-EN ISO 10520:2002.

Acrylamide content. Acrylamide was quantified in the baked cookies by GC-MS/MS after derivatisation according to Mojska (2008), Soares and Fernnandes (2009), and Alves *et al*. (2010).

Sample preparation – The ground samples (2 g) were placed in 50 ml glass Erlenmayer flasks with glass stoppers and supplemented with 100 µl of standard 2,3,3-d_3-acrylamide solution (100 µg/ml). Then 20 ml of distilled water was added to each sample. Acrylamide was extracted for 30 min at ~ 60°C in a shaken water bath (257 rpm). The samples were cooled to the room temperature and centrifuged (20 min, 6000 rpm, 4°C). The precipitate was discarded and the supernatant was de-fatted with hexane (20 ml) through shaking for 5 min (257 rpm). The hexane layer was discarded. This operation was repeated twice with decreasing hexane volumes. Then the de-fatted samples were mixed with 1 ml of Carrez I and II reagents and centrifuged for 20 min (6000 rpm, 4°C). The resulting supernatants were subjected to SPE on BAKERBOND SPE Oktadecyl (C18) column (6 ml, 500 mg).

This extraction was carried out as follows:

(1) Column conditioning – C18 column was washed with 5 ml of methanol and with 2 portions (5 ml) of distilled water at a low flow rate to avoid column dehydration.
(2) The application of 3 ml sample onto the preconditioned column during 5 min (without any vacuum pump).
(3) Acrylamide elution with 2 portions (4 ml) of distilled water

The water extracts were cooled and derivatised with 2.5 g KBr, 0.1 ml HBr (pH 1–3) and 2.5 ml of bromine water. Bromination reaction was carried out for 1 h in the darkness in an ice-water bath (~ 0°C). When it was complete, excess Br_2 was degraded with a few drops of 1M $Na_2S_2O_3$ (until the yellow colour disappeared). Next, the samples were supplemented with ~ 4 g NaCl and extracted with 2 portions (4 ml) of ethyl acetate (with shaking at 257 rpm for 5 min). The organic phases were pooled (the 2,3-dibromo acrylamide derivative is soluble in ethyl acetate), dried over anhydrous sodium sulphate (~1 g of Na_2SO_4), and centrifuged (20 min, 6000 rpm, 4°C). An aliquot of the organic phase (0.5 ml) was evaporated to dryness at 40°C under vacuum and the solids were dissolved in ethyl acetate. The ultimate volume of each sample in dark glass 2 ml vials was ~1 ml.

GC-MS/MS analysis – The 2,3-dibromo derivative of acrylamide was quantified by GC by using Varian 450-GC gas chromatograph equipped with an ion trap mass detector (Varian 220-MS) and a split/splitless injector. The analytical separation was performed on a Varian Factor Four VF-5ms capillary column (0.25 µm film thickness, 30 m, 0.25 mm *i.d.*) (Varian, Lake Forest, USA). The samples (1 µl) were analysed at ionisation energy (EI) of 70 eV. In the first step, the precursor ions with *m/z* of 152 and 155 were derived from 2,3-dibromo acrylamide derivative and 2,3-dibromo derivative of deuterated acrylamide, respectively. Their collisions gave rise to daughter ions with *m/z* of 135 (from the ions with *m/z* of 152) and 137 (from the ions with *m/z* of 155). The calculation of acrylamide concentration in the tested samples was based on the ratio of the surface areas under the peaks corresponding to the ions with *m/z* of 135 and 137 *m/z*.

Gas chromatography conditions:

– temperature was increased from 65°C (at a rate of 15°C/min) to 240°C (23 min),
– temperature of injector 250°C,
– carrier gas: helium, flow rate of 40 ml/s,
– parameters of mass spectrometry: ionisation energy 70 eV; temperature of ion source 180°C; temperature of transfer line 250°C.

Procedure – Quantification was performed by the internal standard method. The calibration curve was constructed by plotting the ratio *A*aa/*A*is against *C*aa/*C*is where *A*aa is the area of unlabelled acrylamide as mass trace *m/z 135* and *A*is is the area of deuterin labelled acrylamide as mass trace *m*/*z 137. C*aa/*C*is means the concentration ratio between acrylamide and 2,3,3-d_3-acrylamide. The calibration curve was prepared in the range of 2.5–500 µg/l. The correlation coefficients were usually higher than 0.998.

The limits of detection (LOD) and limits of quantification (LOQ) of the method were calculated using the calibration curve parameters. In this case, the detection limit was 5 µg/kg and the limit of quantification was set at 15 µg/kg. To determine the reproducibility of this method (characterised by the reproducibility standard deviation), each acrylamide assay was executed in five replicates.

The recoveries were determined by adding 50 µg/l of the acrylamide standard solution to the sample. Average recoveries ranging from 73% to 89% were obtained.

Statistical analysis. All the assays were carried out in triplicate for each sort of flour, dough, and shortcrust cookies and the results were averaged. One-way ANOVA was carried out to find if the differences between the results were statistically significant.

RESULTS AND DISCUSSION

This study aimed at the determination of the interplay between flour type and its amino acid and carbohydrate profiles, and the effect of the latter on acrylamide contents in shortcrust cookies obtained from these flours or their blends.

Physicochemical characteristics of tested flours and their blends

The cookies were made from: wheat Poznań flour type 500, spelt-wheat flour type 630, and flours from rice, chickpea, and *Amaranthus* seeds. The last three flours were mixed with the wheat Poznań flour type 500 in the proportions of 1:1, 1:1, and 1:3 (w/w), respectively. The flours and their blends were tested for: humidity, acidity, total ash, fat, protein, wet gluten, gluten number, and falling number.

Humidity was the highest in the spelt-wheat flour samples (13.71%) and the lowest in the samples of blended wheat and chickpea flours (1:1, w/w) (8.59%) (Table 1). Water content in flours depends on the humidity of grains, milling method, and storage conditions. It is to note that all the tested flours and their mixtures were characterised by humidity below the upper permitted level of 15%. The moisture level in flours determines their baking quality and stability during storage. When it is too high, the quality of flours gradually worsens because of the enzyme catalysed processes and proliferation of contaminating microorganisms.

The acidity and total ash content in the tested flours and their blends were: 3°N and 0.55% for wheat flour, 4.06°N and 0.602% for spelt-wheat flour, 4.87°N and 0.832% for the blend of wheat and rice flours, 9.3°N and 2.14% for the blend of wheat and chickpea flours and 14.2°N and 1.38% for the blend of wheat and amaranth flours, respectively (Table 1).

Protein content in the tested flours and their blends varied between 10.84% and 18.90% solid substance (s.s). It was the highest in the blend of wheat and chickpea flours (1:1, w/w) (18.90% s.s.) (Table 1). Spelt-wheat flour was the richest in gluten (56.60% s.s.) while the mixture of wheat and rice flours (1:1, w/w) contained approximately 5-fold

Table 1. Physicochemical characteristics of tested flours and their blends used to bake shortcrust cookies

	Wheat flour	Spelt-wheat flour	Blend of wheat and		
			rice flours 1:1 (w/w)	chickpea flours 1:1 (w/w)	amaranthus flours 3:1 (w/w)
Humidity (%)	9.57 ± 0.05	13.71 ± 0.04	9.48 ± 0.05	8.59 ± 0.03	9.42 ± 0.03
Acidity (°N)	3.00 ± 0.01	4.06 ± 0.02	4.87 ± 0.01	9.30 ± 0.02	14.02 ± 0.01
Total ash (% s.s.)	0.55 ± 0.05	0.60 ± 0.05	0.83 ± 0.03	2.14 ± 0.11	1.38 ± 0.04
Protein (% s.s.)	13.13 ± 0.01	15.54 ± 0.02	10.84 ± 0.02	18.90 ± 0.01	13.71 ± 0.01
Fat (% s.s.)	1.65 ± 0.02	2.08 ± 0.06	2.37 ± 0.05	4.14 ± 0.05	3.21 ± 0.04
Wet gluten (% s.s.)	43.40 ± 0.01	56.60 ± 0.02	11.00 ± 0.02	–	25.70 ± 0.03
Gluten number (GN)	59.86 ± 0.01	63.71 ± 0.02	17.27 ± 0.03	–	39.02 ± 0.02
Falling number (s)	179.00 ± 5.00	233.00 ± 4.03	252.00 ± 5.09	76.00 ± 3.03	67.00 ± 3.01

s.s. – solid substance content; s – second

less of this substance (11% s.s.). Gluten was not quantified in the blend of wheat and chickpea (1:1, w/w) flours (Table 1). The quantity and quality of gluten that decide on the baking quality of flours are described by the gluten number which characterises the quality and strength of flour as well as its technological quality. Based on the gluten number, the spelt-wheat flour was classified as a strong flour, the wheat flour was classified as a medium quality flour, and blends of wheat flour with either rice or amaranth flours were classified as weak ones (Table 1). The medium class flour can be used to produce the majority of small size baked goods while weak flours are applicable to the production of wafers and biscuits.

Effect of the flour type on the sum of aspartic acid and asparagine contents

The concentrations of free amino acids and the sum of aspartic acid and asparagine were determined in the tested flours and their blends. Asparagine is one of the principal acrylamide precursors apart from carbohydrates and, therefore, the determination of the effect of baking on its concentration was very important. Asparagine is easily converted to aspartic acid and therefore only the sum of the original aspartic acid and aspartic acid derived from asparagine can be quantified (Table 2).

Quantitative analysis of amino acid composition of the flours used to produce the cookies revealed that the sum of aspartic acid and asparagine was flour-dependent. This result is consistent with the finding of Claus *et al.* (2006) who reported that the levels of free asparagine increased with the ash content in the analysed flours. Our tests revealed the highest value of the sum of aspartic acid and asparagine in the mixture of wheat and chickpea flours (1:1, w/w) (417.98 μg/100 g s.s.), and the lowest one in the blend of wheat and rice flours (171.16 μg/100 g s.s.). These results are much lower compared to the data reported by Hamlet *et al.* (2008) (74–664 mg/kg) (Table 2, Figure 1). The discrepancies between the amounts of asparagine, which were determined as the sum of aspartic acid and asparagine in the tested flours, and the results reported by other authors might be a consequence of different conditions during the crop cultivation (Muttucumaru *et al.* 2006) and grain processing (Surdyk 2004).

Effect of flour type on its amino acid profile

The results compiled in Table 2 show that the most abundant amino acids in the tested flours and their blends were aspartic and glutamic acids, alanine, phenylalanine, tyrosine, and valine. The blends of wheat and amaranth flours (3:1, w/w)

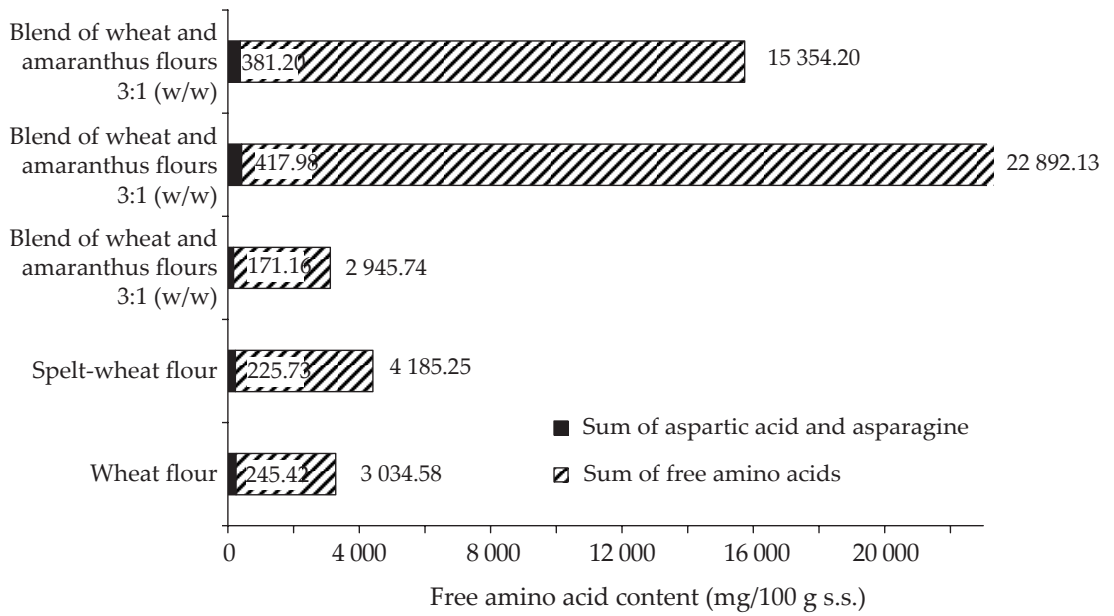


Figure 1. Contents of free amino acids in tested flours and their blends (the remaining amino acids are a sum of Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, and Arg)

Table 2. Free amino acids (μg/100 g s.s) and a sum of aspartic acid and asparagine contained in selected cake ingredients and baked shorcrust cookies

Amino acid	Wheat flour			Spelt-wheat flour			Blend of wheat and rice flours 1:1 (w/w)			Blend of wheat and chickpea flours 1:1 (w/w)			Blend of wheat and amaranthus flours 3:1 (w/w)		
	flour	hen egg yolk	shortcrust cookies	flour	hen egg yolk	shortcrust cookies	flour	hen egg yolk	shortcrust cookies	flour	hen egg yolk	shortcrust cookies	flour	hen egg yolk	shortcrust cookies
Aspartic acid*	245.42 ± 0.02	332.80 ± 0.01	146.82 ± 0.02	225.73 ± 0.01	332.80 ± 0.01	110.16 ± 0.01	171.16 ± 0.02	332.80 ± 0.01	42.14 ± 0.02	417.98 ± 0.01	332.80 ± 0.01	280.29 ± 0.04	381.20 ± 0.02	332.80 ± 0.01	261.90 ± 0.05
Threonine	99.22 ± 0.02	689.43 ± 0.02	195.97 ± 0.02	117.91 ± 0.01	689.43 ± 0.02	310.31 ± 0.01	100.53 ± 0.02	689.43 ± 0.02	169.21 ± 0.02	163.66 ± 0.01	689.43 ± 0.02	415.15 ± 0.03	231.56 ± 0.02	689.43 ± 0.02	284.12 ± 0.02
Serine	144.52 ± 0.03	674.97 ± 0.02	218.86 ± 0.01	188.02 ± 0.02	674.97 ± 0.02	345.96 ± 0.03	107.23 ± 0.01	674.97 ± 0.02	200.54 ± 0.01	138.37 ± 0.04	674.97 ± 0.02	292.93 ± 0.05	361.09 ± 0.03	674.97 ± 0.02	321.24 ± 0.02
Glutamic acid	423.60 ± 0.02	1003.20 ± 0.03	379.55 ± 0.03	333.52 ± 0.04	1003.20 ± 0.03	620.58 ± 0.02	460.51 ± 0.01	1003.20 ± 0.03	421.58 ± 0.02	2585.74 ± 0.04	1003.20 ± 0.03	2655.11 ± 0.02	1361.24 ± 0.03	1003.20 ± 0.03	819.38 ± 0.02
Proline	44.91 ± 0,01	492.05 ± 0.01	310.37 ± 0.02	228.91 ± 0.02	492.05 ± 0.01	310.77 ± 0.02	160.58 ± 0.03	492.05 ± 0.01	243.82 ± 0.02	1328.33 ± 0.02	492.05 ± 0.01	1437.77 ± 0.02	196.98 ± 0.03	492.05 ± 0.01	338.71 ± 0.04
Glycine	136.97 ± 0.01	210.13 ± 0.01	109.31 ± 0.01	236.40 ± 0.02	210.13 ± 0.01	187.85 ± 0.02	131.72 ± 0.02	210.13 ± 0.01	94.26 ± 0.01	177.20 ± 0.02	210.13 ± 0.01	232.08 ± 0.06	202.40 ± 0.03	210.13 ± 0.01	142.05 ± 0.02
Alanine	410.43 ± 0.03	364.41 ± 0.02	251.73 ± 0.01	639.38 ± 0.02	364.41 ± 0.02	415.29 ± 0.02	498.13 ± 0.02	364.41 ± 0.02	241.79 ± 0.01	314.91 ± 0.02	364.41 ± 0.02	371.20 ± 0.04	677.26 ± 0.03	364.41 ± 0.02	332.57 ± 0.03
Cysteine	22.86 ± 0.01	31.25 ± 0.01	14.48 ± 0.01	17.64 ± 0.01	31.25 ± 0.01	23.82 ± 0.01	16.50 ± 0.00	31.25 ± 0.01	13.08 ± 0.00	11.30 ± 0.05	31.25 ± 0.01	–	24.55 ± 0.02	31.25 ± 0.01	14.95 ± 0.01
Valine	228.81 ± 0.02	579.76 ± 0.02	210.68 ± 0.02	313.09 ± 0.01	579.76 ± 0.02	345.20 ± 0.02	188.83 ± 0.02	579.76 ± 0.02	196.80 ± 0.04	2689.99 ± 0.04	579.76 ± 0.02	503.35 ± 0.04	574.72 ± 0.01	579.76 ± 0.02	322.48 ± 0.01
Methionine	11.25 ± 0.01	222.89 ± 0.02	13.83 ± 0.01	21.71 ± 0.01	222.89 ± 0.02	54.96 ± 0.01	5.62 ± 0.01	222.89 ± 0.02	9.64 ± 0.00	33.47 ± 0.05	222.89 ± 0.02	30.82 ± 0.03	98.73 ± 0.01	222.89 ± 0.02	41.24 ± 0.01
Isoleucine	138.61 ± 0.02	484.60 ± 0.03	165.02 ± 0.01	181.37 ± 0.02	484.60 ± 0.03	254.34 ± 0.04	105.88 ± 0.01	484.60 ± 0.03	144.68 ± 0.03	185.96 ± 0.01	484.60 ± 0.03	315.03 ± 0.03	313.77 ± 0.02	484.60 ± 0.03	238.01 ± 0.03
Leucine	180.63 ± 0.02	988.38 ± 0.03	276.53 ± 0.02	235.46 ± 0.02	988.38 ± 0.03	417.37 ± 0.03	119.91 ± 0.03	988.38 ± 0.03	225.28 ± 0.02	166.91 ± 0.03	988.38 ± 0.03	492.97 ± 0.02	283.74 ± 0.04	988.38 ± 0.03	385.37 ± 0.03
Tyrosine	265.53 ± 0.02	300.52 ± 0.03	311.82 ± 0.02	500.83 ± 0.02	300.52 ± 0.03	449.59 ± 0.02	199.06 ± 0.03	300.52 ± 0.03	265.42 ± 0.01	260.19 ± 0.02	300.52 ± 0.03	678.59 ± 0.02	965.61 ± 0.03	300.52 ± 0.03	553.70 ± 0.03
Phenylalanine	341.95 ± 0.02	541.09 ± 0.03	380.01 ± 0.02	649.04 ± 0.02	541.09 ± 0.03	605.51 ± 0.03	394.56 ± 0.02	541.09 ± 0.03	376.31 ± 0.02	330.49 ± 0.02	541.09 ± 0.03	768.77 ± 0.01	1108.52 ± 0.04	541.09 ± 0.03	671.62 ± 0.03
Histidine	69.09 ± 0.01	158.31 ± 0.02	58.45 ± 0.03	71.91 ± 0.01	158.31 ± 0.02	97.93 ± 0.01	61.04 ± 0.01	158.31 ± 0.02	46.08 ± 0.01	188.76 ± 0.02	158.31 ± 0.02	255.23 ± 0.01	336.49 ± 0.02	158.31 ± 0.02	153.96 ± 0.02
Lysine	79.94 ± 0.01	684.55 ± 0.01	85.62 ± 0.02	66.09 ± 0.03	684.55 ± 0.01	137.90 ± 0.01	65.39 ± 0.01	684.55 ± 0.01	62.46 ± 0.03	170.41 ± 0.04	684.55 ± 0.01	226.05 ± 0.01	165.45 ± 0.01	684.55 ± 0.01	134.86 ± 0.01
Arginine	190,78 ± 0.02	nd	214,53 ± 0.01	158,18 ± 0.02	nd	263,66 ± 0.02	13728,38 ± 0.02	nd	128,36 ± 0.01	159,03 ± 0.01	nd	8707,77 ± 0.02	8070,84 ± 0.01	nd	914,78 ± 0.03

nd – not detected; *sum aspartic acid with asparagine

and wheat and chickpea flours (1:1, w/w) were also rich in arginine. These results are consistent with the data published by Mustafa *et al.* (2007). Our analyses showed that the level of free amino acids was the highest in the mixture of wheat and chickpea flours (1:1, w/w) (22 892.13 μg/100 g s.s.) and the lowest in the blend of wheat and rice flours (1:1, w/w) (2945.74 μg/100 g s.s.) (Figure 1). These values were correlated with the ash and protein contents in these flour blends which equalled 2.14% and 18.90% s.s., respectively, for the first blend, and 0.83% and 10.84% s.s., respectively, for the second mixture (Table 1). The different levels of free amino acids in various flours can be a consequence of different activities of proteolytic enzymes which release amino acids from proteins and peptides (Claus *et al.* 2008).

Effect of flour type on its carbohydrate profile

Our analyses showed that the contents of starch, glucose, fructose, ribose, maltose, and sucrose were different in the tested flours and their mixtures (Table 3). The level of reducing sugars and sucrose was the highest in the blend of wheat and rice flours (1:1, w/w) (15.80 mg/g s.s.) while the blend of wheat and chickpea flours (1:1, w/w) contained the lowest amounts of these sugars (6.81 mg/g s.s.) (Table 3). Starch is known to be the main component of flours. Its content in the tested flours varied between 84.79 g/100 g s.s. (the highest value) for the spelt-wheat flour and 63.49 g/100 g s.s. (the lowest content) for the mixture of wheat and chickpea flours (1:1, w/w) (Figure 3).

Also sucrose is a natural component of flours. Its level was the highest in the mixture of wheat and rice flours (1:1, w/w) (7.28 mg/g s.s.) while in the blend of wheat and chickpea flours (1:1, w/w) it was less than half of it (3.39 mg/g s.s.) (Table 3). The amounts of sucrose in the other tested flours and their blends were almost the same (Figure 2). The concentrations of glucose, fructose, and maltose were the highest in the blend of wheat and rice flours (1:1, w/w) and the lowest in the mixture of wheat and chickpea flours (1:1, w/w). Besides, the spelt-wheat flour was free of ribose (Table 3).

Effect of the baking conditions on the changes in amino acid and carbohydrate composition profiles in shortcrust cookies

The amino acid and carbohydrate composition profiles in the baked shortcrust cookies were different from those in flours and their blends. This difference was caused by the reactions between free amino acids (mainly asparagine) and reducing sugars generating various compounds including acrylamide, as well as by partial hydrolysis of sucrose and proteins taking place during baking.

The analyses of amino acid composition profiles of the shortcrust cookies derived from the tested flours and their blends revealed that the amounts of these compounds were lower than their sum in the raw flour and hen egg yolks (Table 2). The largest decrease in free amino acid level was observed when the cookies were derived from blended wheat and amaranth flours (3:1, w/w) (by 61.8%) and the smallest – when the cookies were obtained from the blend of wheat and rice flours (1:1, w/w) (by 2.5%) (Table 4). However, when the cookies were obtained from pure wheat and spelt-wheat flours,

Table 3. Contents of reducing sugars and sucrose (mg/g s.s.) in tested flours, their blends and baked shortcrust cookies

	Wheat flour		Spelt-wheat flour		Blend of wheat and rice flours 1:1 (w/w)		Blend of wheat and chickpea flours 1:1 (w/w)		Blend of wheat and amaranthus flours 3:1 (w/w)	
	flour	cookies	flour	cookies	flour	cookies	flour	cookies	flour	cookies
Glucose	2.19 ± 0.02	1.20 ± 0.01	3.11 ± 0.02	2.15 ± 0.01	4.06 ± 0.02	1.25 ± 0.02	1.20 ± 0.01	0.94 ± 0.01	1.83 ± 0.02	0.83 ± 0.01
Fructose	1.03 ± 0.01	0.01 ± 0.02	1.23 ± 0.02	0.02 ± 0.02	1.07 ± 0.01	0.01 ± 0.02	0.93 ± 0.01	0.02 ± 0.02	0.98 ± 0.01	0.01 ± 0.00
Maltose	2.61 ± 0.02	–	2.29 ± 0.01	–	2.85 ± 0.01	–	0.48 ± 0.02	–	2.27 ± 0.01	–
Ribose	1.06 ± 0.01	0.16 ± 0.02	–	0.31 ± 0.02	0.53 ± 0.02	0.18 ± 0.01	0.81 ± 0.02	1.30 ± 0.02	1.26 ± 0.02	0.12 ± 0.01
Sucrose	5.15 ± 0.01	1.34 ± 0.01	5.78 ± 0.01	1.96 ± 0.02	7.28 ± 0.02	1.13 ± 0.01	3.39 ± 0.02	1.56 ± 0.02	4.53 ± 0.02	0.95 ± 0.01
Sum	12.04 ± 0.03	2.71 ± 0.02	12.41 ± 0.01	4.44 ± 0.01	15.79 ± 0.01	2.57 ± 0.02	6.81 ± 0.01	3.82 ± 0.02	10.87 ± 0.03	1.91 ± 0.02

Table 4. Decrease in a sum of aspartic acid and asparagine and total free amino acids caused by baking (in %)

	Wheat flour	Spelt-wheat flour	Blend of wheat and chickpea flours 1:1 (w/w)	Blend of wheat and rice flours 1:1 (w/w)	Blend of wheat and amaranthus flours 3:1 (w/w)
Aspartic acid*	74.6	80.3	*62.7*	91.6	63.3
Total free amino acids	9.6 (rise)	17.5 (rise)	25.4	2.5	61.8

*a sum of aspartic acid and asparagine; underlined – utmost value

the contents of free amino acids were increased by 9.6% and 17.5%, respectively (Table 4). This increase can be ascribed to the presence of free amino acids in hen egg yolk used to formulate the cookies. Irrespective of the type of flour, all resulting cookies contained less aspartic acid and asparagine compared to the batter ingredients. The decrease in the sum of aspartic acid and asparagine was the highest when the cookies were obtained from the mixture of wheat and rice flours (1:1, w/w) (by 91.6%) and the lowest – when the blend of wheat and chickpea flours (1:1, w/w) was used in the experiment (by 62.7%) (Table 4). This relatively small decrease correlated with the lowest acrylamide level in the latter cookies.

The analyses of the carbohydrate profiles in the tested cookies revealed the highest concentrations of glucose, fructose, and sucrose in the cookies derived from spelt-wheat flour, and the lowest – in the cookies containing amaranth flour (Table 3). This analyses showed that the concentrations of these 3 sugars decreased during baking. Glucose content was reduced by 22.2% and 69.3% when the pastry was produced from the blends of wheat flour with flours from chickpea or rice, respectively (Table 5). Fructose concentration, which was smaller in the flours compared to that of glucose, decreased during baking to approximately 0.4% of the initial level. The cookies made from the mixture of wheat and chickpea flours (1:1, w/w) contained more ribose (by 61%) compared to the batter ingredients. It is to note that the cookies derived from the spelt-wheat flour contained ribose which was absent in this flour. None of the analysed cookies contained maltose which was found in all the flours.

Baking caused hydrolysis of starch. Its quantities in the cookies were approximately 61% lower compared to flours and their blends. The largest decrease in the starch content was observed for the cookies from spelt-wheat flour (by 63.4%) and the smallest – for the pastry derived from the

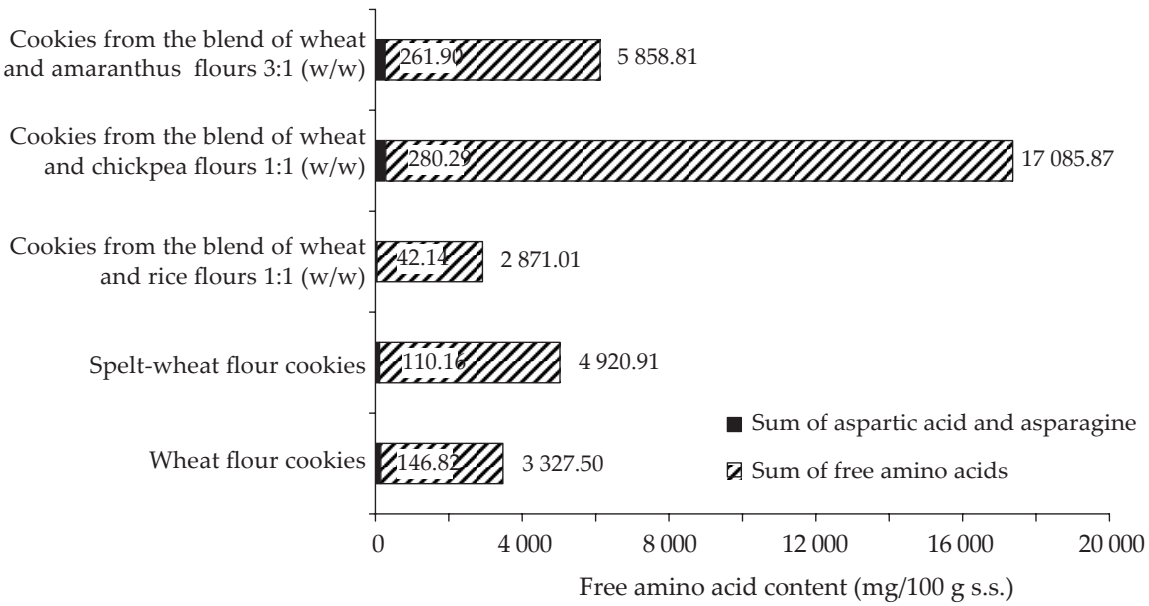


Figure 2. Free amino acids concentrations in shortcrust cookies obtained from tested flours and their blends (the other amino acids are a sum of: Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, and Arg)

Table 5. A decrease in reducing sugars and sucrose contents caused by baking (in %)

	Wheat flour	Spelt-wheat flour	Blend of wheat and rice flours 1:1 (w/w)	Blend of wheat and chickpea flours 1:1 (w/w)	Blend of wheat and amaranthus flours 3:1 (w/w)
Glucose	45.2	30.9	69.3	*22.2*	54.9
Fructose	–	98.0	99.2	*98.1*	99.5
Maltose	–	–	–	–	–
Ribose	84.8	increase	66.9	61.0 (increase)	91.5
Sucrose	73.9	66.0	84.5	*53.9*	78.9
Starch	61.0	63.4	*60.4*	60.5	62.6

underlined – utmost value; italic – bottommost value

mixed wheat and rice flours (1:1, w/w) (by 60.4%) (Figure 4, Table 5).

Sucrose contained in pastries originates not only from the flour because it is also added as a sweetener. Our analysis revealed a decrease in sucrose content by approximately 70% compared to the batter ingredients. This decrease was the highest when the cookies were produced from the blend of wheat and rice flours (by 84.5%) and the least – for the cookies based on the mixture of wheat and chickpea flours (1:1, w/w) (by 53.9%) (Table 5).

Effect of flour type on acrylamide content in cookies

Apart from the determination of the effect of the flour type on physicochemical features of the dough and baked shortcrust cookies, also acrylamide content in these cookies was estimated. The cookies were formulated from wheat Poznań flour, spelt-wheat flour, and mixtures of wheat flour with rice (1:1, w/w), chickpea (1:1, w/w), and amaranth (3:1, w/w) flours that differed in aspartic acid levels and carbohydrate profiles. Baking was conducted at 180°C for 10 minutes. Such conditions stimulate chemical reactions generating toxic acrylamide (Gőkmen *et al*. 2007). The concentrations of this compound ranged between 41.9 μg/kg in the cookies based on wheat flour only, and 5.7 μg/kg in the cookies derived from its mixture with the chickpea flour (1:1, w/w) (Figure 4). The latter acrylamide content was 86.4% lower than the highest level while the cookies obtained from the blend of wheat and rice flours (1:1, w/w) contained only 9.07% less acrylamide than the purely wheat cookies (Figure 5). Asparagine and reducing sugars are known to be acrylamide precursors and the correlation between acrylamide levels in foods and asparagine contents in raw materials is well documented (Mottram *et al*. 2002). However, asparagine can be converted to aspartic

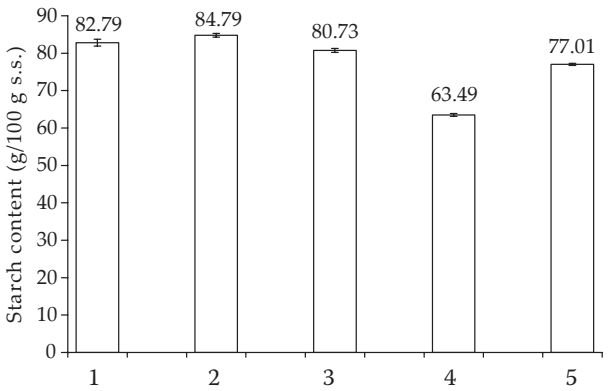
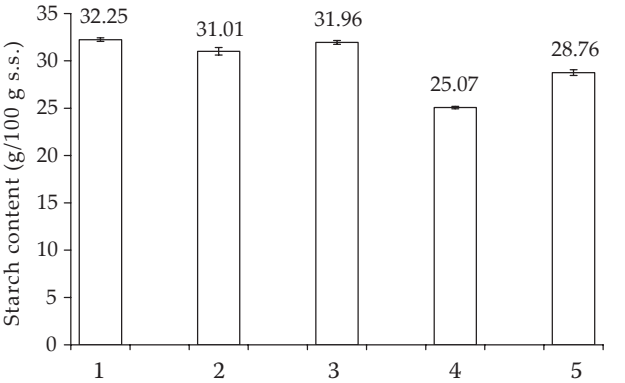
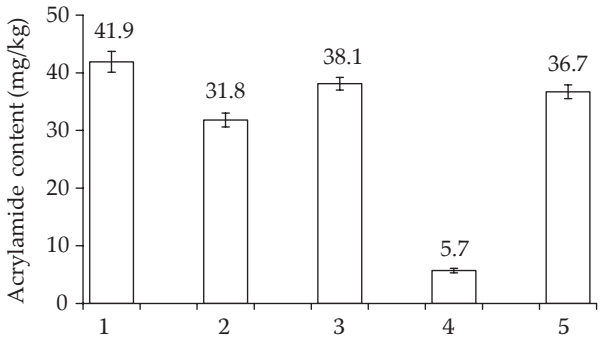


Figure 3. Starch content in tested flours and their blends



1 – wheat flour; 2 – spelt-wheat flour; 3 – blend of wheat and rice flours 1:1 (w/w); 4 – blend of wheat and chickpea flours 1:1 (w/w); 5 – blend of wheat and amaranthus flours 3:1 (w/w)

Figure 4. Starch contentrations in shortcrust cookies obtained from tested flours and their blends



1 – wheat flour; 2 – spelt-wheat flour; 3 – blend of wheat and rice flours 1:1 (w/w); 4 – blend of wheat and chickpea flours 1:1 (w/w); 5 – blend of wheat and amaranthus flours 3:1 (w/w)

Figure 5. Acrylamide concentrations in shortcrust cookies obtained from tested flours and their blends

acid which does not react with reducing sugars to produce acrylamide. The conversion of asparagine to aspartic acid is catalysed by the enzyme asparaginase which is synthesised by many plants to control the level of asparagine which is incorporated into plant proteins. Asparaginase, which is used in food processing to reduce acrylamide levels, is synthesised by *Aspergillus oryzae*. This enzyme is optimally active at 60°C and pH = 7. Its application caused a substantial decrease in acrylamide concentration (by 60–90%) in gingerbread (Amrein *et al*. 2004) and chips (Pedereschi *et al*. 2008) without any changes in the taste and aroma of these food products.

To avoid errors in quantification of the highly reactive asparagine, the flours, their blends, and cookies were tested for the sum of asparagine and aspartic acid. The highest value of this sum was found in the blend of wheat and chickpea flours (1:1, w/w) (Table 2) which provided evidence for asparagine conversion to aspartic acid. The cookies baked from this blend were characterised by the lowest acrylamide level while in the cookies derived from the blend containing 50% rice flour, its concentration was much higher (38.1 μg/kg) (Figure 5). The sum of aspartic acid and asparagine in the latter blend was the lowest (171.16 μg/kg) (Table 2). The mixture of wheat and chickpea flours (1:1, w/w) was characterised by the lowest concentrations of glucose, fructose, and sucrose (Table 3). In consequence, the decrease in glucose and sucrose levels during the baking process was the smallest (Table 5). By contrast, the mixture of wheat and rice flours was characterised by the highest concentrations of these three sugars (Table 3) which were substantially decreased by baking. This corresponded to the formation of relatively large amounts of acrylamide compared to the cookies derived from blended wheat and chickpea flours (1:1) (Figure 5).

The relatively low acrylamide concentration in the cookies produced from the blend of wheat and chickpea flours could also result from the protective effect of chickpea proteins which had been described by Tareke *et al.* (2002) who noticed that acrylamide level was decreased when sliced potatoes were supplemented with chickpea proteins before frying.

CONCLUSION

The study presented here aimed at the characterisation of amino acid and carbohydrate profiles in selected flours and the determination of the effect of these profiles on acrylamide concentration in the shortcrust cookies produced from these flours. Five different flours, that is wheat Poznań flour and flours from spelt-wheat, rice, chickpea, and *Amaranthus* seeds were used to produce the shortcrust cookies. The rice, chickpea, and *Amaranth* flours were mixed with the wheat Poznań flour in the proportions of: 1:1 (w/w), 1:1 (w/w), and 1:3 (w/w), respectively. The highest concentrations of reducing sugars and sucrose were found in the blend of wheat and rice flours while the lowest ones in the blend of wheat and chickpea flours. Other flours tested and their blends contained almost the same amounts of sugars. The tested materials were particularly rich in aspartic acid which dominated over other amino acids. Its level was the highest in the blend of wheat and chickpea flours while the mixture of wheat and rice flours contained the least amount of aspartic acid. The analysis of amino acid and carbohydrate composition profiles in the shortcrust cookies obtained within the scope of this study revealed that the levels of these substances were decreased by baking.

The lowest acrylamide concentration was found in the cookies derived from the blend of wheat and chickpea flours (1:1, w/w) (5.7 μg/kg) while the richest in acrylamide were the cookies produced from wheat Poznań flour (41.9 μg/kg). The former cookies contained 86.4% less acrylamide than the

latter which could result from the protective effect of chickpea proteins. This effect was observed also by other researchers (Tareke *et al*. 2002) who found that fried potato slices contained less acrylamide when chickpea protein was added before frying.

In conclusion, the blend of wheat and chickpea flours (1:1, w/w) is particularly well suited to the manufacturing of shortcrust cookies.

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