



THE INFLUENCE OF CULINARY PROCESSING ON THE CONTENT OF SOME SECONDARY METABOLITES IN FLINT CORN SEEDS

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Received 25th April 2016, accepted 30th May 2016

Abstract. *The influence of culinary processing on the content of phenolic and phytic acid in flint corn seeds, belonging to ten Romanian local populations, was the purpose of this paper.*

*The biological material, used in experiments, was represented by flint corn seeds (*Zea mays* L. var. *indurata*) belonging to ten Romanian local populations. There were conducted three culinary processing of seeds (boiling, steaming and roasting,) followed by the determination of total phenolic content and phytic acid in thermally processed grains and the water used. The culinary processing of corn seed samples has significantly influenced the seeds' content in phenols and the phytic acid. Thus, both the boiling and the steaming process led to the decrease of phenolic compounds and of the phytic acid in corn seeds, through thermal destruction (more) and through releasing into used waters (less), which could be the sources of such metabolites. The roasting process has generally led to significant increase of phenolic compounds, and to decrease of phytic acid.*

Keywords: *culinary, phenolic content, phytic acid, corn seeds*

1. Introduction

Consumption of fruits, vegetables, and unpolished grains is strongly associated with the reduced risk of developing chronic diseases such as cancer and cardiovascular disease [1,2] cited by [5].

Phytochemicals in food are beneficial to health due to their high antioxidant properties, which is related to prevention of cardiovascular disease, diabetes or obesity and mutagenesis or cancer [3,4] cited by [5].

Plant phenolics have potential health benefits mainly due to reactive oxygen species scavenging and inhibition, electrophile scavenging, and metal chelation [6].

The interest in antioxidant activities, bioactive properties and anti-radical

scavenging activities of anthocyanins and phenolics has increased [7,4], being, according to epidemiological and in vitro research [8], inverse relationships between the incidence diseases such as cardiovascular, cancer and cataracts, that come with aging, and consumption of vegetables and fruits containing anthocyanins and phenolics.

Like other secondary metabolites (anthocyanins and carotenoids), phenolics are influenced by plant species and varieties [9,10,11,12,13], and are primarily synthesized through the pentose phosphate pathway (PPP), shikimate and phenylpropanoid pathways [14].

Phenolic compounds exhibit pharmacological properties such as: antitumor, antiviral, antimicrobial, antiinflammatory, hypotensive and

antioxidant activity [15,16], being a relationship between the consumption of phenolic-rich food products and a low incidence of coronary heart disease, atherosclerosis, certain forms of cancer and stroke [17,18,19,20].

The phytic acid (inositol hexaphosphate or phytate) is a major component of all plant seeds [21,22], and in cereal grains, most of the minerals form salts with this acid, that act as a storage compound of phosphorous [23]. Phytate inhibits lipid peroxidation by forming a complex in which all six coordination sites of Fe (III) are occupied by phytate [24].

According to Shamsuddin and Ullah (1989), the phytic acid can have anticancer effects at both the initiation and promotion stages of colon cancer; it significantly reduced colon tumor number, size and mitotic rate, when compared to the control group.

Considered as antinutritional factors, because they interact with food constituents such as minerals which make them unavailable [26,27], phytate and polyphenols can be reduced by processing methods such as: soaking, sprouting, cooking, malting and fermentation [28,27,29,30,31,32].

Corn (*Zea mays* ssp. *Mays*) is a major cereal used to produce grain and fodder that are the basis for a number of foods, feed, pharmaceutical and industrial products. Due to its adaptability and productivity, it is the third most cultivated field crop after wheat and rice [14].

In our diets, corn is a source of macro- and micronutrients [33,34], a rich source of many phytochemicals, including carotenoids [34,35,36,37,38,13,39], phenolic compounds [34,35,40,41,13,39], anthocyanins [35,41,42], tocopherols [34,43] and phytic acid, which have multiple functional roles, for example, as antioxidants [35,11,12,24], as

antimutagens [11], and as inhibitors of colorectal carcinogenesis [11,10,25].

Cooking induces changes in physiological and chemical composition, influencing the concentration and bioavailability of bioactive compounds in food [44]. Turkmen et al. (2005) found that thermal treatments decreased the total phenolics in squash, peas and leek, while in sweet corn cooking led to an increase in the level of phenolic compounds [45].

According to Oliveira et al., 2010 [65], cited by [46], to understand better the effects of cooking on antioxidant levels in food, it is necessary to test real cooking conditions, because the behavior of any food cannot be predicted.

In this paper there was studied to what extent the culinary processing influences the content of phenolic compounds and phytic acid in corn seeds, belonging to ten Romanian local populations.

2. Experimental

2.1. Research materials

The biological material, used in this work, was represented by flint corn seeds (*Zea mays* L. var. *indurata*) belonging to ten Romanian local populations (LP). Working and control samples from each LP grains were made up, using dried seeds (moisture content = 8-10 %) coming from crops of the last two years.

2.2. Procedure and research methods

Three culinary processings were conducted, as follows.

For boiling, there were taken 100 corn seeds of each sample, which have been placed in a stainless steel vessel with three liters capacity. The boiling was done in one liter of tap water in the pot covered, for 30 minutes (timed from the moment when the water began to boil).

For steaming, in each pot, a dense mesh fixed to the walls was mounted, to two-

thirds of the vessel bottom. After pouring of one liter of tap water into the bowl, on the sieve were placed 100 seeds that were steamed, within covered pot, for 30 minutes (timed from the moment when the water began to boil).

For roasting, 100 seeds from each sample were roasted for 20 minutes at 150-190°C, in a covered stainless steel vessel.

In order to determine Total Phenolic Content (TPC), first an extract for each seeds sample was obtained, weighing 1 g of grains, which were finely ground and subjected to extraction with a mixture methanol and water (80/20), by stirring, centrifuging and recovering the supernatant [47]. The estimation of Total Phenolic Contents in seeds extract was carried out through a colorimetric assay, by measuring its reducing capacity with Folin-Ciocalteu reagent. TPC was expressed as mg Gallic Acid Equivalent/g dry seeds ie dry weight (mg GAE/g DW). For this purpose, a standard curve was generated, representing the absorbance values of gallic acid standard solutions in relation to their concentrations [48].

The phytic acid (PA) determination was done using Garcia-Villanova method, based on treating the sample with a ferric salt and titration of Fe³⁺ ions (which has not precipitated with phytic acid), with

EDTA solution, in the presence of sulfosalicylic acid as an indicator [49]. For this assay, 1 g of grains was finely ground, adding HCl-Na₂SO₄ solution for phytic acid extraction. After some operations (filtration, adding of HCl-Na₂SO₄, clorhidric solution of feric clorure, and sulfosalicylic acid solution, boiling, filtration) the mixture obtained was heated at 70°C and Fe³⁺ ions were titrated (la cald), according to the method above mentioned. Phytic acid content was expressed as mg per 100 g dry weight (mg% DW).

2.3. Statistical analysis. The data of experiments, consisting in four replicates for each determination, were statistically processed using SAS Version 8.02 [50]. In order to analyze the significance of differences among samples, generalized linear model analysis was carried out, and for multiple comparisons was used Duncan's multiple range test ($P < 0.05$).

3. Results and discussion

In the Table 1 are rendered the values of the total phenolic content (TPC) in the ten flint corn seed samples, subjected to culinary processing.

Table 1
Comparative values of the total phenolic content in flint corn seeds culinary processed

Corn local populations (LP)	TPC (mg GAE/g DW)					
	Raw material*	Boiled		Steamed		Roasted Seeds
		Seeds	Water	Seeds	Water	
LP1 (white)***	7.5±0.5C**	4.3±0.6E**	0.9±0.07FG	6±0.8D	0.2±0.02G	8.6±0.9C
LP2 (yellow)	8.2±0.7C	4.9±0.3DE	0.8±0.05FG	6.5±0.3D	0.3±0.03G	9.1±0.6BC
LP3 (yellow)	7.9±0.4C	4.7±0.8DE	1.1±0.09FG	6.2±0.7D	0.2±0.01G	9.4±0.7BC
LP4 (red)	8.7±0.9BC	4.9±0.5DE	0.8±0.06FG	7.1±0.5CD	0.3±0.05G	8.5±0.5C
LP5 (purple)	9.1±0.6BC	5.3±0.6DE	0.9±0.06FG	6.9±0.3CD	0.5±0.06G	10.2±0.8B
LP6 (white)	6.5±0.7D	4.1±0.2E	0.6±0.07G	5.3±0.6CD	0.3±0.01G	7.4±0.5CD
LP7 (red)	10.2±0.8B	5.9±0.4D	1.2±0.06FG	8.1±0.7C	0.4±0.02G	11.8±1.2A
LP8 (red)	8.8±0.6BC	5.3±0.6DE	1.1±0.08FG	7±0.4CD	0.2±0.03G	9.7±0.8B
LP9 (yellow)	6.8±0.8CD	3.8±0.5E	0.9±0.07FG	5.6±0.7D	0.2±0.04G	7.5±0.6C
LP10 (purple)	9.7±0.9B	5.9±0.7D	1.2±0.09FG	7.7±0.9C	0.3±0.05G	9.5±0.9BC

*Unprocessed seeds; **Means with different letters are statistically different ($P < 0.05$); ()***kernel colour

As seen from Tab. 1, the total phenolic content (TPC) in seeds of the ten maize local populations (LP) ranged between 6.5 ± 0.7 and 10.2 ± 0.8 mg GAE/g DW, the highest values being found in red and purple grains. According to some authors [13,39,51], cited by [5], the pigmented corn contains (more) anthocyanins, carotenoids, phenolic compounds, and antioxidant activity than non-pigmented corn

Compared with control samples (raw seeds), TPC of seeds has been modified after processing by boiling, steaming or baking (roasting).

Thus, boiling for 30 minutes led to a significant decrease ($P < 0.05$) of the total content of phenolic compounds from the seeds of LP, with percentages between 38% (LP6) and 43% (LP4 and LP9).

Analyzing the boiling water of seeds, the presence of phenolic compounds was found, whose percentages were between 10% (LP2, LP4, LP5 and LP6) and 14% (LP3 and LP9).

Gathering (mathematically) the content of phenolic compounds from the seeds of each boiled sample and from its boiling water and comparing it with the control (unprocessed seeds), significant differences were found ($P < 0.05$), whose explanation is that a part of the phenolic compounds was destroyed by cooking. These losses of total content of phenolic compounds in the analyzed samples ranged between 26% (LP3) and 33% (LP4), i.e. about 2.5 times greater than phenols released in the boiling water. These first results of this work are consistent with some data reported by some researchers.

Thus, studying antioxidant components, antioxidant activity, and their changes during traditional cooking of fresh purple waxy corn, Harakotr et al. (2014a) found a higher content of phenolics in the boiling

water, than in steaming one, attributed, by authors, to the losses of phenolic in cooking water. Since the sum of phenolics in cooked samples and cooking water consistently differed from their content in raw samples, differences in phenolic content could be, by Harakotr et al. (2014a), due to breakdown of phenolics, which was greater than losses due to leaching into cooking water.

Regarding seeds steaming, the Table 1 shows that this thermal processing for 30 minutes significantly reduced ($P < 0.05$) the total phenols from analyzed seed samples, with percentages between 17% (LP9) and 24% (LP5).

The analysis of water used for seeds steaming, showed the presence of phenolic compounds, with percentages ranging from 2% (LP8) and 5% (LP5 and LP6).

Gathering (mathematically) the content of phenolic compounds from the seeds of each steamed sample and from its steaming water, and comparing it with the control (raw seeds), significant differences were ($P < 0.05$) caused by the destruction of phenolic compounds through steaming. The phenolic compounds losses, through this type of thermal processing, have ranged between 13% (LP6) and 19% (LP3 and LP5), i.e. about 4-6 times greater than phenols released into steaming waters.

Comparing the results of the two types of thermal processing, we can see that steaming has caused losses of phenolic compounds significantly lower ($P < 0.05$) within analyzed corn seeds.

Referring to corn seeds, Harakotr et al. (2014a), reported that boiling had a more detrimental effect on phenolic acids than steaming.

The boiling treatment causes of disruption cellular components with the consequent release of these molecules into the cooking water ([52], cited by [46]), while steaming

treatment causes matrix softening and increases extractability of antioxidant components from the raw materials [53]. By Xu and Chang, (2009), the thermal treatment could cause changes in phenolic substances, in the corn, by liberating the bound phenolic compounds into free form. Analyzing the total content of phenolic compounds in roasted corn seeds and comparing them with control samples (raw seeds), it appears that, except three samples (LP1, LP4, and LP10), in all other samples this parameter has registered significant increases ($P < 0.05$), with percentages between 10.2% (LP8) and 19% (LP3).

This result is consistent with observations and data reported in some scientific papers. Thus, Han and Koh (2011), researching antioxidant activity of hard wheat flour, dough and bread prepared using addition of different phenolic acids, found that the

antioxidant activity and residual free phenolic acid content of flour were reduced by mixing, but increased by fermentation and baking.

By Cheng et al. (2006), the thermal treatment causes phytochemical degradation, oxidation, and Maillard reactions resulting in changes in antioxidant property. Maillard reaction products may protect phytochemicals from oxidation [57], and those products, derived from mixtures of glucose or fructose and cysteine or glutathione, greatly inhibit activities of polyphenoloxidases and oxidoreductases [58]. Heat treatment at 150°C for 40 min. liberated bound phenolics in citrus peels having as result a significant increasing of TPC after treatment [59].

In the Table 2 are rendered the values of the phytic acid content in the ten flint corn seed samples, subjected to culinary processing.

Table 2
Comparative values of the phytic acid content in flint corn seeds culinary processed

Corn local populations (LP)	Phytic acid (mg% DW)					
	Raw material*	Boiled		Steamed		Roasted Seeds
		Seeds	Water	Seeds	Water	
LP1 (white)***	0.9±0.02b**	0.6±0.05bc**	0.15±0.01c	0.8±0.09b	0.05±0.002d	0.72±0.09bc
LP2 (yellow)	0.7±0.05bc	0.5±0.07bc	0.1±0.02c	0.6±0.03bc	0.04±0.005d	0.55±0.03bc
LP3 (yellow)	1.8±0.07a	1.2±0.15b	0.4±0.03bc	1.6±0.12ab	0.09±0.01cd	1.31±0.15ab
LP4 (red)	1.3±0.09ab	1±0.09b	0.15±0.02c	1.2±0.08b	0.06±0.004cd	0.97±0.1b
LP5 (purple)	1.2±0.14b	0.9±0.06b	0.2±0.05c	1±0.1b	0.08±0.01cd	0.94±0.08b
LP6 (white)	0.9±0.07b	0.7±0.09bc	0.15±0.01c	0.8±0.05b	0.04±0.006d	0.76±0.05bc
LP7 (red)	1.5±0.08ab	1±0.16b	0.3±0.02c	1.4±0.16ab	0.06±0.002cd	1.14±0.09b
LP8 (red)	1.7±0.06a	1.3±0.11ab	0.3±0.05c	1.5±0.12ab	0.1±0.04c	1.34±0.14ab
LP9 (yellow)	0.8±0.09b	0.6±0.02bc	0.1±0.01c	0.7±0.08bc	0.08±0.005cd	0.64±0.03bc
LP10 (purple)	1.9±0.08a	1.4±0.17ab	0.2±0.04c	1.8±0.2a	0.06±0.007cd	1.46±0.16ab

*Dried seeds; **Means with different letters are statistically different ($P < 0.05$); (***)kernel colour

Tab. 2 shows that, compared with the control samples (raw seed), the boiling process for 30 minutes led to a significant decrease ($P < 0.05$) of the phytic acid content in seeds of eight LP, with percentages between 18% (LP9) and 32% (LP3).

Analyzing the phytic acid content in the boiling water of seeds, the presence of this

compound was found, whose percentages were between 12% (LP4) and 20% (LP3).

Adding the phytic acid content from seeds of each sample and from its boiling water, and comparing it to the control sample (raw seeds), significant differences were found ($P < 0.05$), explained by the destruction of this compound through boiling. These losses (percentage) of

phytic acid in the analyzed samples ranged between 5% (LP9) and 13% (LP5 and LP7), of about 1.5-2.4 times lower than phytic acid released into the boiling waters. Gupta and Sehgal (1991) have observed a decrease in phytic acid contents of cereal grains used for preparing weaning foods as a result of soaking and germination. The decrease in the level of phytic acid during soaking may be attributed to leaching the acid out into soaking water under the concentration gradient [26,32, cited by 61]. According to Sathe and Venkatachalam (2002), being soluble in water, phytate can be reduced if a soaking step is carried out at temperatures above 45°C but below 60°C, a significant percent of phytate hydrolysis can take place due to activation of endogenous phytases and acid phosphatases, during the early part of the cooking phase. If we take into consideration those mentioned by Sathe and Venkatachalam (2002), in the experiment of this work, the content of phytic acid in boiled seeds would be reduced during their immersion, at temperatures below 60°C, through the phytate hydrolysis caused by phytases and endogenous acid phosphatases activated during this part of the cooking process. It is about the phytate identified in the boiling water. But phytic acid losses should be attributed to its destruction by heat treatment (boiling).

From Tab. 2 one can note that, through seeds steaming for 30 minutes, the content of phytic acid was significantly reduced ($P<0.05$) in seeds of only four samples (from ten analyzed), with percentages between 8% (LP3) and 15% (LP9).

The analysis of water used to seeds steaming showed the presence of phytic acid, with percentages between 3% (LP10) and 10% (LP9).

Adding the phytic acid content from the seeds of each steamed sample and from its steaming water, and comparing it with the

control (raw seeds), significant differences were found ($P<0.05$) caused by the phytate destruction. The losses of this compound, by means of this type of thermal processing, have ranged between 3% (LP3) and 8% (LP8), i.e. about 2-2.5 times lower than phytate released into steaming waters. If we compare the content of phytic acid in roasted seeds with control samples (raw seeds), one can see that this parameter has registered significant decrease ($P<0.05$), with percentages between 15% (LP6) and 27% (LP3). This result is consistent with observations and data reported by some authors.

According to Fretzdorff and Brümmer (1992), the pH and cooking temperature are important factors reducing the content of phytic acid, because in doughs with pH 4.3-4.6 phytic acid is more effectively reduced than in doughs with higher pH. In roasted sliced French toast the baking temperature has reduced phytic acid content by 86% [64].

By some authors [66,67,68,69,70,71,72] cited by [73], the possible beneficial effects of food phytates include lowering of serum cholesterol, triglycerides and protection against certain diseases such as cardiovascular diseases, renal stone formation, and certain types of cancers.

Because phytates can chelate minerals (copper, iron, zinc, magnesium etc.), and many of these minerals are essential cofactors for numerous oxidoreductases, phytate may act as antioxidant *in vivo* [74,75,76], whose activity would be responsible for free radical scavenging action *in vivo*, leading to anticancer activity [69]. According to studies on animals and humans, the phytates decrease mineral bioavailability through forming complexes with these minerals [77,78].

Based on the result obtained in the experiments of this work, all culinary processing used on corn seeds (boiling, steaming and roasting) may contribute, by

means of phytate reduction, to the increase of the minerals bioavailability.

4. Conclusions

The culinary processing of corn seeds, belonging to ten local populations of flint corn (*Zea mays* L. var. *indurata*) has significantly influenced the phenols and the phytic acid seeds content.

Thus, as compared to control samples, the thermal processing of corn seeds by boiling and steaming for 30 minutes, resulted in significant losses (mainly through thermal destruction), higher at boiling, both of phenolic compounds and of phytic acid. In cooking water the presence of these compounds was found (more in boiling and less in steaming ones), thus these waters can be exploited as sources of such metabolites.

In the case of roasted corn seeds (20 min. at 150-190°C), compared to the control samples, the phenolic compounds have generally registered significant increases, apparently due to Maillard reaction products, which have protected phenols against oxidation, on the one hand, and due to the release of bound phenols, by the action of high temperatures and exposure time, on the other hand.

Regarding the phytic acid, the roasting of maize seeds, in the same experimental conditions, has led to significant reductions of this metabolite concentration in all maize samples analyzed.

By careful choice of culinary processing methods of maize seeds would retain a higher level of useful metabolites.

5. Acknowledgments

Many thanks to Suceava Genebank and to Suceava Agricultural Research and Development Station for the biological material provided.

6. References

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