Effects Of Seed Pre-Germination On Free Nutraceutical Compounds In Old Italian Open-Pollinated Maize Varieties

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Introduction

Maize-based food is one of the main human dietary component, although with high phytates contents, one of the highest among plants (8,300-22,200 mg kg^{-1}) (Kerovuo, 1998). Phytic acid (i.e., myo-inositol hexaphosphate) is the major component of plant origin containing phosporous. About 75% of the total phosphorus in cereals and legumes is present in the form of phytate, i.e., phytic acid salt, not readily available by monogastric (poultry, pigs and humans) (Wodzinski and Ullah, 1988).

During the processes of transformation and digestion of foods, phytic acid can be partially dephosphorylated to produce products of degradation, such as penta-, tetra- and tri-phosphates, through the action of endogenous phytase, which are found in most of the seeds of higher plants (Sandberg and Anderson, 1998).

Phytic acid is present as a salt of mono and divalent cations (e.g., potassium, calcium and magnesium). It accumulates rapidly in the aleurone layers of the seeds during the period of maturation and is generally considered to be the primary source of inositol and reserve of phosphorus in the seeds of plants used in animal and human nutrition (flour of seeds, cereal grains and legumes) (Maga, 1982).

Within the PSR project GO-SEEDS on pre-germinated seeds for the food industry, financed by the Veneto Region, in this study the contents of phytic acid and free phosporous as well as free phenolic acids before and after seed pre-germination were investigated in different old open-pollinated maize varieties, in order to evaluate the possible variation of bioavailable nutraceutical compounds.

Materials and Methods

Four open-pollinated maize varieties, i.e., Corvino, Marano, Sponcio, Vinaiolo and Rosso Rostrato, were cultivated during the 2021 growing season in open field at the experimental farm of the University of Padova at Legnaro (Padova, NE Italy) at a density of 7 plants m^{-2}. At harvest, the ears were manually harvested and shelled by a stationary plot combine harvester.

The kernels were used to set-up a pre-germination protocol, which consisted in preliminary water soaking (24 hours) and successive germination for 72 hours at 22°C and 90% air humidity within a climatic chamber. In this way the process complessively lasted 96 hours, at the end of which the kernels showed the primary radicle of ~1 cm and the sprout of ~0.5 cm of length.

The content of free phosphorus was determined in non germinated kernels and 48 and 96 hours from beginning of soaking. Free phosphorus was extracted by 12.5% TCA in $MgCl_2$ (25 mM) for 18 hours; the supernatant collected after centrifugation was further treated with the Chen's reagent and measurements were done at 660 nm wavelength.

Free phenolic acids, such as *p*-coumaric acid, caffeic acid, syringic acid, vanillic acid, and *t*-ferulic acid were extracted with 80% v/v chilled acetonitrile (ACN) after 5 min shaking, and centrifugation. The supernatant was analysed by HPLC using an Ultra Tech sphere C18 column (1.5 µm, 33 mm × 4.6 mm; CIL Cluzeau, Sainte-Foy-La-Grande, France) Photodiode Array Detector at 282 nm wavelength.

Results

The total content of phosphorus in non germinated kernels was quite high with similar values among maize varieties (average 3,268 mg kg^{-1}, with maximum of 3,531 mg kg^{-1} in var. Marano, and minimum of 2,976 mg kg^{-1} in var. Rosso Rostrato), whereas the content of free phosphorus varied largely, it ranging from 84 mg kg^{-1} in var. Corvino and ~120 mg kg^{-1} of varieties Rosso Rostrato and Sponcio.
During germination, while the content of phytic acid remained stable, free P decreased rapidly within 48 hours and remained stable during the rest of the processs, with no differences among varieties (finale average of 13.6 mg kg^{-1}) (Figure 1). As regards the content of free phenolic acids, it increased over time during pre-germination, the hierarchy among varieties remaining rather stable: the richest variety was Corvino, and the poorest Marano (Figure 1). The most aboundant phenol was ferulic acid in var. Corvino and Rosso Rostrato, and syringic acid in var. Sponcio. Caffeic acid increased largely in all varieties with germination (Figure 1).

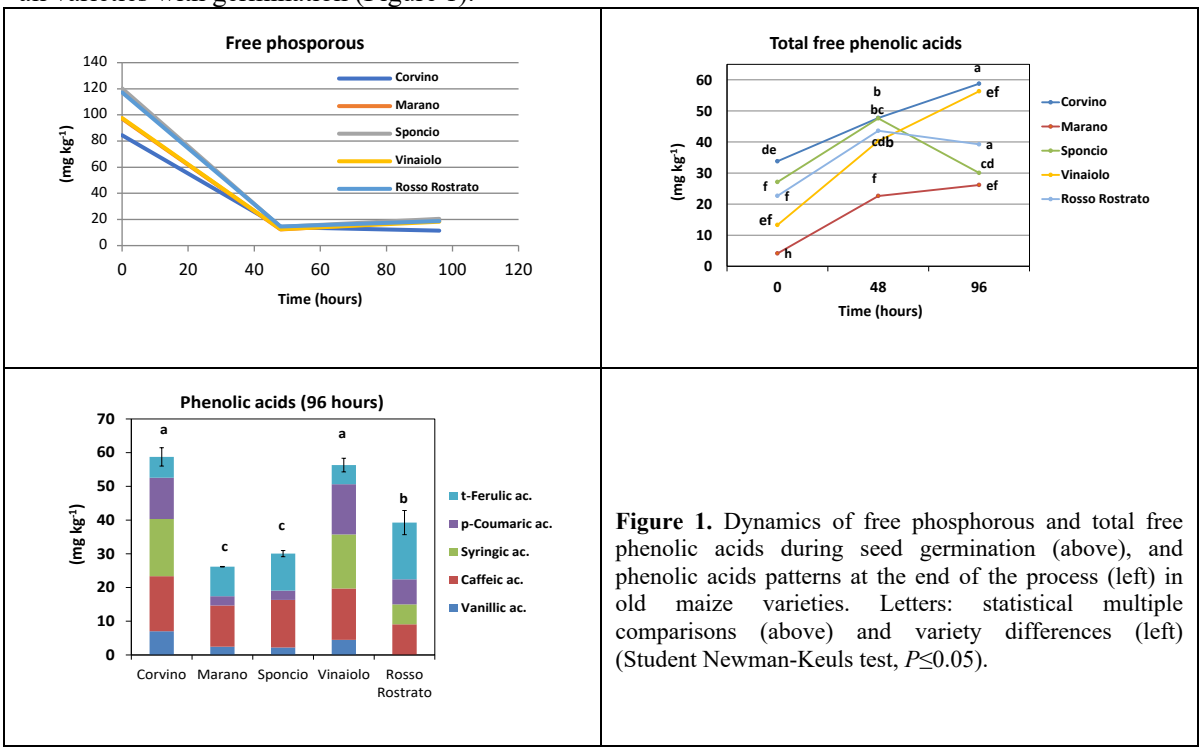

Figure 1. Dynamics of free phosphorous and total free phenolic acids during seed germination (above), and phenolic acids patterns at the end of the process (left) in old maize varieties. Letters: statistical multiple comparisons (above) and variety differences (left) (Student Newman-Keuls test, $P \leq 0.05$).

Conclusions

Old maize varieties represent an important source of genetic variability that can be valorized in the seed pre-germination process for the food chains thanks to their attractive kernels colour and nutraceutical value. Indeed, during the germination process the content of free phosphorous is expected to decrease markedly, probably due to its utilization by the growing sprout, an effect that can reduce the potential renal acid load (PRAL), while the content of phenolic acids often doubled. Seed pre-germination has proved in the past to increase microelement bioavailability at intestinal level, particularly iron, and these results further proved its efficacy in improving also the content of antioxidant compounds.

Literature

Adom, K. K., and Liu, R. H. (2002). Antioxidant activity of grains. J. Agric. Food Chem. 50, 6182–6187.

Kerovuo, J. Lauraeus, M., Nurminen, P., Kalkinnen, N. & Apajalahti, J. (1998). Isolation, characterization, molecular gene cloning and sequencing of a novel phytase from Bacillus subtilis. Appl. Environ. Microbiol. 64, 2079-2085

Maga, J. A. (1982). Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30. 1-9.

Sandberg, A.S. & Andersson, H. (1988). The effect of dietary phytase on the digestion of phytate in the stomach and small intestine of human. Journal of Nutrition. 118, 469-473.