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Effects of alkaline cooking and sprouting on bioactive compounds, their bioavailability and relation to antioxidant capacity of maize flour

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Summary

The contents of total and available niacin, carotenoids, tocopherols, as well as soluble free, conjugated and insoluble bound phenolic compounds was determined in untreated, alkaline-cooked and sprouted kernels of white, sweet and yellow maize. Antioxidant capacity of untreated maize kernels and processed flours was evaluated, too. There was a considerable increase in soluble free and conjugated phenolic compounds contents of maize flour after alkaline cooking and sprouting. The content of total soluble free and conjugated phenolics in maize masa flour and sprouted flour was increased by 35% to 56% and by 46% to 92% as compared to the contents originally present in untreated kernels, respectively. Contrary, conditions of alkaline cooking had strong effect on the loss of bound phenolic compounds, as well as niacin, carotenoids and tocopherols in maize flour. Despite of this, our findings imply that both alkaline cooking and sprouting enhance the antioxidant capacity of maize kernels. According to our results, sprouting is a more effective method to release bound phenolics as well as to enhance the nutritional and functional value of the maize flour. Therefore, sprouted maize, as a rich source of bioavailable phytochemicals, can be used to develop functional maize-based foods.

Keywords

maize; alkaline cooking; sprouting; phenolic compounds; niacin; tocopherols; carotenoids; antioxidant capacity

Due to its high nutritional value, maize is a widely consumed cereal that provides food to the large part of the world's population. Besides, maize contains bioactive phytochemicals such as carotenoids, tocopherols and phenolic compounds. The most important chemical property of these bioactive compounds is their ability to act as antioxidants. Phytochemicals of maize kernels are located mainly in the embryo, aleurone and pericarp. However, only a small percentage of these compounds are present in the free form. More than 80% of maize phenolic compounds are bound to cell wall polysaccharides [1], while lipophilic compounds such as carotenoids form strong complexes with proteins, which greatly reduce their extractability [2]. Although niacin does not belong to bioactive compounds, it is interesting to note that some diseases such as pellagra are related to deficit of niacin. Similar to bioactive components, this vitamin occurs mainly in a nutritionally unavailable form incorporated in large molecules, probably glycoproteins of maize kernels [3].

Poor availability of bioactive compounds is one of the major problems associated with maize-based foods. Some of the methods for improving the nutritional quality and functional value of maize are sprouting and nixtamalization. The biochemical processes occurring during sprouting can generate bioactive components and also increase their availability [4]. Besides, sprouting has been suggested as an inexpensive method to enhance

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the antioxidant capacity of flour through the increase of low-molecular-weight antioxidants [5]. Nixtamalization is the process of thermal-alkaline cooking of maize with lime. The important effects of nixtamalization include increased bioavailability of niacin, improved protein quality and increased calcium content in maize masa products [6].

The aim of this study was to investigate the effects of sprouting and alkaline cooking on the content of bioactive compounds, such as phenolics, carotenoids and tocopherols, as well as their contribution to the total antioxidant capacity of white, sweet and yellow maize flours. We also investigated how sprouting and alkaline cooking affected the chemical forms of phenolic compounds and niacin, which is important for absorption in the human body. The content of tryptophan as the precursor in the synthesis of niacin was also analysed. According to our best knowledge, there are no papers that have compared the effect of alkaline and enzymatic hydrolysis on bioactive compounds of maize. Although the applied processes or treatments have been known for a very long time, our results may be useful for functional food industry.

MATERIALS AND METHODS

Plant materials

The maize genotypes with white, yellow and sweet kernels, developed at Maize Research Institute (MRIZP) Belgrade, Serbia, were used as the initial material. The genotypes were chosen on the basis of kernel colour, as well as their differences in basic chemical composition of the kernels. The kernels of white and yellow genotypes belonged to a semi-flint type. The content of total proteins in kernels of white, yellow and sweet maize was 13.9%, 11.8% and 14.5%, respectively. The kernels of these genotypes at full maturity were used for sprouting, as well as alkaline cooking, i.e. for the preparation of whole-sprouted maize flour and maize masa flour, respectively. Untreated whole maize kernels were used as control samples.

Preparation of whole-sprouted maize flour

The maize kernels were washed and wetted with distilled water in the flow for 60 min with air continuously bubbling through the system. The kernels were then immersed in distilled water (100 g in 150 ml) and steeped for 24 h at room temperature (21 °C). After steeping, water was decanted. The imbibed maize kernels were transferred into a plastic container and incubated in the dark for 5 days in an incubator at temperature of 20 °C and humidity of 50%, and than were air-dried at 55 °C for 8 h to the moisture content of approximately 11%. Sprouted whole maize kernels were produced in a mill company (Bread Line, Belgrade-Zemun, Serbia). All sprouted samples were milled into whole-kernel flour using a Perten 120 lab mill (Perten, Hägersten, Sweden) to a fine powder (particle size < 500 μm).

Preparation of maize masa flour – alkaline cooking or nixtamalization

Maize masa flours were prepared by an imitation of traditional Mexican practice from the whole maize kernels by cooking it at 85 °C for 40 min in a solution of 1% CaO (10 g in 20 ml) [7]. After being left to stand over night at room temperature, the liquor (pH 11.7) was discarded, and the maize kernels were washed five times with tap water. This nixtamal was ground by a food processor (Multipractic; Braun, Kronberg, Germany) into a fine masa. The maize masa was dried in a ventilated oven at 40 °C for 6 h and milled into maize masa flour using a Perten 120 lab mill to a fine powder (particle size < 500 μm).

Analysis of tryptophan

Tryptophan content was determined according to NURIT et al. [8]. The content of tryptophan was expressed as milligrams per kilogram of dry matter.

Analysis of total and available niacin

Total niacin was extracted according to the method described by ŽILIĆ et al. [9]. For the detection of available niacin, extracts were prepared by continuous shaking of 0.5 g of maize flour in 10 ml of 50% (v/v) aqueous ethanol for 60 min at room temperature. Total and available extracted niacin were detected by high performance liquid chromatography (HPLC) at conditions described by ŽILIĆ et al. [9].

Extraction of free soluble, soluble conjugated and insoluble bound phenolic compounds

Free soluble, soluble conjugated and insoluble bound phenolic compounds in maize samples were extracted according to the procedure described by ANTOINE et al. [10]. Twenty millilitres of acetone/methanol/water mixture (7:7:6, v/v/v) were used to extract free and soluble conjugated phenolic compounds from 0.5 g of flour. Insoluble phenolic compounds in solid residues were released by alkaline hydrolysis for 4 h at room temperature using 4 mol·l^{-1} NaOH before extraction. After the pH was adjusted to 1.5–2.0 by 6 mol·l^{-1} HCl, free-conjugated supernatants and hydrolysates were ex-

tracted with ethyl acetate and diethyl ether (1:1, v/v) for four times. Five millilitres of combined extracts were evaporated under N_2 stream at 30 °C to dryness. The final residues were redissolved in 1.5 ml of methanol. After filtering through a nylon filter (pore size 0.45 μm), samples were kept at –40 °C until the HPLC analysis. Such prepared methanolic solutions of phenolic fractions (phenolic extracts) were used for the analyses of phenolic acids, total phenolic and total flavonoid compounds.

Analysis of total phenolics

The total phenolic content was determined according to SINGLETON et al. [11], and expressed as milligrams of gallic acid equivalent (GAE) per kilogram of dry matter.

Analysis of total flavonoids

The flavonoid content was determined according to EBERHARDT et al. [12], and expressed as milligrams of catechin equivalent (CE) per kilogram of dry matter.

Analysis of individual phenolic acids

Chromatographic analyses were performed on a Thermo Scientific Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) consisting of photodiode array detector, quaternary pump, autosampler and column oven. Phenolic acids were separated on a Thermo Scientific Hypersil GOLD aQ C18 column (150 mm × 4.6 mm, particle size 3 μm) using a linear gradient elution programme with a mobile phase containing solvent A (formic acid/H_2O, 1:99, v/v) and solvent B (pure methanol) at a flow rate of 0.8 ml·min^{-1}. The solvent gradient was programmed as described by ŽILIĆ et al. [1]. The chromatograms were recorded at 280 nm by monitoring spectra within the wavelength range 190–400 nm. Amounts of detected compounds were estimated from calibration curves built for each compound.

Analysis of carotenoids

Carotenoids were extracted and analysed according to a method described by ŽILIĆ et al. [1]. The carotenoid extracts were filtered through a nylon syringe filter (pore size 0.45 μm) and analysed in a Thermo Scientific Ultimate 3000 HPLC system equipped with a photodiode array detector and a C18 column (Thermo Scientific ODS Hypersil, 250 mm × 4.6 mm, particle size 3 μm) using solvent A (acetonitrile), solvent B (5 mmol·l^{-1} Tris buffer pH 8.0), and solvent C (tetrahydrofuran) (60:5:35 v/v/v) as the mobile phase at a flow rate of 1.0 ml·min^{-1}. The chromatograms were recorded at 454 nm by monitoring spectra within the wavelength range 300–500 nm. Identified zeaxanthin, lutein and β-carotene peaks were confirmed and quantified by data acquisition and spectral evaluation using the Thermo Scientific Dionex Chromeleon 7.2. chromatographic software.

Analysis of tocopherols

The content of tocopherols was determined by the HPLC method, using extraction by *n*-hexane. The maize flour sample (0.5 g) was mixed with 5 ml of *n*-hexane and the mixture was rigorously shaken at 4 °C for 30 min. After centrifugation at 10 000 ×*g* for 15 min, the upper layer was separated and evaporated under nitrogen gas. The dried sample was then dissolved in 2.5 ml of methanol and filtered through a membrane filter (pore size 0.45 μm). The Thermo Scientific Ultimate 3000 HPLC system was used with Thermo Scientific ODS Hypersil C18 column (250 mm × 4 mm, particle size 3 μm) at a flow rate of 1.0 ml·min^{-1}. The mobile phase consisted of solvent A (methanol), solvent B (water) and solvent C (acetonitrile) (75:5:20, v/v/v). The chromatograms were recorded at 295 nm. Identified α-, γ- and δ-tocopherol peaks were confirmed and quantified by data acquisition and spectral evaluation using the Thermo Scientific Dionex Chromeleon 7.2. chromatographic software.

Analysis of total antioxidant capacity

The antioxidant capacity of maize samples was measured based on the QUENCHER method described by SERPEN et al. [13] using 7 mmol·l^{-1} aqueous solution of ABTS (2,2-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) with 2.45 mmol·l^{-1} $K_2O_8S_2$ as the stock solution. The working solution of $ABTS^{\bullet+}$ was obtained by diluting the stock solution in water/ethanol (50:50, v/v). Finely ground sample (10 mg) was mixed with 20 ml of $ABTS^{\bullet+}$ working solution and the mixture was rigorously shaken for 25 min in a cold room at 4 °C. After centrifugation, absorbance was measured at 734 nm. The total antioxidant capacity was expressed as Trolox equivalent antioxidant capacity (TEAC) in millimoles of Trolox per kilogram of dry matter.

Statistical analysis

The analytical data are reported as mean ± standard deviation of at least two independent extractions. Significance of differences between maize samples was analysed by Fisher's least significant differences test, after the analysis of variance for trials set up according to the randomized complete block

design. Differences with $p < 0.05$ were considered significant. Correlations between parameters were examined using the Pearson's coefficient of correlation.

RESULTS AND DISCUSSION

Contents of tryptophan, total and available niacin

In the present study, content of tryptophan, as the precursor in the synthesis of niacin as well as contents of total and available niacin in untreated and processed maize flours were assessed (Tab. 1). The content of tryptophan in kernels of yellow maize was significantly higher than that in white and sweet maize. Although kernels of yellow maize had the lowest content of total niacin (99.44 mg·kg^{-1}, dry matter basis), it had the highest content of available niacin (42.61 mg·kg^{-1}, dry matter basis), which constituted 42.9% of the total. It is possible that maize with high content of tryptophan may have less bound niacin than normal maize, which is in accordance with results of HASSEN et al. [14]. The percentage contribution of available niacin to the total of white and sweet maize kernels was low and amounted to 14.4% and 9.6%, respectively. According to WALL and CARPENTER [6], raw maize kernels contained 26 mg·kg^{-1} of niacin but only 0.4 mg·kg^{-1} as the free form. It was found that, in addition to the deficit of tryptophan, severe deficiency of available niacin in the diet of developing nations causes the disease pellagra [15]. Alkaline treatment has been shown to make niacin nutritionally available and reduce the chance of developing pellagra [6]. However, according to our results, maize cooking at a temperature of 85 °C for 40 min in a solution of 1% CaO resulted in the reduction of available niacin content, as well as of total niacin and tryptophan contents in the masa flour. Analysis indicated that 8–65% of total niacin was liberated from a chemical linkage by alkaline treatment. However, niacin was not retained after washing the alkali-treated kernels. In contrast to alkaline cooking, sprouting significantly improved the content of available niacin in flour by about 3.25-, 6.49- and 1.28-fold compared to the untreated white, sweet and yellow maize kernels, respectively. The total niacin content was also increased by 15.1% and 19.9% after sprouting of white and sweet maize kernels, respectively, whilst that in sprouted yellow maize flour remained unchanged. These results are in agreement with ONGOL et al. [16].

Contents of total soluble free, conjugated and insoluble bound phenolics and flavonoids

Contents of total phenolics and flavonoids of maize kernels and processed maize flour samples are given in Tab. 2. Among the tested genotypes, the highest total phenolic content was detected in sweet maize. This genotype also contained especially high amounts of soluble free and conjugated phenolic compounds having the value of 684.65 mg·kg^{-1} (dry matter basis; expressed as GAE), which constituted about 20% of the total content. To assess the importance of polyphenols in human health, it is essential to know their bioavailability. In our study, alkaline cooking of maize significantly changed the ratio between soluble and insoluble phenolics in masa flour compared with untreated maize kernels. On average, soluble free + conjugated and insoluble bound phenolics constituted about 13% and 87% of the total content in untreated maize kernels, and about 67% and 33% of the total in masa flour, respectively (Tab. 2). The insoluble bound phenolics present

Tab. 1. Contents of tryptophan, total and available niacin in untreated maize kernels and processed flour.

Maize	Samples	Tryptophan [mg·kg^{-1}]		Total niacin [mg·kg^{-1}]	Available niacin [mg·kg^{-1}]	
White maize	Kernel	833.89 ± 7.91^{c}	(0.6%)	120.36 ± 5.21^{d}	17.29 ± 0.06^{d}	(14.4%)
	Maize masa flour	790.36 ± 7.93^{de}	(0.6%)	101.05 ± 5.11^{e}	5.10 ± 0.02^{f}	(5.0%)
	Sprouted maize flour	923.27 ± 56.77^{ab}	(0.7%)	138.51 ± 5.95^{b}	56.37 ± 0.39^{b}	(40.7%)
Sweet maize	Kernel	835.56 ± 23.63^{c}	(0.6%)	125.55 ± 7.74^{cd}	12.08 ± 0.02^{e}	(9.6%)
	Maize masa flour	836.59 ± 39.43^{c}	(0.5%)	44.43 ± 4.33^{g}	5.91 ± 0.08^{f}	(13.9%)
	Sprouted maize flour	942.21 ± 24.22^{a}	(0.6%)	150.61 ± 4.82^{a}	78.46 ± 0.23^{a}	(52.1%)
Yellow maize	Kernel	954.41 ± 10.16^{a}	(0.8%)	99.44 ± 4.12^{e}	42.61 ± 0.25^{c}	(42.9%)
	Maize masa flour	745.60 ± 7.93^{e}	(0.6%)	91.75 ± 3.45^{f}	39.58 ± 0.14^{c}	(43.2%)
	Sprouted maize flour	904.82 ± 16.20^{b}	(0.8%)	99.94 ± 7.73^{e}	54.57 ± 0.25^{b}	(54.7%)

Means followed by the same letter within the same column were not significantly different ($p > 0.05$). Values in parentheses represent the percentage contribution of tryptophan to the total protein content, as well as available niacin to its total content. Values are related to dry matter.

Tab. 2. Contents of total soluble free, conjugated and insoluble bound phenolic compounds and flavonoids in untreated maize kernels and processed flour.

Maize	Samples	Total phenolics [mg·kg^{-1}]					
		Free and conjugated		Insoluble bound		Total	
White maize	Kernel	302.45 ± 6.11^{f}	(10.9%)	2315.53 ± 20.59^{c}	(89.1%)	2599.10 ± 26.71^{d}	
	Maize masa flour	472.46 ± 40.12^{e}	(61.0%)	302.27 ± 16.95^{ef}	(39.0%)	774.73 ± 43.24^{f}	
	Sprouted maize flour	580.74 ± 12.29^{d}	(22.4%)	2007.76 ± 11.11^{d}	(78.6%)	2588.51 ± 1.15^{d}	
Sweet maize	Kernel	684.65 ± 41.10^{c}	(19.7%)	2835.69 ± 199.57^{a}	(80.4%)	3484.34 ± 99.67^{b}	
	Maize masa flour	925.84 ± 60.06^{b}	(69.0%)	416.80 ± 7.19^{e}	(31.0%)	1342.64 ± 67.25^{e}	
	Sprouted maize flour	1000.19 ± 73.80^{a}	(26.0%)	2849.85 ± 11.14^{a}	(75.0%)	3850.04 ± 84.94^{a}	
Yellow maize	Kernel	316.76 ± 12.27^{f}	(11.2%)	2501.33 ± 66.19^{b}	(88.8%)	2818.10 ± 78.46^{c}	
	Maize masa flour	462.75 ± 33.17^{e}	(70.0%)	198.51 ± 1.79^{f}	(30.0%)	661.26 ± 34.96^{g}	
	Sprouted maize flour	474.52 ± 12.20^{e}	(16.8%)	2358.87 ± 7.30^{c}	(83.3%)	2833.40 ± 4.90^{c}	

Maize	Samples	Total flavonoids [mg·kg^{-1}]					
		Free and conjugated		Insoluble bound		Total	
White maize	Kernel	14.69 ± 1.34^{e}	(11.0%)	119.42 ± 0.59^{d}	(89.1%)	134.11 ± 0.75^{e}	
	Maize masa flour	31.12 ± 2.13^{bc}	(62.7%)	18.55 ± 1.78^{g}	(37.4%)	49.67 ± 2.00^{g}	
	Sprouted maize flour	35.39 ± 6.54^{b}	(24.9%)	106.92 ± 0.93^{e}	(75.1%)	142.31 ± 7.47^{d}	
Sweet maize	Kernel	19.15 ± 5.91^{de}	(12.3%)	136.52 ± 5.32^{b}	(87.7%)	155.67 ± 11.23^{c}	
	Maize masa flour	43.26 ± 2.99^{a}	(65.3%)	22.96 ± 1.43^{f}	(34.7%)	66.22 ± 0.56^{f}	
	Sprouted maize flour	42.10 ± 3.89^{a}	(24.4%)	130.30 ± 0.26^{c}	(69.9%)	172.40 ± 2.75^{a}	
Yellow maize	Kernel	21.05 ± 2.69^{d}	(12.7%)	144.96 ± 2.65^{a}	(87.3%)	166.01 ± 0.04^{b}	
	Maize masa flour	29.44 ± 3.65^{c}	(69.3%)	13.02 ± 0.48^{h}	(30.7%)	42.46 ± 2.51^{h}	
	Sprouted maize flour	33.44 ± 6.59^{bc}	(20.3%)	131.64 ± 0.13^{bc}	(79.8%)	165.08 ± 3.31^{b}	

Means followed by the same letter within the same column were not significantly different ($p > 0.05$). Values in parentheses represent the percentage contribution of each phenolic fraction to its total.
Total phenolics are expreesed as gallic acid equivalents per kilogram of dry matter. Total flavonoids are expreesed as catechin equivalents per kilogram of dry matter.

in masa flour were only about 13.1%, 14.7% and 7.9% of that present in untreated kernels of white, sweet and yellow maize, respectively. Our results also indicated a significant decrease in the total phenolics content after alkaline cooking. These results are in good accordance with literature data [17, 18]. Nixtamalization affects polyphenolic derivatives by breaking down ester linkages, consequently releasing free phenolic forms into the cooking solution [19]. According to the results of GUTIÉREZ-URIBE et al. [17], total phenolics of wastewater (nejayote) were about twice as much compared to maize kernels and masa. According to our study, sprouting significantly increased the contents of total soluble free and conjugated chemical forms of phenolics, whilst slightly decreased the bound form (Tab. 2). Compared to untreated maize kernels, the content of free and conjugated phenolic compounds that may be declared bioavailable increased by about 92%, 46% and 50% after sprouting for 5 days of white, sweet and yellow maize kernels, respectively. This result could be caused by hydrolysis of bound phenolic compounds that contribute to the increase of free phenolics content after sprouting. As regards the 1.10-fold increase in total phenolic compounds found in sprouted sweet maize flour compared to the untreated, it can be explained by biosynthesis of phenols de novo in the embryonic axis of sprouted kernels. Based on the results shown in Tab. 2, changes in the contents of individual chemical forms of total flavonoids caused by the applied treatments were similar to changes of the content of total phenolics.

Contents of total soluble free, conjugated and insoluble bound phenolic acids

Phenolic profiles of maize kernels and processed maize flours obtained by on HPLC analysis showed 7–10 compounds of which three were identified as caffeic, *p*-coumaric and ferulic acids. The contents of different chemical forms of the identified phenolic acids are given in Tab. 3. Ferulic acid was the major component, followed by *p*-coumaric and caffeic acids. The combined effects of alkaline and thermal processing during nixtama-

lization, together with leaching into the cooking solution, increased losses of total *p*-coumaric and ferulic acids in masa flour but enhanced the liberation of free + conjugated moieties. These findings are in accordance with De La Parra et al. [18]. In our study, bound *p*-coumaric and ferulic acids found in masa flour were approximately in the range from 14.3% to 31.2%, and from 12.5% to 20.3% of that present in whole kernels, respectively. On the other hand, content of soluble free + conjugated ferulic acid was at least 10 to 12 times higher in masa flour than in raw kernels, while the contents of soluble free + conjugated *p*-coumaric acid were about 4.96-, 1.82- and 3.75-fold higher in masa flour as compared to those in white, sweet and yellow maize kernels, respectively. Interestingly, the wastewater contained approximately 125 and 10 times more ferulic, and 15 and 53 times more bound ferulic acid compared to kernels and masa, respectively [17]. Several previous studies reported on an increase in free phenolic acids of sprouted cereals [9, 18]. In our study, there was a considerable increase (from 279% to 364%) in soluble free and conjugated ferulic acid content of

Tab. 3. Contents of soluble free, conjugated and insoluble bound phenolic acids in untreated maize kernels and processed flour.

Maize	Samples	Caffeic acid [mg·kg^{-1}]				
		Free and conjugated		Insoluble bound		Total
White maize	Kernel	nd		nd		–
	Maize masa flour	nd		nd		–
	Sprouted maize flour	nd		nd		–
Sweet maize	Kernel	33.31 ± 2.70^{c}	(77.3%)	9.73 ± 0.98^{a}	(22.7%)	43.07 ± 3.68^{b}
	Maize masa flour	37.32 ± 0.37^{b}	(88.0%)	5.10 ± 0.28^{b}	(12.0%)	42.42 ± 0.10^{b}
	Sprouted maize flour	69.11 ± 1.55^{a}	(94.5%)	4.03 ± 0.28^{c}	(5.5%)	73.15 ± 1.83^{a}
Yellow maize	Kernel	nd		nd		–
	Maize masa flour	nd		nd		–
	Sprouted maize flour	nd		nd		–

Maize	Samples	*p*–coumaric acid [mg·kg^{-1}]				
		Free and conjugated		Insoluble bound		Total
White maize	Kernel	0.73 ± 0.01^{h}	(0.5%)	140.69 ± 3.94^{b}	(99.5%)	141.42 ± 3.94^{bc}
	Maize masa flour	3.62 ± 0.09^{d}	(7.6%)	43.88 ± 0.80^{d}	(92.4%)	47.50 ± 0.89^{d}
	Sprouted maize flour	1.65 ± 0.01^{f}	(1.2%)	139.54 ± 5.07^{b}	(98.8%)	141.19 ± 5.07^{bc}
Sweet maize	Kernel	6.01 ± 0.32^{c}	(1.2%)	236.01 ± 17.34^{a}	(98.8%)	242.06 ± 17.67^{a}
	Maize masa flour	10.97 ± 0.62^{a}	(24.3%)	34.21 ± 1.28^{de}	(75.7%)	45.18 ± 0.66^{d}
	Sprouted maize flour	7.84 ± 0.42^{b}	(3.1%)	243.32 ± 2.05^{a}	(96.9%)	251.17 ± 1.63^{a}
Yellow maize	Kernel	1.03 ± 0.01^{g}	(0.8%)	133.08 ± 2.16^{bc}	(99.2%)	134.11 ± 2.16^{c}
	Maize masa flour	3.86 ± 0.10^{d}	(16.9%)	18.98 ± 1.31^{e}	(83.1%)	22.84 ± 1.22^{e}
	Sprouted maize flour	2.15 ± 0.01^{e}	(1.7%)	124.88 ± 4.27^{c}	(98.3%)	127.03 ± 4.27^{c}

Maize	Samples	Ferulic acid [mg·kg^{-1}]				
		Free and conjugated		Insoluble bound		Total
White maize	Kernel	4.40 ± 0.01^{h}	(0.2%)	2150.73 ± 4.17^{c}	(99.8%)	2155.13 ± 4.22^{c}
	Maize masa flour	54.02 ± 0.39^{c}	(12.1%)	390.84 ± 2.16^{f}	(87.9%)	444.86 ± 2.55^{f}
	Sprouted maize flour	16.96 ± 0.05^{f}	(0.9%)	1936.72 ± 11.74^{d}	(99.1%)	1953.68 ± 11.74^{d}
Sweet maize	Kernel	10.60 ± 0.58^{g}	(0.4%)	2684.94 ± 92.22^{a}	(99.6%)	2695.54 ± 92.80^{a}
	Maize masa flour	107.26 ± 3.64^{a}	(16.4%)	545.33 ± 6.79^{e}	(83.6%)	652.59 ± 3.15^{e}
	Sprouted maize flour	49.26 ± 0.26^{d}	(1.8%)	2623.72 ± 11.74^{a}	(98.2%)	2672.98 ± 9.83^{a}
Yellow maize	Kernel	5.16 ± 0.03^{h}	(0.2%)	2406.93 ± 21.31^{b}	(99.8%)	2412.09 ± 21.31^{b}
	Maize masa flour	65.23 ± 3.39^{b}	(17.8%)	301.62 ± 10.66^{g}	(82.2%)	366.85 ± 7.27^{g}
	Sprouted maize flour	20.35 ± 0.05^{e}	(0.9%)	2168.50 ± 40.21^{c}	(99.1%)	2188.85 ± 40.21^{c}

Means followed by the same letter within the same column were not significantly different ($p > 0.05$). Values in parentheses represent the percentage contribution of each phenolic acid fraction to its total. Values are related to dry matter.
nd – not detected.

Tab. 4. Contents of carotenoids in untreated maize kernels and processed flour.

Maize	Samples	Zeaxanthin [mg·kg^{-1}]	Lutein [mg·kg^{-1}]	β-carotene [mg·kg^{-1}]	Total detected carotenoids [mg·kg^{-1}]
White maize	Kernel	nd	0.20 ± 0.01^{g}	nd	0.20 ± 0.01^{g}
	Maize masa flour	nd	0.17 ± 0.01^{g}	nd	0.17 ± 0.01^{g}
	Sprouted maize flour	nd	0.22 ± 0.01^{g}	nd	0.22 ± 0.01^{g}
Sweet maize	Kernel	3.87 ± 0.23^{d}	4.04 ± 0.18^{d}	0.31 ± 0.03^{b}	8.22 ± 0.46^{d}
	Maize masa flour	1.82 ± 0.01^{e}	1.96 ± 0.01^{f}	nd	3.78 ± 0.02^{f}
	Sprouted maize flour	3.68 ± 0.07^{d}	3.53 ± 0.03^{e}	0.29 ± 0.03^{b}	7.50 ± 0.14^{e}
Yellow maize	Kernel	12.54 ± 0.06^{a}	10.89 ± 0.10^{a}	0.60 ± 0.02^{a}	24.03 ± 0.19^{a}
	Maize masa flour	5.23 ± 0.03^{c}	6.04 ± 0.04^{c}	0.20 ± 0.01^{c}	11.46 ± 0.06^{c}
	Sprouted maize flour	8.72 ± 0.13^{b}	8.50 ± 0.16^{b}	0.24 ± 0.01^{c}	17.46 ± 0.29^{b}

Means followed by the same letter within the same column were not significantly different ($p > 0.05$). Values are related to dry matter. nd – not detected.

Tab. 5. Contents of tocopherols in untreated maize kernels and processed flour.

Maize	Samples	α-tocopherol [mg·kg^{-1}]	γ-tocopherol [mg·kg^{-1}]	δ-tocopherol [mg·kg^{-1}]	Total detected tocopherols [mg·kg^{-1}]
White maize	Kernel	3.67 ± 0.95^{e}	32.35 ± 2.97^{d}	nd	36.02 ± 2.92^{d}
	Maize masa flour	nd	14.75 ± 0.38^{f}	nd	14.75 ± 0.38^{f}
	Sprouted maize flour	5.34 ± 0.06^{d}	31.79 ± 0.20^{d}	nd	37.13 ± 0.26^{d}
Sweet maize	Kernel	11.70 ± 0.70^{b}	42.40 ± 2.79^{a}	nd	54.10 ± 2.49^{a}
	Maize masa flour	nd	12.37 ± 1.19^{g}	nd	12.37 ± 1.19^{g}
	Sprouted maize flour	20.60 ± 0.13^{a}	34.52 ± 1.20^{c}	nd	55.12 ± 1.07^{a}
Yellow maize	Kernel	3.85 ± 0.30^{e}	36.85 ± 0.86^{b}	nd	40.70 ± 1.16^{c}
	Maize masa flour	3.87 ± 0.19^{e}	27.81 ± 0.52^{e}	nd	22.22 ± 0.33^{e}
	Sprouted maize flour	6.87 ± 0.26^{c}	36.14 ± 1.05^{bc}	nd	43.01 ± 0.62^{b}

Means followed by the same letter within the same column were not significantly different ($p > 0.05$). Values are related to dry matter. nd – not detected.

maize flour after sprouting, respectively, while the increase in soluble free and conjugated caffeic and *p*-coumaric acid contents was to a lesser extent (Tab. 3). It can be concluded that accumulation of soluble phenolic acids was due to hydrolysis of phenolics bound to cell walls.

Content of carotenoids

Total carotenoids content of maize kernels ranged from 0.20 mg·kg^{-1} (dry matter basis) in white maize to 24.03 mg·kg^{-1} (dry matter basis) in yellow maize (Tab. 4). Lutein and zeaxanthin were major carotenoid species in sweet and yellow maize kernels, accounting for about 97% of total carotenoid content, while the rest being β-carotene. White maize kernels contained only lutein. It is well-known that carotenoids are highly sensitive to light, heat and air. According to our results, alkaline cooking was the process that considerably affected the content of carotenoids. During this process, more than 50% of carotenoids were lost into the cooking liquor. Our results are in accordance with data reported by De La Parra et al. [18]. According to Kean et al. [20], some loss of lutein and zeaxanthin is often explained by isomerization during thermal processing. Regardless of the reduction, due to the high carotenoid content of raw kernels, the masa flour prepared from the yellow maize had higher contents of zeaxanthin and lutein than those of untreated kernels of sweet maize. Compared with the alkaline cooking treatment, sprouting decreased the content of each of the three carotenoids but to a much lesser extent. This process did not result in significant losses of zeaxanthin and β-carotene of sweet maize. On the other hand, the carotenoid losses during sprouting of yellow maize were 30.5% for zeaxanthin, 22.0% for lutein and 60% for β-carotene.

Content of tocopherols

The content of tocopherols in maize kernels and processed maize flour samples is given in Tab. 5. The highest content of total tocopherols

was detected in kernels of sweet maize. Compared with this genotype, the content in kernels of white and yellow maize was lower by 33.4% and 24.8%, respectively. In our study, the losses of tocopherols occurred for all maize genotypes during alkaline cooking. The loss was more pronounced for the sweet maize, which lost about 77% of its initial total tocopherol content, while white and yellow maize lost about 59% and 45%, respectively. Complete α-tocopherol losses were observed for both white and sweet maize when processed into masa flour. However, the content of α-tocopherol was not significantly affected by alkaline cooking of yellow maize. This is congruent to the data of BARRERA-ARELLANO et al. [21] who reported that α-tocopherol is less stable than other tocopherol forms. In addition, alkaline cooking decreased the content of γ-tocopherol in flour by 2.19-, 3.43- and 1.33-fold compared to that in untreated white, sweet and yellow maize kernels, respectively. In accordance with the results of ŽILIĆ et al. [9], the content of α-tocopherol was significantly affected by sprouting, being increased on average for about 1.5-fold. In contrast, sprouting reduced the contents of γ-tocopherol, but the reduction was not significant for white and yellow maize flours. Although the changes of α- and γ-tocopherols during sprouting caused increase of total tocopherols content in flour samples, only the change of the ratio of α- to γ-tocopherol (1 : 10 versus 2 : 10) in sprouted yellow maize flour significantly increased the total content. According to ZHANG et al. [22], during the first days of germination, a decrease in γ-tocopherol is offset by an increase in α-tocopherol, indicating the interconversion of these isomers. After that time period, a net increase in α-tocopherol and total tocopherols suggested the synthesis of new tocopherols.

Tab. 6. Pearson's correlation coefficients between antioxidant capacity of maize flours and content of bioactive compounds.

	Antioxidant capacity	
	Alkaline cooking	Sprouting
Phenolics		
Soluble, free and conjugated	0.99**	0.87*
Insoluble bound	–0.23	0.19
Total phenolics	–0.04	0.57
Flavonoids		
Soluble, free and conjugated	0.66	0.86*
Insoluble bound	–0.29	–0.26
Total flavonoids	–0.21	0.39
Caffeic acid		
Soluble, free and conjugated	0.95**	0.61
Insoluble bound	0.82*	0.34
Total caffeic acid	0.93**	0.60
p-Coumaric acid		
Soluble, free and conjugated	0.97**	0.72*
Insoluble bound	–0.05	0.52
Total *p*-coumaric acid	–0.01	0.54
Ferulic acid		
Soluble, free and conjugated	0.65	0.80*
Insoluble bound	–0.21	0.15
Total ferulic acid	–0.19	0.20
Carotenoids		
Zeaxanthin	–0.19	–0.25
Lutein	–0.17	–0.22
β-carotene	–0.21	–0.17
Total carotenoids	–0.18	–0.23
Tocopherols		
α-tocopherol	0.08	0.71*
γ-tocopherol	–0.33	0.02
Total tocopherols	–0.18	0.57

* – significant at $p < 0.05$; ** – significant at $p < 0.01$.

Antioxidant capacity

The direct or QUENCHER method was used to determine the antioxidant capacity of maize kernels and of processed maize flours, and these values were compared to the amounts of bioactive compounds (Fig. 1, Tab. 6). Given the highest total phenolics content of the sweet maize kernels, this genotype exhibited a higher antioxidant capacity in relation to the white and yellow genotypes. In contrast to the results of DEL POZO-INSFRAN et al. [23], as well as DE LA PARRA et al. [18], our results clearly showed that total antioxidant capacity was increased by 1.21-fold following alkaline processing of all three maize genotypes, despite the dramatic decline in total phenolics and flavonoid contents, as well as carotenoids and tocopherols contents. The increase in total antioxidant capacity of the alkaline-processed white, sweet and yellow maize could be explained by the increased release of other bound phenolics. The results of ŽILIĆ et al. [9] showed that wheat grain phenolic compounds in the bound form, as dominant, exerted lower antioxidant capacity in comparison with its hydrolysed and isolated free forms in extracts. In addition to the soluble free and conjugated phenolics, the formation of Maillard reaction products could be the reason of the enhanced total antioxidant capacity of maize masa flour. Our findings imply that sprouting also enhanced the antioxidant activity of maize kernels. After 5 days of sprouting, the total antioxidant capacities of white, sweet and yellow maize flour were 29.42 mmol·kg^{-1},

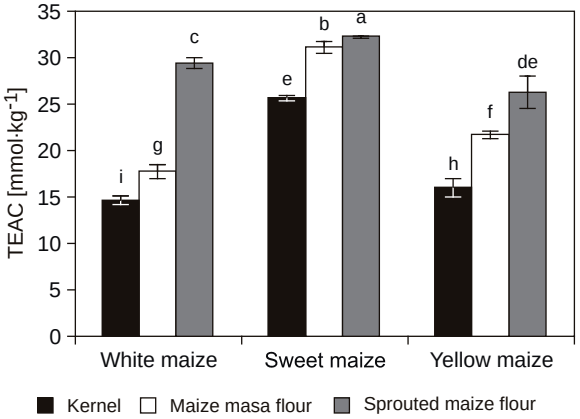

Fig. 1. Total antioxidant capacity of untreated maize kernels and processed flour obtained by QUENCHER method.

The vertical bars represent standard deviations. Bars with different letters are statistically significantly different ($p < 0.05$). Antioxidant activity is expressed as Trolox equivalents per kilogram of dry matter.

32.21 mmol·kg^{-1} and 26.26 mmol·kg^{-1} (dry matter basis; expressed as Trolox equivalents) with significant increases by 101.2%, 26.3%, and 64.5% as compared to those of untreated maize kernels, and by 65.5%, 4.2% and 35.4% as compared to those of masa flour, respectively.

CONCLUSIONS

The present study shows that alkaline hydrolysis has great potential to release phenolics associated with cell walls. However, this process includes harsh heat treatment and chemical modifications. Therefore, conditions of alkaline cooking had strong effect on the loss of bound phenolic compounds, as well as niacin, carotenoids and tocopherols in maize kernels. In contrast to alkaline cooking, sprouting enhanced the phenolic compounds by either biosynthesis de novo or release of these compounds by induced enzymatic hydrolysis, contributing to the increase in antioxidant capacity of whole maize flour. In addition, the present study shows that sprouting enhanced the nutritional value of maize flours through biosynthesis of niacin and tocopherols.

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