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Production of ready-to-eat lentil sprouts with improved antioxidant capacity: Optimization of elicitation conditions with hydrogen peroxide

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ABSTRACT

This study evaluates the optimal conditions for elicitation with H_2O_2 for improving the antioxidant capacity of lentil sprouts. Generally, except for 3-day-old sprouts, elicitation increased phenolic content (in respect to the control). The highest phenolic content was determined for 2-day-old sprouts treated with 15 mM H_2O_2 (0.71 mg/g f.m.). All the studied modifications increased the antioxidant potential of sprouts. The highest elevation (3.2-fold) was found for 5-day-old sprouts (single 15 mM H_2O_2 treatment). A significant increase was also found on the 2nd and 4th days (2.13- and 2.14-fold, respectively). Elicitation induced tyrosine and phenylalanine ammonia-lyases activities. H_2O_2 treatments induced the activity of catalase – especially for 2-day-old sprouts treated with 150 mM H_2O_2 (597 U/g f.m.). Elicitation with H_2O_2 is a useful tool for designing some features of sprouts. Phenolic content and antioxidant capacity are strongly affected by concentration of the elicitor, and time and intervals of its application.

1. Introduction

A rapid increase in demand for preparations improving functioning and the quality of life has been observed in recent years in highly-developed countries. This demand has focused mainly on functional foods of natural origin and nutraceuticals. A properly composed diet may have significant impact in the prevention of numerous diseases, the improvement of the quality of life and the attenuation of symptoms accompanying aging process (Zhao, 2007).

Interest in the possibility of food modification at each stage of production (plant and animal breeding, technological processes, and conditions of product storage) has increased over recent years. The pro-health properties of food of plant origin are strongly determined by secondary metabolite content, including polyphenols (Zhao, 2007). Polyphenols belong to a group of compounds with well-documented antioxidant, antitumor, and anti-inflammatory properties. Sprouting seems to be an effective process for improving the nutritional and nutraceutical quality of legume food (López-Amorós, Hernández, & Estrella, 2006; Silva et al., 2013). Unfortunately, during the germination of a seed, a decrease in phenolic antioxidant content is observed, which results in a subsequent decrease in the antioxidant potential of food (Cevallos-Casals & Cisneros-Zevallos, 2010; Świeca & Baraniak, 2013; Świeca, Gawlik-Dziki, Kowalczyk, & Złotek, 2012).

Elicitation leads to oxidative stress through an increase in reactive oxygen (ROS) and nitrogen species (NOS) levels. On the one hand, ROS and NOS damage attacks the most sensitive biological macromolecules. These species also act as signaling compounds. An elicitor is a factor stimulating any type of plant defense and causing the induction of phenolics biosynthesis. Elicitors might be of either biotic or abiotic origin. In plants, polyphenols act as defense (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing animals), as well as protect plants from oxidation. Usually, oxidative damage and increased resistance under environmental stresses can be correlated with the efficacy of the antioxidative defense system and increased stress tolerance (Zhao, Lawrence, & Verpoorte, 2005).

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Hydrogen peroxide (H_2O_2) is a strong oxidizing agent that is commonly used in medicine, agriculture, and the food industry where it is used as a bleaching agent in wheat flour, edible oil, egg white, etc. It may also be used as an antimicrobial agent in food, e.g. milk, and as a sterilizing agent for food packaging materials. In plants, ROS, including hydrogen peroxide, also contribute in the stress signaling cascade, and thus may be used for the induction (elicitation) of plant resistance (Vasconsuelo & Boland, 2007; Zhao et al., 2005).

Elicitation is an effective technique used in bioreactor systems for the overproduction of metabolites with potential biological activity e.g. phenolics (Matkowski, 2008). Phenolics are primarily produced through the pentose phosphate (PPP), shikimate and phenylpropanoid pathways. The oxidative PPP provides precursor erythrose-4-phosphate for the shikimate pathway. The shikimate pathway converts these sugar phosphates into aromatic amino acids such as phenylalanine and tyrosine, which become the precursors for the phenylpropanoid pathway that synthesizes phenolics (Shetty, 2004). In plants, these amino acids are transformed into trans-cinnamic acid and *p*-coumaric acid *via* phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase, respectively. The synthesis of phenolic compounds is accompanied by the stimulation of these enzymes.

Plants modify their metabolism to adjust to variable environmental conditions. This enables the modification of their composition and consequent changes of the activity of plant-origin food (Feng, Song, Lee, & Huang, 2010; Gawlik-Dziki, Świeca, Dziki, & Sugier, 2013; Gawlik-Dziki, Świeca, & Sugier, 2012; McCue, Zheng, Pinkham, & Shetty, 2000; Pérez-Balibrea, Moreno, & García-Viguera, 2011; Randhir, Lin, & Shetty, 2004; Złotek, Świeca, & Jakubczyk, 2014; Świeca & Baraniak, 2013; Świeca, Baraniak, & Gawlik-Dziki, 2013; Świeca et al., 2012). Despite the analysis of final effects (changes in bioactive component levels and bioactivity itself), the cited papers lack data concerning the mechanisms for acquiring new features. Currently, there is no study reporting the selection of the optimal concentration, time of exposure and intervals of elicitor treatments considering the effectiveness of these biotech treatments in the creation of some features of low-processed food.

The purpose of this study was to evaluate the optimal conditions of elicitation using hydrogen peroxide for improving the antioxidant capacity of ready-to-eat lentil sprouts. We focused on the activities of enzymes involved in plant defense and phenolic synthesis and metabolism.

2. Materials and methods

2.1. Plant material and growth conditions

Lentil seeds var. Tina were purchased from PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilized in 1% (v/v) sodium hypochloride for 10 min, then drained and washed with distilled water until they reached neutral pH (6.8). After that, they were placed in distilled water and soaked for 6 h at 25 °C. Seeds were dark germinated (25 °C, 85% relative humidity) for 5 days in a growth chamber on Petri dishes (ϕ 125 mm) lined with absorbent paper (approximately 150 seeds per dish). Seedlings were watered with 5 ml of Milli-Q water daily.

For the experiments, 15 mM and 150 mM H_2O_2 were selected as abiotic elicitors. All solutions were freshly prepared before each application. For Ox1 treatment, 1-day-old seedlings were watered only once with 5 ml of 15 or 150 mM H_2O_2 (single treatment; Ox1–15 and Ox1–150, respectively) and then cultivated under standard conditions (watered with distilled water). For Ox2 treatments, 1-day-old seedlings (since the first day of cultivation) were watered daily (to the end of sprouting) with 5 ml of 15 mM and 150 mM H_2O_2 (continuous treatment; Ox2–15 and Ox2–150, respectively). Sprout samples were gently collected, weighed and rapidly frozen and kept in polyethylene bags at −20 °C. Three independent experiments were carried out.

2.2. Growth analysis

In order to determine the influence of elicitation on sprout growth the morphological characteristic (length of roots and stalk) and biomass accumulation (10 sprouts mass) were determined.

2.3. Phenolics content

Lentil flours (0.2 g) were extracted three times with 4 ml of acetone/water/ hydrochloric acid (70:29:1, v/v/v). After centrifugation (10 min., 6800×*g*) fractions were collected, combined and used for further analysis.

The amount of total phenolics was determined using Folin–Ciocalteau reagent (Singleton, Orthofer, & Lamuela-Raventos, 1974). To 0.5 ml of the sample, 0.5 ml H_2O, 2 ml Folin–Ciocalteau reagent (1:5 H_2O) were added, and after 3 min, 10 ml of 10% Na_2CO_3 and the contents were mixed and allowed to stand for 30 min. Absorbation at 725 nm was measured in a UV–vis spectrophotometer. The amount of total phenolics was calculated as a gallic acid equivalent (GAE) in mg per g of fresh mass (f.m.).

2.4. Antioxidant activities

Antiradical activity was carried out using an improved ABTS decolorization assay (Re et al., 1999). Free radical scavenging ability was expresses as Trolox equivalent in mg per g of fresh mass (f.m.).

Reducing power was determined by the method of Oyaizu (1986). Reducing power was expressed as Trolox equivalent in mg per g of fresh mass (f.m.).

Chelating power was determined by the method of Decker and Welch (1990). Chelating power was expressed as EDTA equivalent (EDTA) in mg per g of fresh mass (f.m.).

The inhibition of the hemoglobin-catalyzed peroxidation of linoleic acid was determined according to Goupy, Vulcain, Caris-Veyrat, and Dangles (2007). The activity was expressed as quercetin equivalent (Q) in mg per g of fresh mass (f.m.).

Four complementary antioxidant methods were intergraded to obtain the total antioxidant activity index (IA) (1). The index may be useful for evaluation total antioxidant potential of sprouts from different germination conditions in respect to control. The IA was calculated as the sum of relative activities (RA) (2) for each antioxidant chemical methods divided by a number of methods (Świeca & Baraniak, 2013).

$$\mathrm{IA} = \frac{\Sigma \mathrm{RA}_{(n)}}{4} \tag{1}$$

RA was calculated as follows:

$$\mathrm{RA} = \frac{A_x}{A_c}, \tag{2}$$

where: A_x-activity of modified sprouts for the method, A_c-activity of control sprouts determined for the method

2.5. Oxidative damage

The degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, as described by Dhindsa, Plumb-dhindsa, and Thorpe (1981). Samples (0.2 g) were homogenized in 2 ml of 5% trichloroacetic acid (TCA) solution and centrifuged at 13,500×*g* for 15 min at room temperature. The