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The effect of fruit extracts with polyphenol oxidase (PPO) activity on the in vitro accessibility of iron in high-tannin sorghum

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Abstract

Dephytinized high-tannin sorghum flour was incubated with crude extracts from pear, banana or avocado, respectively, followed by investigation of the effects on the phenolic content and on in vitro accessible iron. All fruits contained polyphenol oxidase (PPO) activity and incubation resulted in significant reduction of phenolic compounds. Incubation with avocado extract resulted in the lowest levels of phenolic compounds, as well as the highest amount of in vitro accessible iron. Peroxidase activity and some organic acids in the fruit extracts might also have contributed to the positive effect on iron accessibility. Nevertheless, incubation of the sorghum flour with the fruit extracts under conditions enabling the PPO to oxidize phenolic compounds, resulted in the highest accessibility of iron. The results from this study suggest that the PPO activity in simple fruit extracts can be utilized to increase the accessibility of iron in dephytinized polyphenol-containing cereal foods.

Keywords: Oxidation; Polyphenol oxidase; PPO; Fruits; Sorghum; Tannin; Polyphenol; Iron accessibility; Bioavailability; Peroxidase; Organic acids

1. Introduction

Polyphenol oxidase (PPO) is often associated with deterioration of foods because of its involvement in browning reactions. It oxidizes a number of phenolic compounds to the corresponding quinones, which easily undergo secondary reactions with amino acids, proteins or other phenols, to form melanin pigments (Friedman, 1996; Whitaker & Lee, 1995). The ability of PPO to oxidize phenolic compounds may, however, be utilized to increase the bioavailability of iron in polyphenol-containing plant foods. Coloured cereals, for example, are known to contain large amounts of phenolic compounds, such as condensed tannins. High-tannin sorghum is used as a staple food in many arid areas of the world and the tannins contribute strongly to the low bioavailability of iron in the vegetable diet eaten by the people in these areas. It is mainly the *ortho*-dihydroxyl groups present in condensed tannins that have been shown to bind iron (III) (Gust & Suwalski, 1994; Slabbert, 1992), making it unavailable for absorption in the gastrointestinal tract (Brune, Rossander, & Hallberg, 1989). Oxidation of phenolic compounds may result in a reduced iron-binding capacity and a higher availability of iron. We have shown, in an earlier study, that the in vitro accessibility of iron in phytate-reduced high-tannin varieties of sorghum and millet increased with polyphenol oxidase (mushroom tyrosinase) treatment (Matuschek, Towo, & Svanberg, 2001).

PPO may not be able to oxidize complex phenolics such as oligomers and polymers, except in the presence of a simpler phenolic compound (Cheynier & da Silva, 1991; Haslam et al., 1992). A hydroxycinnamic acid, for example, can trigger the oxidation of the more complex polyphenol by a coupled redox reaction. This has been shown, e.g., in procyanidins (Cheynier & da Silva, 1991) and theaflavins (Opie, Clifford, & Robertson, 1993). In addition to condensed tannins, high-tannin sorghum also contains flavonoids and phenolic acids (Hahn, Rooney, & Earp, 1984), which probably can be used as initial substrates by PPO and thereby contribute to oxidation of the tannins.

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Many fruits and vegetables, such as pear, banana and avocado, have a high PPO activity (Almeida & Nogueira, 1995). In addition, fruit extracts also contain significant amounts of organic acids, which may have a positive effect on iron absorption (Ballot et al., 1987; Gillooly et al., 1983). However, fruit juices are often ingested at the same time as a meal, which will not enable the PPO to reduce the phenolic content in the food. The purpose of this study was to investigate how incubation of dephytinized high-tannin sorghum with fruit extracts containing PPO activity affects the phenolic content and the in vitro accessible iron.

2. Materials and methods

2.1. Materials

A high-tannin sorghum (*Sorghum bicolor* L. Moench) variety, locally called udo, was purchased at a local market in Dodoma, Tanzania. The grains were sorted and washed several times with tap water, followed by repeated rinsing in deionized water. After washing, the grains were dried and milled in a disc-type mill (Laboratory Mill 3300) to obtain a fine flour (80% passing a 250 μm sieve). Ripe fruits of pear (*Pyrus communis*), banana (*Musa* sp.) and avocado (*Persea americana*) were purchased at a local store in Göteborg, Sweden. Wheat phytase (EC 3.1.3.26, P1259), pepsin (EC 3.4.23.1, P6887), pancreatin (P1750), bile extract (B8631), (±)-catechin (C1788), 4-methylcatechol (M34200) and 2,3-dihydroxynaphthalene (D116009) were purchased from Sigma–Aldrich, Stockholm, Sweden.

2.2. Phytase incubation

A portion of sorghum flour was suspended in H_2O (10 ml/g flour) and the pH was adjusted to 4.8 with HCl before addition of lyophilized phytase (2.0 U/g flour). The mixture was incubated in a shaking water bath at 55 °C for 24 h, followed by freeze-drying.

2.3. Crude fruit extracts

PPO was extracted by mixing 100 g fruit pulp in pieces with 100 ml ice cold H_2O (150 ml H_2O/100 g for avocado) and 1 g polyvinylpyrrolidone in a Braun 4262 food processor (Braun, Kronberg, Germany) for 2 min. The extract was centrifuged at 12,000*g* and 4 °C for 15 min and filtered trough a double cotton cloth. The filtrate was dialyzed (Spectra/Por 4, MWCO 12–14 kDa, Spectrum Laboratories, Rancho Dominguez, US) against H_2O at 4 °C for 48 h and kept at −20 °C until used.

2.4. Determination of PPO activity

The PPO activity in the fruit extracts was determined by both polarographic and spectrophotometric methods. The polarographic determinations were made on a Hansatech Oxygraph system equipped with a Clark-type oxygen electrode fitted in a pre-heated cell (30 °C) with a gas-tight plunger (Techtum Lab, Umeå, Sweden). The fruit extract (50 μl) was added to 2 ml of 20 mM 4-methylcatechol in 0.1 M phosphate buffer at pH 6.0. Prior to the measurement, 4-methylcatechol was dissolved in a small amount of methanol and added to the buffer solution, which had been bubbled with air at 30 °C for 1 h. The PPO activity was expressed as nmol O_2 consumed per min and per ml of enzyme solution. The spectrophotometric determinations were made at 500 nm and the activity was calculated from the initial change in absorbance. The substrate (300 μl of 0.2 M 4-methylcatechol in methanol) was mixed with 2.6 ml 0.1 M phosphate buffer at pH 6.0 and 100 μl fruit extract were added immediately before the measurement. One unit of PPO activity was defined as equal to a change of 0.001 in absorbance per min and per ml of enzyme solution.

2.5. Detection of peroxidase activity

Hydrogen peroxide (0.5%, 100 μl) and guaiacol (1% in 95% ethanol, 100 μl) were added to 500 μl of the fruit extracts. Peroxidase activity was estimated visually after 3.5 min by the formation of a red–brown reaction product. A strong colour reaction was interpreted as a high peroxidase activity.

2.6. Incubation with fruit extracts

A sample of dephytinized sorghum flour was suspended in fruit extract (10 ml/g flour). The pH was adjusted to 6.5 with 1 M NaOH and the slurry was incubated in a shaking water bath at 30 °C for 16 h. The sample was freeze-dried before analysis of phenolic compounds. A control sample for in vitro accessible iron was made by incubation of dephytinized sorghum with fruit extract containing 30 mM 2,3-dihydroxynaphthalene (2,3-DHN), which was dissolved in a small amount of methanol before addition to the extract.

2.7. Determination of organic acids

The amount of ascorbic acid in the fruit extracts was determined on an HPLC system equipped with a Kromasil C18 column (5 μm, 150 mm × 2.1 mm, Akzo Nobel, Bohus, Sweden). The mobile phase consisted of A: 4 mM tetrabutyl ammonium hydroxide with a pH value adjusted to 4.1 with formic acid and B: 25% ace-