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THE INFLUENCE OF THERMAL PROCESSING ON TOTAL PHENOLIC CONTENT IN POPCORN SEEDS

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Abstract. The aim of this paper was to search how much the thermal processing can influence the total phenolic content in popcorn seeds. The biological material, used in this work, was represented by popcorn seeds (Zea mays L. var. everta) belonging to eight Romanian local populations. The experiment consisted in three different thermal processings (boiling and steaming for 30 minutes, and roasting for 20 minutes at 150-190°C), followed by determination of total phenolic content in processed grains and in used waters. 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. For this purpose, a standard curve was generated, representing the absorbance values of gallic acid standard solutions in relation to their concentrations. As compared to control samples (raw material), the thermal processing of popcorn seeds, by boiling and steaming, resulted in significant losses of phenolic compounds, higher through boiling. The presence of phenols was also found within cooking waters, more in boiling, and less in steaming ones. Compared to the control samples, in the roasted popcorn seeds the phenolic compounds have registered significant increases, probably due to the release of bound phenols by the action of high temperatures and exposure time.

INTRODUCTION

Consumption of fruits, vegetables, and un-polished grains is strongly associated with the reduced risk of developing chronic diseases such as cancer and cardiovascular disease (*Isabelle et al., 2010; Liu, 2004,* cited by *Harakotr et al., 2014b*).

Plant phenolics have potential health benefits mainly due to reactive oxygen species scavenging and inhibition, electrophile scavenging, and metal chelation (*Huang et al., 1992*).

Corn (*Zea mays* L.) 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 (*Randhir and Shetty, 2005*).

In our diets, corn is a source of macro- and micronutrients (*Gonzalez et al., 2005; Chander et al., 2008*), a rich source of many phytochemicals, including carotenoids (*Chander et al., 2008; De La Parra et al., 2007; Kopselld et al., 2009; Kean et al., 2008, Li et al., 2007a; Lopez-Martinez et al., 2009; Montilla et al., 2011*), phenolic compounds (*Chander et al., 2008; De La Parra et al., 2007; Santiago et al., 2007; Pedreschi and Cisneros-Zevallos, 2007; Lopez-Martinez et al., 2009; Montilla et al., 2011*), anthocyanins (*De La Parra et al., 2007; Pedreschi et Cisneros-Zevallos, 2007; Li et al., 2008*), tocopherols (*Chander et al., 2008, Ibrahim and Juvik, 2009*), and phytic acid, which have multiple functional roles, for example, as antioxidants (*De La Parra et al., 2007; Pedreschi and Luis, 2006; Li et al., 2007b; Graf and Eaton, 1990*), as antimutagens (*Pedreschi and Luis, 2006*), and as inhibitors of colorectal carcinogenesis (*Pedreschi and Luis, 2006; Zhao et al., 2005; Shamsuddin and Ullah, 1989*).

Due to their high antioxidant properties, anthocyanin pigments in corn have anti-inflammatory effects (*He and Giusti, 2010*), can prevent diabetes and obesity (*Tsuda et al., 2003*), heart ischemia–reperfusion injury and hyperlipidemia (*Toufektsian et al., 2008*), and potentially reduce the risk of colon cancer (*Hagiwara et al., 2001*).

Phenolic compounds exhibit pharmacological properties such as: antitumor, antiviral, antimicrobial, antiinflammatory, hypotensive and antioxidant activity (*Cowan, 1999; Shetty, 1997*), 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 (*Hertog et al., 1993; Diaz et al., 1997; Ito and Hirose, 1989; Ness and Powles, 1997*).

Like other secondary metabolites (anthocyanins and carotenoids), phenolics are influenced by plant species and varieties (*Abdel-Aal and Hucl, 1999; Zhao et al., 2005; Pedreschi and Luis, 2006; Li et al., 2007b; Lopez-Martinez et al., 2009*), and are primarily synthesized through the pentose phosphate pathway (PPP), shikimate and phenylpropanoid pathways (*Randhir and Shetty, 2005*).

By *Turkmen et al.* (*2005*), cooking induces changes in physiological and chemical composition, influencing the concentration and bioavailability of bioactive compounds in food, the thermal treatments decreasing the total phenolics in squash, peas and leek. In sweet corn cooking led to an increase in the level of phenolic compounds (*Dewanto et al., 2002*).

In this paper it has studied if and to what extent the thermal processing can modify the total phenolic content in popcorn seeds, belonging to eight Romanian local populations.

MATERIALS AND METHODS

Research materials. The biological material was represented by popcorn seeds (*Zea mays* L. var. *everta*) belonging to eight Romanian local populations (LP). Dried seeds (moisture content = 8-10 %), coming from crops of the last two years, were used to prepare working and control samples from each LP.

Procedure and research methods. The experiment consisted in three thermal processings, as follows. *For boiling*, they have taken 100 corn seeds of each sample, which have been placed in a stainless steel vessel of three liters capacity. The boiling has 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 (*Adom and Liu, 2002*)

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. For this purpose, a standard curve was generated, representing the absorbance values of gallic acid standard solutions in relation to their concentrations (*Moore and Yu, 2008*). TPC was expressed as mg Gallic Acid Equivalent/g dry seeds i.e. dry weight (mg GAE/g DW).

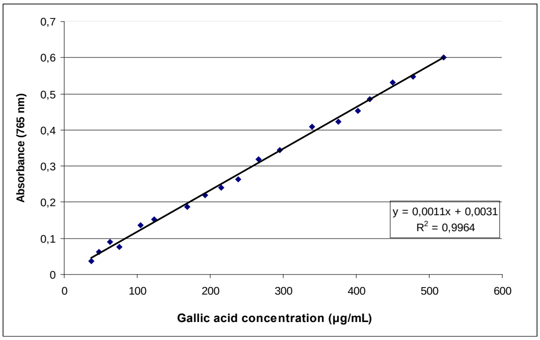

Fig. 1. Standard curve for TPC, using Gallic Acid

Statistical analysis. The data of experiments, coming from four replicates for each determination, were statistically processed using SAS Version 8.02 (*SAS Institute, 2005*). 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).

RESULTS AND DISCUSSIONS

In the Table 1 are rendered the values of the total phenolic content (TPC) in the eight popcorn seed samples, subjected to thermal processing.

Table 1. Comparative values of TPC in popcorn seeds thermal processed

Popcorn local populations (LP)	TPC (mg GAE/g DW)					
	Raw material*	Boiled		Steamed		Roasted Seeds
		Seeds	Water	Seeds	Water	
LP1 (red)***	8.2±0.7BC**	4.2±0.3DE**	0.8±0.04G	5.8±0.6D	0.4±0.05G	9.9±0.6B
LP2 (purple)	7.8±0.4BC	4.5±0.3DE	0.9±0.07FG	6.8±0.5CD	0.2±0.04G	8.6±0.3BC
LP3 (red)	9.7±0.5B	4.7±0.5DE	1.1±0.05FG	7.9±0.4BC	0.3±0.05G	10.7±0.9A
LP4 (red)	8.5±0.4BC	4.7±0.5DE	0.9±0.06FG	6.8±0.5CD	0.2±0.07G	8.7±0.5BC
LP5 (orange)	6.9±0.5CD	3.8±0.7EF	1.2±0.07FG	5.9±0.3D	0.1±0.03G	8.6±0.7BC
LP6 (white)	5.7±0.5D	4.2±0.5E	0.5±0.08G	4.8±0.6DE	0.2±0.03G	6.9±0.3CD
LP7 (yellow)	6.5±0.3C	3.9±0.8EF	0.8±0.05G	5.8±0.6D	0.1±0.04G	7.8±0.7BC
LP8 (white)	6.2±0.7CD	2.6±0.5F	0.9±0.04FG	4.6±0.3DE	0.1±0.07G	6.5±0.4C

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

As seen from Tab. 1, the total phenolic content (TPC) in seeds of the eight maize local populations (LP) ranged between 5.7±0.5 and 9.7±0.5 mg GAE/g DW, the highest values being found in red and purple grains.

According to some authors (*Lopez-Martinez et al., 2009; Montilla et al., 2011; Žilić et al., 2012, cited by Harakotr et al., 2014b*), the pigmented corn contains (more) anthocyanins, carotenoids, phenolic compounds, and antioxidant activity than non-pigmented corn.

TPC of seeds was modified after thermal processing (boiling, steaming or roasting), as compared to the control samples (raw seeds). ***Thus, boiling for 30 minutes*** led to a significant decrease (*P<0.05*) of the total content of phenolic compounds in all popcorn seeds, with percentages between 26.3% (LP6) and 51.5% (LP3).

Analyzing the boiling water of seeds, TPC (reported to raw material) was between 8.7% (LP6) and 17.4% (LP5). Significant differences (*P<0.05*) were between LP1, LP6 and LP7 (with close values), on one hand, and LP2, LP3, LP4, and LP5 (with close values), on the other hand. A part of the phenolic compounds was destroyed in each seeds sample by boiling. This fact one can see gathering (mathematically) the content of phenolic compounds from each boiled sample and from its boiling water and comparing it with the control (unprocessed seeds). These losses of total content of phenolic compounds in the analyzed samples ranged between 17.5% (LP6) and 43.5% (LP8), i.e. between 2 and 2.5 times greater than phenols released in the boiling water. These results are consistent with the data reported by some researchers.

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

As to seeds steaming, the Table 1 shows that 30 minutes of this thermal processing caused a significant reducing of TPC in all seed samples (*P<0.05*), with percentages between 10.7% (LP7) and 29.2% (LP1).

The analysis of water from seeds steaming, showed the presence of TPC, whose percentages (reported to raw material) ranged from 1.5% (LP5 and LP7) to 4.8% (LP1). As can be seen in the Table 1, there are no significant differences between TPC values of water from seed steaming.

Gathering (mathematically) the content of phenolic compounds from each steamed seeds sample and from its steaming water, and comparing it with the control (raw seeds), one can see significant differences (*P<0.05*), caused by the destruction of phenolic compounds through steaming. The phenolic compounds losses, through this type of thermal processing, ranged between 9.2% (LP7) and 27% (LP1), i.e. about 6 times greater than phenols released into steaming waters.

Comparing the two types of thermal processing, it can see that steaming has caused losses of phenolic compounds significantly lower than boiling (*P<0.05*).

According to *Harakotr et al. (2014a),* corn seeds boiling had a more detrimental effect on phenolic acids than steaming. The boiling treatment causes disruption of cellular components with the consequent release of these molecules into the cooking water (*Miglio et al., 2008,* cited by *Harakotr et al., 2014a*). By *Huang et al. (2006),* steaming treatment causes matrix softening and increases extractability of antioxidant components from the raw materials. The thermal treatment could cause changes in phenolic substances, in the corn, by liberating the bound phenolic compounds into free form (*Xu and Chang, 2009*).

Comparing with control samples (raw seeds), the total content of phenolic compounds in ***roasted corn seeds*** has registered significant increases (*P<0.05*) in all samples (except LP2 and LP4), with percentages between 2.3% (LP4) and 24.6% (LP5). This results are consistent with observations and data reported in some scientific works.

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.

During thermal treatment**,** Maillard reaction products may protect phytochemicals from oxidation (*Lin et al., 2008*), and according to *Jeong et al (2004),* heat treatment at 150°C for 40 min. liberated bound phenolics in citrus peels having as result a significant increasing of TPC after treatment.

CONCLUSIONS

The thermal processing of corn seeds, belonging to eight local populations of popcorn (*Zea mays* L. var. *everta*) has significantly influenced the total phenolic content of seeds.

As compared to control samples (raw material), the thermal processing of popcorn seeds by boiling and steaming for 30 minutes, resulted in significant losses of phenolic compounds, higher through boiling. The presence of these compounds was found within cooking waters, more in boiling, and less in steaming ones.

Compared to the control samples, in the roasted popcorn seeds (20 min. at 150-190°C), the phenolic compounds have 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.

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