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ORIGINAL PAPER

Formation of α-dicarbonyl compounds in cookies made from wheat, hull-less barley and colored corn and its relation with phenolic compounds, free amino acids and sugars

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Abstract Cookies are baked at elevated temperatures to obtain a desirable browning and flavor development, which are provided by Maillard reaction and caramelization. The drawback here is the formation of α-dicarbonyl compounds, and they are associated with many metabolic disorders besides involving in the development of the desired flavor and browning. Cookies are one of the major sources of α-dicarbonyl compounds in diet. This study was performed to evaluate the formation of α-dicarbonyl compounds in cookies prepared from different cereal flours consisting of diverse natural phenolic compounds. Flours of white wheat, hull-less barley and yellow, dark-red, blue, dark-blue colored corns containing different amount of phenolic compounds were selected as raw materials. A negative correlation was observed between total phenolic compounds and glyoxal, methylglyoxal and diacetyl concentrations after baking. α-Dicarbonyl compound-trapping ability of phenolic compounds was attributed to this reduction during baking of cookies. On the other hand, higher amounts of 3-deoxyglucosone and 1-deoxyglucosone were observed with increasing total phenolic compounds, which was in accordance with the higher sucrose hydrolysis.

Keywords Cereal cookies · α-Dicarbonyl compounds · Phenolic compounds · Free amino acids · Sucrose degradation

Introduction

The negative effects of reactive carbonyl compounds on health have been better understood in recent years. The imbalance formation and metabolism of dicarbonyl compounds in vivo and also excessive intake from foods cause dicarbonyl stress [1]. The relationship between α-dicarbonyl compounds formed in vivo and aging along with many metabolic disorders such as diabetes, obesity, cardiovascular, neurological and renal diseases have been proposed [2]. α-Dicarbonyl compounds are responsible for glycation of several biomolecules in vivo [1]. Although having adverse effects on health, α-dicarbonyl compounds play an important role on flavor and browning development, which are desirable consequences of thermal processing of certain foods [3, 4]. Therefore, studies about their formation, quantitation and elimination have a critical importance both in biochemical and food research.

Maillard reaction, caramelization and lipid oxidation are responsible for the formation of α-dicarbonyl compounds in foods during thermal processing [5–8]. Dehydration of hexose sugars mainly leads to 3-deoxyglucosone formation and, to a smaller extent, to 1-deoxyglucosone, while major oxidation product of hexoses is glucosone [9, 10]. Glyoxal, methylglyoxal and diacetyl (dimethylglyoxal) are

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formed from fragmentation of reactive intermediates during Maillard reaction and also from degradation of lipid oxidation products [11–14]. Progresses of Maillard reaction, caramelization and thermal oxidation highly depend on time–temperature profile of the thermal process. Additionally, interaction of precursors with many other food components may alter the formation of these reactive intermediates.

α-Dicarbonyl compounds accumulate particularly in bakery products and fruit juices during both processing and storage as these products contain high amounts of sugars, honey or high-fructose corn syrup [15–18]. Arribas-Lorenzo et al. [19] reported glyoxal and methylglyoxal concentrations in cookies ($n = 26$) ranging between 4.8–26.0 and 3.7–81.4 mg/kg, respectively. They also indicated that cookies made from ammonium bicarbonate and fructose contained higher amount of methylglyoxal. In addition to that, baking time was linearly correlated with methylglyoxal and glyoxal formation during baking of cookies [19]. Degen et al. [15] reported that concentrations of 3-deoxyglucosone, 3-deoxygalactosone and methylglyoxal were in the range of 8.5–385, 0–88 and 1.8–68 mg/kg in cookies ($n = 13$), respectively. Since cookies are one of the main sources of α-dicarbonyl compounds in the diet, their mitigation or elimination is critically important during thermal processing.

Totlani et al. [20] have shown that epicatechin can react with α-dicarbonyl compounds in model Maillard reaction system. Trapping of α-dicarbonyl compounds under physiological conditions has been studied widely, and it has been shown that α-dicarbonyl compounds react with certain positions on the phenol ring via electrophilic aromatic substitution [21, 22]. Catechins [22, 23], curcumin [24], daidzein [25], genistein [25, 26], theaflavins [23], phloretin [27], phenolic acids [28], fruit and vegetable seed extracts [29] have been shown to trap dicarbonyl compounds. Effect of phenolic compounds on trapping α-dicarbonyl compounds during food processing was focused mainly on flavor development [30–33]. α-Dicarbonyl compounds react with several food components, and they involve in the formation of melanoidins [34].

In this study, to evaluate α-dicarbonyl formation in cookies, flours of white wheat, hull-less barley and yellow, dark-red, blue, dark-blue colored corns containing different amount of phenolic compounds were selected as raw materials. 3-Deoxyglucosone, 1-deoxyglucosone, glucosone, glyoxal, methylglyoxal and diacetyl concentrations were measured with LC–MS with precolumn derivatization. Additionally, to evaluate the effect of Maillard reaction and caramelization on the concentration of α-dicarbonyl compounds, the content of free amino acids in the flour and sugar contents in cookies were analyzed.

Materials and Methods

Chemicals and consumables

3-Deoxyglucosone (75 %), glyoxal (40 %), methylglyoxal (40 %), quinoxaline (99 %), 2-methylquinoxaline (97 %), 2,3-dimethylquinoxaline (97 %), o-phenylenediamine (98 %), diethylenetriaminepentaacetic acid (98 %), Folin–Ciocalteu reagent (2 N), methanol (≥99.9 %) and acetonitrile (≥99.9 %) and water (CHROMASOLV grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Gallic acid (>99 %), glucose (>99 %), fructose (>99 %), sucrose (>99 %) and all amino acids (>98 %) were also purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid (98 %) was purchased from JT Baker (Deventer, Holland). Potassium hexacyanoferrate, zinc sulfate, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate dihydrate, sodium hydroxide, sodium carbonate, hydrochloric acid (37 %), pure ethyl acetate and pure diethyl ether were purchased from Merck (Darmstadt, Germany). Nylon syringe filters (pore size 0.45 μm) and OASIS HLB cartridges were supplied by Waters (Milford, MA, USA).

Sodium chloride, ammonium bicarbonate, sodium bicarbonate, sucrose, nonfat dry milk and high-fructose corn syrup used in the formulation of cookies were obtained from local producers.

Flour samples

Blue popcorn (*Zea mays* L. spp. Everta), dark-red popcorn (*Zea mays* L. spp. Everta), blue-standard corn (*Zea mays* L.) and hull-less barley (*Hordeum vulgare* L. var. nudum Hook. f.) genotypes were developed in the Maize Research Institute (MRIZP) in Belgrade, Serbia. The flours of these corn genotypes and the hull-less barley were characterized by high content of anthocyanins and proanthocyanidins, respectively [35, 36]. The anthocyanins are concentrated in the aleurone of blue corn genotypes and pericarp of red corn genotype. Spring two-rowed hull-less barley variety "Apolon" used in this study is characterized by high content of beta-glucans [37]. Whole grain flours (integral flour) were produced by Perten 120 laboratory mill (Perten, Sweden) (particle size < 500 μm). Commercial soft wheat flour type 400 and yellow corn flour were purchased from a local supermarket.

Preparation of cookies

The dough was prepared according to the recipe described in AACC Methods 10–54 (2000) with some modifications [38]. The recipe contained 40.0 g of flour, 16.8 g of

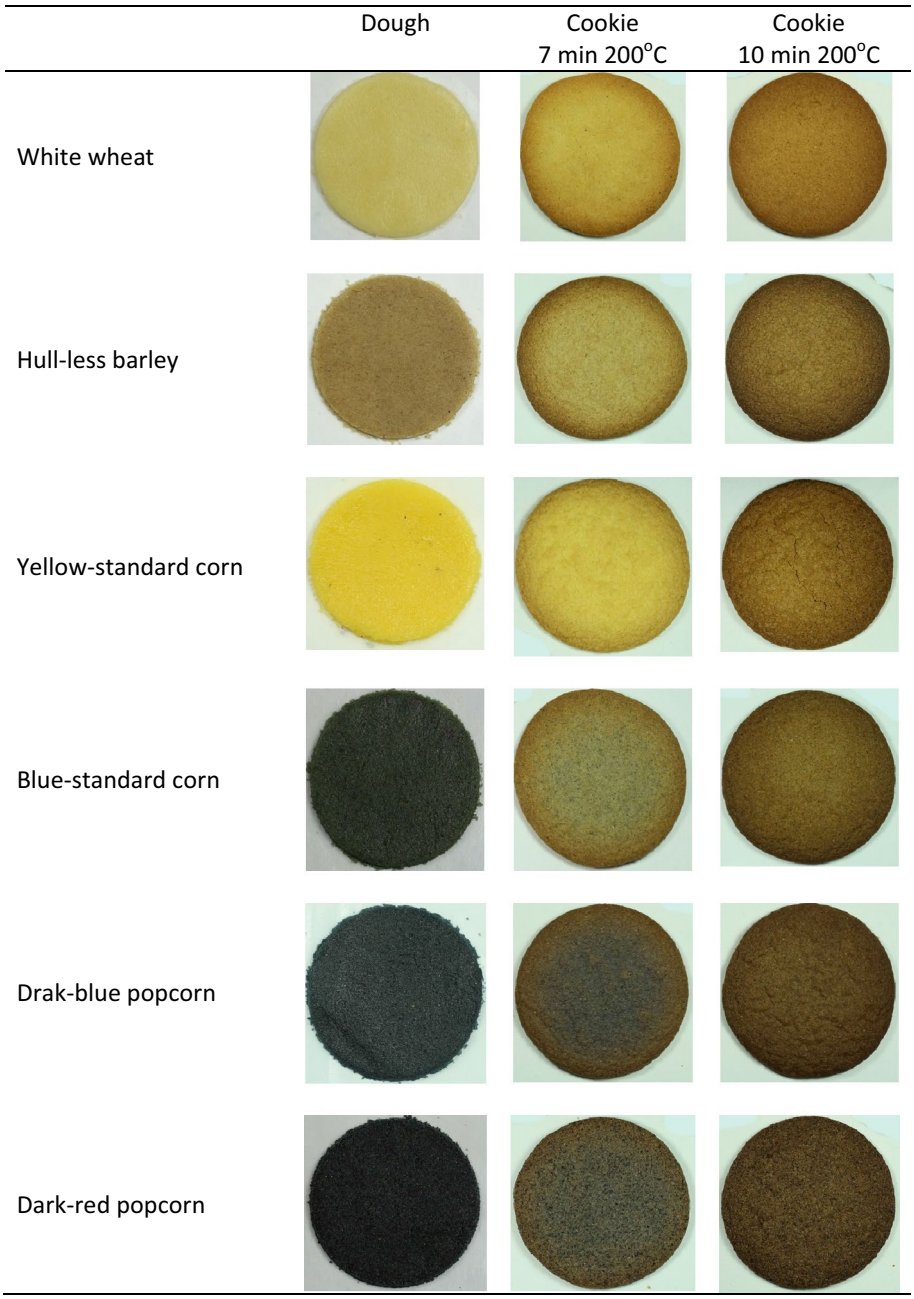

Fig. 1 Photographs of dough and cookies baked at 200 °C for 7 and 10 min

sucrose, 16.0 g of shortening (refined palm oil), 0.5 g of sodium chloride, 0.2 g of ammonium bicarbonate, 0.4 g of sodium bicarbonate, 0.6 g high-fructose corn syrup, 0.4 g of nonfat dry milk and 8.8 ml of water. All ingredients were mixed thoroughly in accordance with the AACC Method 10–54 procedure using the Kitchen Aid 5KSM150 dough mixer. The dough was rolled out to disks with a diameter of 5 cm and a height of 3 mm, and baked in the oven (Memmert, UNE 400) at 200 °C for 7 and 10 min. All baking experiments were performed in duplicate. Dough and cookies are shown in Fig. 1.

Extraction of total phenolic compounds from flours

Phenolic compounds in the wheat, hull-less barley and corn flour samples were extracted according to the procedure described by Antoine et al. [39]. Total phenolics in 500 mg of samples were released by alkaline hydrolysis for 4 h at room temperature using 4 M NaOH. After the pH was adjusted to 2.0 by 6 M HCl, all the hydrolyzates were extracted with ethyl acetate and diethyl ether (1:1, v/v) for four times. Five milliliter of combined extracts was evaporated under N_2 stream at 30 °C to dryness. Final residues

were redissolved in 1.5 ml of methanol. The extracts were kept at −70 °C prior to analyses. All extractions were performed in duplicate.

Analysis of total phenolic compounds in flours

The total phenolic compound content was determined by a Folin–Ciocalteu assay as described by Singleton et al. [40] and expressed as mg of gallic acid equivalent (GAE) per kg of dry matter (d.m.).

Analysis of free amino acids in flours

One gram of flour sample was extracted with 20 ml water in three stages (10, 5, 5 ml) by mixing for 3 min in a shaker. After centrifugation at 5000×*g* for 5 min in each step, the extract was collected in a test tube. Two hundred microliter of the combined extract was mixed with 300 µl water and 500 µl acetonitrile, and then, it was centrifuged at 8000×*g* for 5 min. The clear supernatant was filtered through 0.45 µm nylon syringe filter into an autosampler vial. Samples were extracted in duplicate.

The analysis of free amino acids was carried out according to the method described previously [41]. The samples were analyzed by using Waters Acquity UPLC system coupled to a TQ detector operated in ES + mode. Chromatographic separations were performed on Atlantis HILIC column (2.1 × 150 mm, 3 µm) by using a gradient mixture of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B) at flow rate of 0.4 ml/min. The eluent composition starting with 15 % of A linearly increased to 40 % in 4 min and held for 3 min. Then, it was linearly decreased to its initial conditions (15 % of A) in 1 min. The total chromatographic run was completed in 8 min. The column equilibrated at 40 °C and Waters ACQUITY FTN autosampler was held at 10 °C during the analysis. The electrospray source had the following settings: capillary voltage of 3.5 kV; cone voltage of 20 V; extractor voltage of 3 V; source temperature of 120 °C; desolvation temperature of 350 °C; and desolvation gas (nitrogen) flow of 900 l/h. Quantification was performed by means of external calibration curves built for all amino acids in a range between 0.05 and 2.0 mg/l.

Extraction of sugars and α-dicarbonyl compounds from cookies

One gram of ground cookie was used for extraction with distilled water. The extraction was done in three stages (10, 5, 5 ml) by vortexing for 3 min at room temperature, followed by centrifugation at 5000×*g* for 5 min. The combined extracts were kept for sugars and α-dicarbonyl compounds analysis. All cookie samples were extracted in duplicates.

Analysis of sugars in cookies

One milliliter water extract was mixed with 50 µl Carrez I and 50 µl Carrez II solutions that were prepared by dissolving 15 g potassium hexacyanoferrate in 100 ml water and 30 g zinc sulfate in 100 ml water, respectively. After stirring, the mixture was centrifuged at 8000×*g* for 3 min, and then, supernatant was separated and filtered through a preconditioned Oasis HLB cartridge. The cartridges were preconditioned by passing 1 ml methanol and 1 ml water subsequently. The first 8 drops of the sample eluent was discarded and the rest was collected into an autosampler vial.

Analysis of sugars was performed on Agilent 1200 HPLC system consisting of a quaternary pump, an autosampler, a column oven and a refractive index detector. An isocratic elution with a mobile phase consisting 0.1 % phosphoric acid in water (v/v) at a flow rate of 1.2 ml/min was used. Five microliter of sample was injected into a Shodex RSpak KC-811 column (300 mm × 8 mm i.d.) (Tokyo, Japan) conditioned to 40 °C. Quantification of fructose, glucose and sucrose was according to the external calibration curves built between the concentrations of 0.005–1 %.

Analysis of α-dicarbonyl compounds in cookies

The preparation of the α-dicarbonyl compounds extracts was carried out as described above. The co-extracted colloids were precipitated by mixing with acetonitrile. Two hundred microliter of the extract was diluted with 800 µl of the mixture of acetonitrile/water (5:3, v/v), and centrifuged at 8000×*g* for 5 min. The clean supernatant was used for derivatization. Derivatization of α-dicarbonyl compounds was carried out with *o*-phenylenediamine (*o*-PDA) according to the procedure published previously [42]. Quinoxaline derivatives were formed by the reaction of α-dicarbonyl compounds with 0.2 % *o*-PDA solution containing 11 mM diethylenetriaminepentaacetic acid. Five hundred microliter of supernatant was mixed with 150 µl of *o*-PDA and 150 µL of 0.5 M sodium phosphate buffer (pH 7). The mixture was immediately filtered through 0.45-µm nylon syringe filter and kept at room temperature in the dark for 2 h prior to HPLC–ESI–MS measurement.

α-Dicarbonyl compounds were determined by using an Agilent 1200 series HPLC system coupled with an Agilent 6130 single-quadrupole mass spectrometer. The chromatographic separation was performed on a Merck Purospher Star RP-18e column (150 mm × 4.6 mm i.d., 5 µm) at a flow rate of 1 ml/min at 30 °C using a gradient mixture of (A) 1 % formic acid in water and (B) 1 % formic acid in methanol as the mobile phase. The gradient mixture was started from 30 % B and increased to 60 %

B in 10 min; then, it was decreased to 30 % B in 2 min and the 30 % B remained for 3 min. The chromatographic run was completed in 15 min. MS data were acquired in the positive mode, and α-dicarbonyl compounds were identified by selected ion monitoring (SIM) mode. The SIM ions $[M + H]^+$ of the quinoxaline derivatives of α-dicarbonyl compounds were used for quantitation. The concentrations of quinoxaline, 2-methylquinoxaline and 2,3-dimethylquinoaxaline were estimated from calibration curves built for each compound in the range between 0.01 and 0.2 mg/l. The acetonitrile/water (50:50, v/v) working solutions of 3-deoxyglucosone in the concentration range between 0.01 and 2.0 mg/l were derivatized and analyzed as described above to build its external calibration curve. The same calibration curve was used for semi-quantitation of glucosone and 1-deoxyglucosone quinoxaline derivatives since both have same proton-accepting groups. 3-Deoxygalactosone was not resolved in this chromatographic run, and thus, 3-deoxyglucosone concentrations reported here contained both epimers. The chromatograms obtained from standard compounds and a cookie sample are given in the supplementary file (Online Resource 1). The percentage recoveries of 3-deoxyglucosone, glyoxal and methylglyoxal were determined by analyzing the spiked cookie samples (in concentration of 2, 5, 20 mg/kg). The mean recoveries of 3-deoxyglucosone, glyoxal and methylglyoxal were found to be 101.3, 104.3 and 98.1 %.

Measurement of pH

Ground cookies (0.4 g) were mixed with 20 ml of distilled water and vortexed for 3 min. The mixture was held at ambient temperature for 1 h to separate solid and liquid phases. After centrifugation, the pH of supernatants was measured using a pH meter (MeterLab PHM210, France).

Statistical analysis

The analytical data are reported as average ± standard deviation of at the least two independent extractions. Significance of differences between flour and cookie samples were analyzed by the Fisher's least significant difference test (LSD), after the analysis of variance for trials set up according to the complete randomized block design. Differences with $p < 0.05$ were considered significant. The degree of linear dependence between two variables was examined by using the Pearson's correlation coefficient, r, giving a value between $+1$ and -1. The value $+1$ represents total positive correlation, while -1 represents total negative correlation, and zero means no correlation.

Results and discussion

Content of total phenolic compounds

The lowest content of total phenolic compounds was found in white wheat flour (69 mg GAE/kg). Yellow-standard corn flour had considerably lower total phenolic compounds (944 mg GAE/kg) than those from blue-standard corn, dark-blue and dark-red popcorn that had 2801, 3210 and 3476 mg GAE/kg total phenolic compounds, respectively. Hull-less barley flour had 1311 mg GAE/kg total phenolic compounds, being 1.4-fold higher than yellow-standard corn flour. Colored corns are characterized by high amount of anthocyanins, which are responsible for the highest phenolic compounds content in the samples [36]. Pigmented cereals such as wheat, rice, corn, maize, sorghum, barley and their products (as natural colorants, functional food ingredients and dietary supplements) have been proposed to play significant role in healthy and nutrition [43]. Color and stability of anthocyanins depend on its structure, pH, temperature, oxygen, metal ions and intermolecular association with other compounds. The color of dough and the color changes in cookies are shown in Fig. 1.

Content of free amino acids in cereal flours

Concentrations of free amino acids in flour samples are given in Table 1. Total free amino acids in hull-less barley was found to be 1822.9 mg/kg, which is the highest. Among the used corn flours, the highest content of total free amino acids was detected in yellow-standard corn flour (1594.7 mg/kg) followed by blue-standard corn flour, dark-blue and dark-red popcorn flour (1416.7, 1341,7 and 1176.5 mg/kg, respectively). White wheat flour was found to be containing 1372.5 mg/kg total free amino acids, not significantly different from blue-standard corn and dark-blue popcorn flour. Proline content of corn flours was threefold to fivefold higher than hull-less barley and 13- to 17-fold higher than white wheat flour.

Sucrose, glucose and fructose concentration and pH of cookies

There were no significant differences of sucrose and invert sugar concentrations in cookies baked at 200 °C for 7 min (Table 3). It was reported that the sucrose hydrolysis became apparent after 8 min of baking at 200 °C for cookies made from wheat flour [44]. Compared with cookies baked at 200 °C for 7 min, the content of sucrose was decreased by 10.6, 7.0 and 17.4 % after baking for 10 min in cookies made from blue, dark-blue and dark-red corn flours, respectively. In barley and yellow corn cookies,

Table 1 Concentration of free amino acids (mg/kg) in wheat, barley and corn flour samples

Amino acids	White wheat	Hull-less barley	Yellow corn	Blue-standard corn	Dark-blue popcorn	Dark-red popcorn
Ala	55.4 ± 1.1^{e}	113.1 ± 2.4^{a}	84.1 ± 2.5^{b}	60.9 ± 1.6^{c}	57.5 ± 1.3^{de}	48.9 ± 1.2^{f}
Arg	87.5 ± 1.8^{b}	181.5 ± 4.7^{a}	41.5 ± 1.2^{f}	80.8 ± 2.0^{c}	71.9 ± 2.1^{d}	55.0 ± 1.3^{e}
Asn	306.5 ± 26.2^{b}	370.9 ± 14.8^{a}	223.8 ± 7.1^{d}	275.3 ± 5.5^{c}	267.2 ± 8.5^{c}	268.2 ± 10.2^{c}
Asp	244.2 ± 7.3^{b}	186.2 ± 7.4^{d}	401.1 ± 8.0^{a}	216.8 ± 6.9^{c}	148.7 ± 5.3^{e}	129.9 ± 2.6^{f}
Cys	3.5 ± 0.2^{ab}	3.7 ± 0.3^{a}	3.3 ± 0.0^{bc}	3.1 ± 0.1^{c}	3.8 ± 0.1^{a}	3.4 ± 0.1^{bc}
Gln	28.6 ± 0.6^{b}	97.0 ± 3.5^{a}	12.4 ± 0.3^{c}	14.9 ± 0.5^{c}	15.6 ± 0.6^{c}	15.5 ± 0.6^{c}
Glu	168.9 ± 4.2^{c}	148.7 ± 5.3^{d}	273.6 ± 10.9^{a}	190.8 ± 6.5^{b}	193.8 ± 3.9^{b}	165.3 ± 5.3^{c}
Gly	24.3 ± 0.8^{b}	32.0 ± 1.7^{a}	13.0 ± 0.4^{d}	12.1 ± 0.3^{d}	16.2 ± 0.6^{c}	11.2 ± 0.3^{d}
His	19.5 ± 0.3^{d}	59.6 ± 1.9^{a}	20.3 ± 0.8^{d}	36.3 ± 1.4^{b}	25.5 ± 0.9^{c}	20.0 ± 0.7^{d}
Leu/Ile	37.6 ± 0.9^{bc}	106.3 ± 7.3^{a}	43.1 ± 1.2^{b}	31.5 ± 1.3^{cd}	31.6 ± 1.1^{cd}	23.9 ± 1.0^{d}
Lys	27.0 ± 0.3^{d}	83.3 ± 3.2^{a}	36.1 ± 1.1^{c}	60.0 ± 2.4^{b}	36.1 ± 1.4^{c}	27.4 ± 0.5^{d}
Met	7.5 ± 0.1^{c}	21.5 ± 1.8^{a}	12.5 ± 0.5^{b}	6.4 ± 0.3^{c}	7.4 ± 0.3^{c}	5.8 ± 0.2^{c}
Phe	17.8 ± 0.1^{cd}	61.2 ± 4.1^{a}	24.8 ± 1.0^{b}	19.5 ± 0.7^{c}	15.1 ± 0.3^{cd}	13.0 ± 0.2^{d}
Pro	20.7 ± 0.9^{f}	73.6 ± 2.6^{e}	265.0 ± 7.9^{d}	283.4 ± 8.9^{c}	344.5 ± 12.2^{a}	299.2 ± 8.1^{b}
Ser	17.1 ± 0.5^{f}	53.4 ± 1.2^{a}	34.7 ± 0.7^{b}	25.6 ± 1.0^{c}	23.4 ± 0.7^{d}	20.1 ± 0.3^{e}
Thr	15.1 ± 0.1^{d}	44.9 ± 1.1^{a}	21.5 ± 0.6^{b}	19.1 ± 0.3^{c}	18.0 ± 0.3^{c}	14.8 ± 0.2^{d}
Trp	246.6 ± 6.5^{a}	72.2 ± 2.2^{b}	11.1 ± 0.2^{c}	16.5 ± 0.7^{c}	14.8 ± 0.3^{c}	12.4 ± 0.2^{c}
Tyr	18.1 ± 0.5^{f}	38.4 ± 1.0^{b}	41.3 ± 1.2^{a}	35.7 ± 0.9^{c}	23.8 ± 0.8^{d}	20.8 ± 0.4^{e}
Val	26.3 ± 0.6^{d}	75.3 ± 2.1^{a}	31.2 ± 1.1^{b}	28.0 ± 1.0^{cd}	26.8 ± 0.7^{d}	21.7 ± 0.4^{e}
Total	1372.5^{c}	1822.9^{a}	1594.7^{b}	1416.7^{c}	1341.7^{c}	1176.5^{d}

Superscript letter in each row indicates statistically significant difference ($p < 0.05$)

Table 2 Concentration of the sugars (%) and pH of wheat, barley and corn cookies

	Time (min)	Sucrose (%)	Glucose (%)	Fructose (%)	pH
White wheat	7	25.9 ± 0.1^{a}	0.63 ± 0.03^{e}	0.39 ± 0.03^{g}	7.85^{b}
	10	25.2 ± 0.4^{a}	0.69 ± 0.04^{e}	0.48 ± 0.03^{ef}	7.76^{b}
Hull-less barley	7	25.9 ± 0.2^{a}	0.64 ± 0.04^{e}	0.49 ± 0.01^{ef}	7.73^{b}
	10	24.8 ± 0.5^{ab}	0.80 ± 0.06^{d}	0.55 ± 0.02^{de}	7.46^{a}
Yellow corn	7	26.1 ± 0.9^{a}	0.67 ± 0.03^{e}	0.44 ± 0.02^{fg}	7.54^{a}
	10	25.2 ± 0.1^{a}	0.82 ± 0.02^{d}	0.58 ± 0.03^{d}	7.38^{a}
Blue-standard corn	7	25.9 ± 0.5^{a}	0.70 ± 0.06^{e}	0.49 ± 0.01^{ef}	7.73^{b}
	10	23.1 ± 1.5^{b}	1.22 ± 0.10^{b}	0.81 ± 0.07^{b}	7.39^{a}
Dark-blue popcorn	7	26.0 ± 0.1^{a}	0.69 ± 0.02^{e}	0.46 ± 0.01^{fg}	7.54^{a}
	10	24.2 ± 0.8^{d}	1.10 ± 0.10^{c}	0.65 ± 0.02^{c}	7.40^{a}
Dark-red popcorn	7	25.9 ± 0.3^{a}	0.70 ± 0.01^{e}	0.47 ± 0.01^{ef}	7.85^{b}
	10	21.4 ± 0.8^{c}	1.61 ± 0.11^{a}	0.99 ± 0.07^{a}	7.43^{a}

Superscript letter in each column indicates statistically significant difference ($p < 0.05$)

concentration of sucrose was decreased by 4.3 and 3.4 %, respectively, while it was not statistically different in wheat cookies. Invert sugar concentrations were increased significantly in all cookies except glucose in wheat flour cookie.

The pH values of cookies were neutral to slightly alkaline and ranged from 7.54 to 7.85 after 7-min baking (Table 2). There were no remarkable decreases in pH values of cookies after 10-min baking. The pH values of cookies baked for 10 min were not significantly different from each other except white wheat cookie. Therefore, higher sucrose hydrolyses in colored corn cookies cannot be attributed to changes in pH.

Content of α-dicarbonyl compounds and their correlation with phenolic compounds, free amino acids and sugars

In all cookies, 3-deoxyglucosone was the predominating α-dicarbonyl compound except the cookies from wheat flour. The highest formation of 3-deoxyglucosone was

Table 3 Concentration of α-dicarbonyl compounds (mg/kg) in wheat, barley and corn cookies

Cookie samples	Time (min)	3-Deoxyglucosone	1-Deoxyglucosone[δ]	Glucosone[δ]	Glyoxal	Methylglyoxal	Diacetyl
White wheat	7	18.5 ± 1.5^{g}	0.74 ± 0.34^{f}	0.26 ± 0.02^{e}	26.8 ± 0.7^{a}	21.8 ± 2.3^{b}	1.66 ± 0.14^{de}
	10	28.3 ± 2.2^{ef}	2.49 ± 0.15^{d}	0.19 ± 0.03^{f}	17.9 ± 0.1^{b}	37.8 ± 2.1^{a}	3.51 ± 0.33^{a}
Hull-less barley	7	33.8 ± 0.6^{de}	2.51 ± 0.26^{d}	0.84 ± 0.01^{a}	12.7 ± 0.4^{d}	17.4 ± 1.1^{d}	0.94 ± 0.07^{fg}
	10	53.2 ± 4.9^{c}	3.94 ± 0.14^{ab}	0.58 ± 0.03^{b}	16.5 ± 1.0^{c}	19.6 ± 1.1^{bcd}	2.13 ± 0.29^{cd}
Yellow corn	7	24.3 ± 1.7^{fg}	1.75 ± 0.01^{e}	n.d.	11.9 ± 0.6^{d}	11.2 ± 0.4^{f}	1.51 ± 0.11^{ef}
	10	32.8 ± 3.1^{de}	1.83 ± 0.05^{e}	n.d.	12.3 ± 0.4^{d}	20.7 ± 1.4^{bcd}	3.28 ± 0.30^{a}
Blue-standard corn	7	31.6 ± 2.2^{de}	3.23 ± 0.01^{bc}	0.33 ± 0.02^{d}	8.9 ± 0.8^{fg}	10.4 ± 1.9^{f}	0.94 ± 0.01^{g}
	10	60.8 ± 5.0^{b}	2.71 ± 0.13^{cd}	0.26 ± 0.05^{e}	9.2 ± 0.3^{f}	14.5 ± 1.1^{e}	2.31 ± 0.21^{bc}
Dark-blue popcorn	7	30.9 ± 2.0^{de}	4.52 ± 0.13^{a}	0.38 ± 0.04^{cd}	7.6 ± 0.1^{h}	10.4 ± 1.3^{f}	0.76 ± 0.03^{g}
	10	61.8 ± 5.4^{b}	3.11 ± 0.31^{c}	0.35 ± 0.02^{cd}	10.0 ± 0.3^{ef}	13.1 ± 1.0^{ef}	1.73 ± 0.16^{de}
Dark-red popcorn	7	36.8 ± 1.7^{d}	4.46 ± 0.09^{a}	0.57 ± 0.03^{b}	8.0 ± 0.8^{gh}	11.8 ± 0.0^{f}	1.04 ± 0.02^{fg}
	10	82.3 ± 6.4^{a}	3.24 ± 0.15^{bc}	0.40 ± 0.01^{c}	8.9 ± 0.7^{fg}	18.7 ± 0.9^{cd}	2.14 ± 0.02^{cd}

* Superscript letter in each column indicates statistically significant difference ($p < 0.05$). *n.d.* not detected

[δ] 1-Deoxyglucosone and glucosone were semi-quantitated by using 3-deoxyglucosone calibration curve

observed in dark-red popcorn cookies, and it was increased from 36.8 to 82.3 mg/kg upon baking for 7 and 10 min (Table 3). Similarly, baking of other colored corn cookies for 10 min increased the 3-deoxyglucosone concentrations by more than the double in comparison with 7 min. Higher increase rates in 3-deoxyglucosone concentration of colored corn cookies were in accordance with their sucrose hydrolysis rates. The lowest amount of 3-deoxyglucosone formed in cookies of wheat and yellow corn, which were 18.5 and 24.3 mg/kg after 7-min baking, and the levels were not significantly different from each other. After 10 min of baking, levels of those were increased almost 1.5-fold, which were also the lowest among others. Moreover, a correlation analysis was performed between total phenolics and α-dicarbonyl compound formation in the cookies. High positive correlations were estimated between total phenolic compounds and 3-deoxyglucosone ($r = 0.81$ and 0.93) after baking 7 and 10 min, respectively ($p < 0.05$). However, there is no such mechanistic study that explains how phenolic compounds or anthocyanins could affect the formation of 3-deoxyglucosone or sucrose hydrolyses.

Glucosone and 1-deoxyglucosone were semi-quantitated according to calibration curve of 3-deoxyglucosone as no standard compounds were available for unequivocal identification. Based on the semi-quantitation of glucosone and 1-deoxyglucosone, their concentrations were rather low in all cookies, which is in accordance with several food groups and model reaction systems [10, 42, 45]. Glucosone and 1-deoxyglucosone have reductone structure, and they are readily degraded by oxidation [10]. Therefore, they do not accumulate during thermal processing. 1-Deoxyglucosone concentrations were increased in wheat, barley and yellow corn cookies after baking 10 min in comparison with 7 min. Higher concentrations of 1-deoxyglucosone were formed in colored corn cookies baked at 7 min, and in contrast to other cookies a decrease was observed after 10-min baking. High positive correlations were estimated between total phenolic compounds and 1-deoxyglucosone ($r = 0.98$) after baking 7 min ($p < 0.05$). No correlation ($r = 0.35$) was observed after 10-min baking, as 1-deoxyglucosone tended to degrade. No correlation was observed also for glucosone at 7-min or 10-min baking ($r = 0.28$ and 0.37, respectively).

Fragmentation of the hexodiuloses (C_6) results with the formation of new α-dicarbonyl compounds along with other degradation products. Cleavage of the carbon skeleton especially proceeds in the alkaline conditions as in the case of bakery products. Glyoxal (C_2), methylglyoxal (C_3) and diacetyl (C_4) are the major fragmentation products containing α-dicarbonyl moiety. They are formed in Maillard reaction, caramelization and lipid oxidation during heating. Glyoxal formed in blue corn, dark-blue and dark-red popcorn cookies were 8.9, 7.6 and 8.0 mg/kg, respectively. These glyoxal concentrations were found to be 1.5-fold to threefold lesser compared with wheat, yellow corn and hull-less barley cookies after 7 min of baking. Methylglyoxal concentrations of wheat cookies were found to be 21.8 and 37.9 mg/kg, which were two times higher than colored corn cookies, for baking times of 7 and 10 min. Concentration of diacetyl was found to be lower than the concentration of glyoxal and methylglyoxal in all cookies. Diacetyl concentration doubled when the baking time was increased from 7 to 10 min. High negative correlations between total phenolic compounds content and concentration of glyoxal ($r = -0.85$ and -0.89), methylglyoxal ($r = -0.77$ and -0.80), and diacetyl ($r = -0.77$ and -0.85) were estimated for 7-min and 10-min baking,

respectively ($p < 0.05$). Formation of dicarbonyl compounds originated from sucrose degradation was expected to be higher in colored corn cookies. Contrary to the higher concentration of hexodiuloses, lower concentrations of glyoxal, methylglyoxal and diacetyl were observed in colored corn cookies. This reduction could be attributed to fact that phenolic compounds trap α-dicarbonyl compounds. The same effect has not been reported for hexodiuloses since they are found in cyclic molecular structures limiting their reactivity toward the phenolic ring. Additionally, prevention of lipid oxidation by phenolic compounds could also play role on the formation of α-dicarbonyl compounds. On the other hand, there could be other factors effecting α-dicarbonyl formation and elimination such as redox reactions, reaction of dicarbonyl compounds with proteins and other macromolecules.

Concentration of reducing sugars in cookies baked at 200 °C for 7 min was sixfold to tenfold higher than total free amino acid contents of flour samples, and it was eightfold to 22-fold when the baking time was 10 min. Depending on the low content of free amino acids in the cereal flours and high content of reducing sugars in the cookies, caramelization could be considered as the dominant mechanism in the formation of α-dicarbonyl compounds during baking of cookies.

Although high level of α-dicarbonyl compounds can be found in thermally processed sugar-rich foods, it is not well known whether they bear a health risk. Occurrence of dicarbonyl stress is attributed mainly to endogenous formation of α-dicarbonyl compounds and its imbalance with respect to detoxifying systems. Studies about the bioavailability and intestinal absorption of α-dicarbonyl compounds are limited. Glyoxal and methylglyoxal are mainly metabolized by glyoxalase system, and also aldehyde reductase and aldehyde dehydrogenase systems involve in a lesser extent [46]. It has been shown that dietary methylglyoxal do not contribute to urinary excretion levels of methylglyoxal and its metabolite D-lactate [47]. In addition to that, methylglyoxal formation in vivo may account ca. 3 mmol/day, which substantially exceeds the levels taken by foods (0.1–0.3 mmol/day) [1, 15]. 3-Deoxyglucosone has shown to transform 3-deoxyfructose by aldoketo reductases and 3-deoxy-2-keto-gluconic acid by aldehyde dehydrogenases, which are less reactive and secreted in urine [48, 49]. Degen et al. [50] demonstrated that only 10–15 % of the dietary 3-deoxyglucosone recovered as metabolites in urine. However, the fate of the remaining is unknown. In the very low pH of the stomach, 3-deoxyglucosone is most probably dehydrated to HMF, which is another concern due to its metabolite 5-sulfoxymethyl-2-furfural. 3-Deoxyglucosone might also react with proteins and amino acids during gastrointestinal digestion. α-Dicarbonyl compounds from foodstuffs may induce dicarbonyl stress in the gastrointestinal lumen [1].

Considering the closer amounts of reactants (reducing sugars and amino acids) in the cookies prepared from different cereal flour samples containing varying quantities and diversity of phenolic compounds, there is a considerable effect of phenolic compounds on α-dicarbonyl formation and elimination. This study showed that a reduction in glyoxal, methylglyoxal and diacetyl content could be achieved by natural phenolic compounds during thermal processing of bakeries, especially those of containing ammonium bicarbonate, which creates alkaline conditions. Colored corn flour could be the source of natural dietary antiglycation agents due to good abilities of their phenolic compounds to trap C_2, C_3 and C_4 α-dicarbonyl compounds. Nevertheless, higher concentrations of 3-deoxyglucosone formation in colored corn cookies baked at 200 °C for 7 and 10 min were the drawback. Higher sucrose hydrolysis and 3-deoxyglucosone formation in cookies containing elevated amounts of phenolic compounds need further clarification. In addition, this study recommends investigating in detail the effects of milder processing conditions on the occurrence of α-dicarbonyl compounds in cereal cookies.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

Compliance with Ethics requirements This article does not contain any studies with human or animal subjects.

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