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Antioxidant power as biochemical endpoint in bread for screening and early managing quality and toxicant-related safety anomalies in food production

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ABSTRACT

Flaxseeds are both a food ingredient and a natural source of antioxidants (e.g. lignans, PUFAs) and pro-oxidant contaminants (e.g. cadmium): the variable mixture of anti- and pro-oxidant substances may impact on the redox homeostasis of flaxseed-enriched foods. The antioxidant power is studied here as biochemical activity of flaxseeds in white wheat bread and as endpoint for possible screening of anomalous variations of bioactive mixtures (antioxidants *vs*. prooxidants) in food matrices.

A bioprobe assay based on the superoxide dismutase (SOD) enzyme (6 channels of the multiprobe bioelectronic platform *BEST*) was performed on white wheat bread with and without flaxseeds. Nine *BEST* channels were simultaneously used for validation and monitoring of measuring conditions (temperature, pH, conductivity). Findings were compared with quantitative analysis of antioxidants and pro-oxidant contaminants. Organic and aqueous extracts of both bread types were examined in parallel.

The SOD-probe detected the difference in antioxidant power given by 10% flaxseed, thus supporting the use of antioxidant power detected by bioenzymatic screening as sensitive biochemical endpoint. Mixtures of bioactive molecules in foods generate biochemical activities that can be monitored as time-effective indicators of invariability, which is pivotal in the daily control of anomalies in food production and therefore in the protection of consumers' health.

1. Introduction

Biochemical activities can be monitored via biochemical endpoints measured by biosensors (Dragone et al., 2009; Dragone and Grasso, 2012; Frazzoli et al., 2007; Mello and Kubota, 2002). As biochemical endpoint, the antioxidant power of a specific food results from the balance between pro-oxidant and antioxidant molecules; any shift in the balance, such as a depletion of antioxidants, would alter redox homeostasis. Therefore it is need measure the net effect of 'whole food', that takes into account the pro-oxidant/antioxidant molecules.

In Europe, wheat is the most common grain used for the preparation of bread, also owing to its high levels of gluten giving the dough sponginess and elasticity that suit consumers taste; other wheat species (e.g. durum) or other grains (e.g. rye, corn) are also used, especially in certain countries, either alone or in combination with wheat flour. Bread types include "white bread", made from flour containing only the central core of the grain (endosperm), "whole wheat bread" containing the whole wheat grain

Abbreviations: Cd, cadmium; DMSO, dimethyl sulfoxide; FB, flaxseed bread; FBa, aqueous extracts of flaxseed bread; FBo, organic extracts of flaxseed bread; HACCP, Hazard Analysis and Critical Control Points; HPLC, high pressure liquid chromatography; ICPMS, inductively coupled plasma mass spectrometry; ISIS, integrated sample introduction system; LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometry; LoD, limit of detection; PUFAs, polyunsaturated fatty acids; ROS, Reactive Oxygen Species; SOD, superoxide dismutase; WB, white wheat toast bread; WBa, aqueous extracts of white wheat toast bread; WBo, organic extracts of white wheat toast bread.

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(endosperm and bran), "wheat germ bread" enriched with wheat germ, and "whole grain bread" that is whole wheat bread added with whole grains to increase fibre content. Bread is increasingly enriched with seeds. A major representative of such seeds is flaxseed, which has antioxidant activity, due to its content in lignans (Touré and Xueming, 2010), diphenolic compounds and omega-3 alpha-linolenic fatty acid. Flaxseeds are also known for their vulnerability to the accumulation of cadmium (Cd) (Oomah et al., 2007). This toxic element has pro-oxidant potential (Stasenko et al., 2010) by both counteracting the bioactivity of cellular antioxidants and stimulating the exhalation of Reactive Oxygen Species (ROS) by mitochondria (EFSA, 2009): the presence of Cd at certain concentrations, thus, might reduce the natural antioxidant power of food ingredients such as flaxseeds.

Mycotoxins are widespread contaminants of cereals and oilseeds; although these substances possess different structures and exert different effects, most of them share the capacity to alter the cellular redox balance by interfering with redox signalling pathways (El Golli-Bennour and Bacha, 2011).

Therefore, biochemical activities may be usefully applied to evaluate the balance between undesired pro-oxidants and nutrients in order to monitor the maintenance of food production parameters and support Hazard Analysis and Critical Control Points (HACCP).

The present work has been performed within the *Bread project* of the 6th FP network of excellence CASCADE (http://cascade.projectcoordinator.net/) pointing at assessing biological activities in bread through the application of multiple methods. In particular, we applied the superoxide dismutase (SOD) bioprobe to detect the superoxide radical as a marker for i) testing the "net" antioxidant power, and ii) assessing the antioxidant power sensitivity as biochemical endpoint. We quantified bioactive molecules naturally present in bread and known to affect redox homeostasis, namely, mycotoxins and Cd (pro-oxidant substances stimulating ROS-producing pathways, see Corcuera et al., 2012; Stasenko et al., 2010; EFSA, 2009), and polyunsaturated fatty acids (PUFAs) and lignans (antioxidant nutrients with radical scavenging capacity that can significantly mitigate or neutralize the ROS-related effects depending on the ratio radical scavangers/ROS, see Fu et al., 2011; Kasote, 2013; Rodriguez-Leyva et al., 2010). In order to assess the sensitivity of antioxidant power as biochemical endpoint, we tested the net antioxidant power in white wheat bread samples with and without milled flaxseed; the SOD results were, then, evaluated face to those of conventional quantitative analyses. Finally, we tested the performance of the probe as integrated in the bioelectronic system of the patented technological platform *BEST* (Frazzoli et al., 2010, 2014).

2. Materials

2.1. Bread samples composition and preparation

One hundred kg each of white wheat toast bread (WB) and white wheat toast bread +10% milled flaxseed (FB) were baked at Fazer Bakeries Ltd. in Finland The same ingredients and baking procedures were used for both bread types with the only difference of milled flaxseed. The breads were analyzed for macronutrient composition by Lantmännen Analyzen (Tampere, Finland) with accredited methods. Energy content, total fibre and moisture were 263 and 242 kcal/100 g, 3.1 and 6.9 g/100 g, and 33.0 and 37.3 g/100 g in WB and FB respectively. As expected, FB showed a higher concentration of crude fat (5.4 *vs.* 3.0 g/100 g in WB).

Fifty Kg of bread were stored at −20 °C at the Functional Foods Forum, University of Turku −Finland, whereas the remaining was used for extraction. Wheat bread and flaxseed bread were processed by INRA (Toulouse, France) into aqueous (FBa and WBa) and organic (FBo and WBo) extracts soluble respectively in water and acetone and/or dimethyl sulfoxide (DMSO).

Finally, aqueous extracts and slurry organic extracts of WB and FB were provided for the present work, along with the solvents. Bread extracts used in this work corresponded to material extracted from 100 g of bread (1 mL corresponding to 10 g of bread). After dissolution by appropriate solvent (H_2O, DMSO), the 6 sample solutions (namely FBa, WBa, FBo, WBo, organic solvent, water) were stored at −20 °C.

2.2. Bread samples quantitative analysis

Extracts (evaporated to dryness) with corresponding solvent blanks analyzed for their content in: Cd, mycotoxins (ochratoxin A, zearalanone, nivalenol, deoxynivalenol, T-2, HT-2), and lignans (secoisolariciresinol, matairesinol, pinoresinol, lariciresinol). The possible presence of the so-called "mammalian" lignans, enterodiol and enterolactone, was measured as well, in order to verify their possible presence from exogenous sources such as humic water and plant (including wheat) tissues (Smeds et al., 2007).

For the instrumental analysis, dried organic extracts were added with 10 mL of DMSO whereas dried aqueous extracts were dissolved in 10 mL sterile water.

Organic extracts in DMSO were diluted 1:5 with MilliQ water before injection (10 μL) in the liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). The high pressure liquid chromatography (HPLC) system comprised an Agilent 1200 series pumps and an autosampler. A GEMINI C18 column 50 mm × 2 mm i.d., 5 μm particle size (Phenomenex, Torrance, CA, USA) was employed for the chromatographic separation. For the LC-MS/MS analysis, an Agilent 6410 triple quadrupole mass spectrometer was used. Conditions were as follows (negative ionization mode experiments): source temperature, 350 °C; capillary voltage, 4000 V; gas (nitrogen) flow, 10 L/min; nebulizer gas, 50 psi. For the positive ionization mode conditions were: capillary voltage, 4400 V; gas flow, 4 L/min; nebulizer gas, 30 psi. Mass spectrometry analyses were done in the Multiple Reaction Monitoring (MRM) mode. Calibration curves were built by spiking 100 μL of methanol, subsequent drying and dissolving in MilliQ water (by ultra-pure water system, Millipore, Milford, MA, USA); internal standards were added thereafter before injection. The mobile phases were 5 mM CH_3COONH_4 in MilliQ water (A) and acetonitrile (B) with a gradient from 1% B to 100% B in 20 min at 200 μL/min. The linearity of the method was calculated in the range of 0.01 and 100 ng/μL and correlation coefficients (R^2) were in the 0.9932–0.9998 range of for mycotoxins and lignans.

The limits of quantification (LoQs) were calculated on the basis of a "signal to noise" ratio of 10, in relation to the response produced by the injection of the standard solutions; the methods' LoQ ranged 0.056–0.070 ng/μL for most mycotoxins (ochratoxin A, zearalanone, nivalenol, deoxynivalenol) except for T2 and HT2 mycotoxins for which LoQs were higher than 0.6 ng/μL; the methods' LoQ for lignans ranged 0.007–0.023 ng/μL. Percent relative standard deviation (%RSD) from the analyses of three sample replicates was below 6%.

The accuracy of the method was evaluated as mean recoveries calculated through spiked samples: in the case of FB the recoveries ranged from 71.1% to 126.3% for lignans and the mammalian lignans, except for pinoresinol which had a lower recovery of 63.2% and for mycotoxins the recoveries ranged from 79.7% to 130.3%, except for zearalenone which had a lower recoveries of 64.2%; in the case of WB the recoveries ranged from 68.2% to 106.9% for lignans and the mammalian lignans, except for secoisolariciresinol (55.2%) and lariciresinol (60.4%), while the recoveries for