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ORIGINAL ARTICLE

Elicitation with abiotic stresses improves pro-health constituents, antioxidant potential and nutritional quality of lentil sprouts

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Abstract Phenolic content and antioxidant potential of lentil sprouts may be enhanced by treatment of seedlings in abiotic stress conditions without any negative influence on nutritional quality.

The health-relevant and nutritional quality of sprouts was improved by elicitation of 2-day-old sprouts with oxidative, osmotic, ion-osmotic and temperature stresses. Among the sprouts studied, those obtained by elicitation with osmotic (600 mM mannitol) and ion-osmotic (300 mM NaCl) shocks had the highest total phenolic content levels: 6.52 and 6.56 mg/g flour, respectively. Oxidative stress significantly enhanced the levels of (+)-catechin and *p*-coumaric acid. A marked elevation of the chlorogenic and gallic acid contents was also determined for sprouts induced at 4 °C and 40 °C. The elevated phenolic content was translated into the antioxidant potential of sprouts, especially the ability to reduce lipid oxidation. A marked elevation of this ability was determined for seedlings treated with 20 mM, 200 mM H_2O_2 (oxidative stress) and 600 mM mannitol (osmotic stress); about a 12-fold, 8-fold and 9.5-fold increase in respect to control sprouts. The highest ability to quench free radicals was observed in sprouts induced by osmotic stress (IC_{50}- 4.91 and 5.12 mg/ml for 200 mM and 600 mM mannitol, respectively). The highest total antioxidant activity indexes were determined for sprouts elicited with 20 mM H_2O_2 and 600 mM mannitol: 4.0 and 3.4, respectively. All studied growth conditions, except induction at 40 °C, caused a significant elevation of resistant starch levels which was also affected in a subsequent reduction of starch digestibility.

Improvement of sprout quality by elicitation with abiotic stresses is a cheap and easy biotechnology and it seems to be an alternative to conventional techniques applied to improve the health promoting phytochemical levels and bioactivity of low-processed food.

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1. Introduction

Food quality and functionality may be modified at each stage of its production including plant and animal breeding, technological processing, endogenous ingredients and/or by modification

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of storage conditions (Francis et al., 2012). In the case of low processed food, such as sprouts, the quality and potential bioactivity are mainly determined by seed quality and conditions of germination (Świeca et al., 2014a; Świeca and Baraniak, 2014b; Swieca et al., 2013a; Pérez-Balibrea et al., 2011; Tsurunaga et al., 2013).

Plants subjected to environmental stresses produce reactive oxygen species (ROS), thus antioxidant activity is of fundamental importance to their life. ROS can damage vital cellular macromolecules, e.g. proteins, DNA, and lipids; however, on the other hand, they play an important role in the regulation of plant metabolism, acting as regulators of auxin transport and/or signaling compounds during response to stress conditions (Fujita et al., 2006; Shetty, 2004). To reduce excess ROS, plants have developed an antioxidant defense system, which comprises enzymatic and non-enzymatic components. Non-enzymatic response is mainly linked with overproduction of antioxidants e.g. phenolics. Phenolics are primarily produced through the pentose phosphate, the shikimate and the phenylpropanoid pathways (Shetty, 2004). The antioxidant potential of phenolics is bound with: (a) radical-scavenging abilities; (b) the ability to chelate metal transition ions; (c) reducing power; (d) prevention of lipids and other biomolecules against oxidation; (e) inhibition of prooxidant enzymes; (f) activation of enzymatic defense system (Fernandez-Panchon et al., 2008; Gawlik-Dziki et al., 2012).

Numerous studies have indicated the positive correlation between consumption of phenolic-rich food and health. Legume phenolics exhibit therapeutic benefits such as hypoglycemic, anticancer, antioxidant, anti-inflammatory, antimicrobial and anticholesterol effects (Zhao, 2007). Germination is one of the most common and effective processes for improving the quality of legumes (Świeca et al., 2012; Pająk et al., 2013; Ghavidel and Prakash, 2007; Cevallos-Casals and Cisneros-Zevallos, 2010). There are several reports about the effect of germination methods on the nutraceutical value of legumes including, soybeans, mung beans or lentils. Among these strategies those employing biotic and abiotic elicitors have been widely studied (Świeca et al., 2014a,b, 2013a; Pérez-Balibrea et al., 2011; Gawlik-Dziki et al., 2013; Oh and Rajeshekar, 2009; Baenas et al., 2014). These techniques effectively increase production of phenolics and other compounds involved in plant response to stress and, although not yet applied to large scale production, have proved to be a very efficient procedure on a laboratory scale (Ahmed and Baig, 2014). Thus, elicitation of sprouts seems to be a promising alternative to other conventional biotechnological techniques used for improving the yield of plant secondary metabolites and the nutraceutical potential of low-processed food. Additionally, the application of minimal processing methods may preserve the shelf-life of sprouts and reduce food microorganisms without affecting the sensory and nutritional quality (Peñas et al., 2009).

The hypothesis proposed by these studies is that the phenolic content and antioxidant potential of ready-to-eat lentil sprouts may be enhanced by elicitation of seedlings in abiotic stress conditions. Cross-talk between different signaling pathways is very common in plant defense responses; thus we suppose that all the studied factors will be effective inductors of phenolic synthesis (Fujita et al., 2006). On the other hand, on the basis of studies concerning plant responses to abiotic stresses we assumed that some qualitative and quantitative differences between them would be found. The parameters measured to characterize the effect of these elicitors were biomass yield, phenolic content, and antioxidant activities (the abilities to prevent lipids and scavenge free radicals, chelating and reducing power).

2. Material and methods

2.1. Materials and preparation of sprouts

2.1.1. Sprouting

Lentil seeds var. Tina were purchased from PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilized in 1% (v/v) sodium hypochloride (Sigma-Aldrich, USA) for 10 min, then drained and washed with distilled water until they reached neutral pH. They were placed in distilled water and soaked for 6 h at 25 °C. Seeds were dark germinated for 8 days in a growth chamber (SANYO MLR-350H) on Petri dishes (ϕ 125 mm) lined with absorbent paper. Seedlings were watered with 5 ml of Milli-Q water daily. Sprout (8-day-old) samples were gently collected, weighed (fresh mass), rapidly frozen and kept in polyethylene bags at −20 °C. For each treatment, three replicates were performed.

2.1.2. Elicitation

Elicitation conditions were selected in previous screening studies. For the experiments, temperature (4 °C and 40 °C – TC and TH, respectively), H_2O_2 (20 mM and 200 mM – Ox1 and Ox2, respectively), mannitol (200 mM and 600 mM – Os1 and Os2, respectively) and NaCl (100 mM and 300 mM – S-Os1 and S-Os2, respectively) were selected as abiotic elicitors. All solutions were freshly prepared before each application. Mannitol (Os1, Os2), NaCl (S-O1, S-O2) and H_2O_2 (Ox1) treatments were applied by watering daily (not soaking) 2-day-old sprouts with 5 ml of test solution. For Ox2 (200 mM H_2O_2) treatment 2-day-old seedlings were only once watered with 5 ml of 200 mM H_2O_2 and then cultivated under standard conditions. For temperature conditioning treatment, 2-day-old sprouts were incubated at 4 °C and 40 °C (TC and TH, respectively) for 1 h and then cultivated under standard conditions. Sprout (8-day-old) samples were gently collected, weighed (fresh mass), rapidly frozen and kept in polyethylene bags at −20 °C.

2.1.3. Growth analysis

In order to determine the influence of elicitation on sprout vigor the growth ratio was proposed. The growth ratio was defined as an amount of fresh weight obtained from 1 g of dormant seeds after germination.

2.1.4. Flour preparation

Sprouts were dried in a forced-air oven at 50 °C for 12 h (Ghavidel et al., 2007). After that sprouts were grounded in a labor mill, and sieved (60 mesh). Sprout flours were stored at 4 °C.

2.2. Phenolic content and antioxidant activities

2.2.1. Extract preparation

Lentil flours (0.2 g) were extracted three times with 4 ml of acetone/water/hydrochloric acid (70:29:1, v/v/v). After