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Method improvement for the determination of total phenolics content in yellow maize flour*

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Polyphenols are the most abundant antioxidants in human diet. These plant secondary metabolites are useful also as radical scavenging, with positive biomedical effects against cardiovascular diseases, cancer growth and diseases related to aging. Polyphenols are present in cereals including maize (Dykes and Rooney, Cereal Foods World, 52(3): 105–111, 2007; Gorinstein *et al.*, Eur. Food Res. Technol., 225: 321–328, 2007; Guo and Beta, Food Res. Intern., 51: 518–525, 2013).

Maize (Zea mays L.) is a potential crop for bio-fortification through breeding programs, because of the presence of considerable natural variability for the main components of the grains (Liu, J. Cereal Sci., 46: 207–219, 2007; Nuss and Tanumihardjo, Compr. Rev. Food Sci. Food Safety, 9: 417-436, 2010), and because it contains a higher total phenolic content (TPC) as compared to other cereals grains (Adom and Liu, J. Agric. Food Chem., 50: 6182-6187, 2002).

With respect to the content of phenols, the data available in the literature are mostly related to colored maize (Del Pozo-Insfran *et al.*, Cereal Chem., 84(2):162–168, 2007; Lopez-Martinez *et al.*, LWT - Food Sci. and Technol., 42: 1187–1192, 2009; Mora-Rochin *et al.*, J. of Cereal Sci. 52: 502–508, 2010; Žilić *et al.*, J. Agric. Food Chem., 60(5): 1224–1231, 2012). At present, there are scarce references about total phenolics content in yellow maize, which is commonly used both as food or feed. In addition, in the papers that report the determination of total phenolics content, the methods cited always refer to Singleton and Rossi (Am. J. Enol. Vitic., 16: 144–158, 1965), who set up this analysis in wine; eventual modifications to this method are often not specified, and do not allow to replicate the analysis elsewhere.

The aim of the present work is to provide details on the modifications to the method by Singleton and Rossi (1965) for total phenolics content analysis in maize flour; nevertheless, the method has proved suitable for any type of flour.

The extraction of phenolic compounds from plant materials is influenced by several factors such as temperature, light, type of solvent used and the vegetable matrix itself. The extraction of phenolic compounds from plant materials may also be influenced by other factors, such as solvent-to-solid

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ratio and the particle size of the sample. Increasing solvent-to-solid ratio was found to work positively for enhancing phenol yields. However, an equilibrium between the use of high and low solvent-to-solid ratios, involving a balance between high costs and solvent wastes and avoidance of saturation effects, has to be found in order to obtain an optimized value (Dai and Mumper, Molecules, 15(10): 7313–7352, 2010).

In the literature, solvent-solid ratios from 1:100 to 1:10,000 were reported; therefore, to limit the use of organic solvents, very small quantities of sample were sometimes used (e.g. 50 µg), raising doubts about the representativeness of the sample analyzed. The search for a good compromise between solvent-solid ratio and representativeness of the sample, as well as a balanced use of organic solvents, has led to the choice to use 1 gram of maize flour, extracted with 10 mL of solvent (5 mL x 2). Furthermore, the developed method has also other advantages, such as costs reduction, a limited number of operating steps and a relative speed of analysis.

Maize flour samples (1.0 g) were extracted with 5.0 mL of extraction solution (methanol: distilled water: hydrochloric acid, 80:20:1) and stirred for 2 hours at 37°C in the dark. After centrifugation at 4,000 rpm for 15 min at 4°C, the supernatants were transferred in a new tube and the pellets were extracted again for 15 min with 5.0 mL of extraction solution. The extracts were centrifuged a second time, and the supernatants were pooled. All samples were extracted in duplicate.

A stock solution of gallic acid (GA) was prepared by dissolving 0.1 g of the standard in 10.0 mL of 95% methanol. The stock solution can be stored at −20°C for two to three weeks. The working solution was prepared mixing 2.5 mL of GA stock solution and 22.5 mL of 95% methanol. Also this solution can be stored at −20°C for two to three weeks.

For the calibration curve we used the following concentrations of GA: 50 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL and 400 µg/mL. Each concentration of GA was calculated as the average of three readings at 760 nm in a Perkin Elmer UV-VIS spectrophotometer (Lambda 2). The coefficient of determination of the obtained equation was $r^2 = 0.9988$. The mean of the standard deviations for all the mean values calculated was 0.04±0.03 g_{GAE}/Kg_{dm}, and indicated that the method is easily replicable.

The developed method is therefore simple, cheap, relatively quick and can be used, with the addition of one step of acidification of the final extract, for the analysis of the content of total anthocyanins, as already carried out in fruit samples (*unpublished data*).

The improved method was used for the analysis of flours of local maize varieties and lines, mainly with yellow kernel, belonging to the germplasm collection maintained at CRA-MAC. In previous studies, the traditional Italian maize germplasm resulted to be interesting from the nutritional point of view, being rich in carotenoids (Berardo *et al.*, J. Agric. Food Chem., 57(6): 2378–2384, 2009; Alfieri et al., Tecnica Molitoria Intern., 63(13/A): 82–89, 2012; Alfieri et al., J. Cereal Science, *in press*) and showing a high total antioxidant capacity (Redaelli *et al.*, Proc. IX Convegno Biodiversità, Valenzano (BA) 6-7 Sept. 2012, pp. 263-269, 2013).

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In the whole set of data, the values of TPC presented a discrete variability, from 0.82±0.01 g_{GAE}/Kg_{dm} (VA74) to 1.92±0.12 g_{GAE}/Kg_{dm} (Oh43), and the general mean value was 1.16±0.22 g_{GAE}/Kg_{dm}. Among the local genotypes analyzed, Lo295 and Lo457 had the highest TPC values (1.67±0.02 g_{GAE}/Kg_{dm}).

These data allow to consider the developed method as a reliable tool for further screening of other materials, with particular attention to the lines developed from breeding programs.

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