Food Chemistry 161 (2014) 288–295

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts

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A R T I C L E   I N F O

Article history:
Received 4 December 2013
Received in revised form 25 February 2014
Accepted 2 April 2014
Available online 13 April 2014

Keywords:
Elicitation
Bioaccessibility
Precursor feeding
Sprouting
Antioxidant activity
Phenolic overproduction

A B S T R A C T

This study considered the efficacy of UV-B treatment and precursor feeding with phenolic synthesis precursors in relation to the production of polyphenol-rich sprouts. The used modifications of sprouting caused an increase in the activities of tyrosine ammonia-lyase and phenylalanine ammonia-lyase. The biotechnological approaches used allowed for a significant enhancement of the antioxidant potential of sprouts that was strongly and positively correlated with total phenolic content. The highest increase of flavonoids content was found for the sprouts obtained with phenylalanine treatment (2.41 mg/g FM, 1.6-fold with respect to the control). The highest increase in the antioxidant capacity was found for the sprouts obtained with phenylalanine (an increase of 27% after solid–liquid extraction) and combined UV-tyrosine treatments (an increase of 44% for potentially bioaccessible fractions). The results of this work may suggest the use of elicitation supported by precursor feeding as an easy and cheap tool for improving the nutraceutical potential of low-processed food.

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1. Introduction

The pro-health action of food of plant origin is strongly determined by their secondary metabolite content, including polyphenols. Polyphenols are a group of compounds with well documented antioxidant, anti-tumour and anti-inflammatory abilities (Zhao, 2007). In recent years, an increase in low-processed food consumption has been observed. Sprouting appeared to be an effective process for improving the nutritional and nutraceutical quality of legume foods (López-Amorós, Hernández, & Estrella, 2006). Unfortunately, during seed germination a decrease in the phenolic antioxidant content is observed, which results in a subsequent decrease in the antioxidant potential of the sprouts (Świeca, Gawlik-Dziki, Kowalczyk, & Złotek, 2012).

Phenolics are primarily produced through the pentose phosphate, the shikimate and the phenylpropanoid pathways (Shetty, 2004). Polyphenols apparently act as defense (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing animals), as well as protecting the plant from oxidation (Zhao, Lawrence, & Verpoorte, 2005). Plant resistance against pathogens/environmental shocks is performed by both pre-existing (constitutive) and induced defense systems. As a response to variable environmental conditions, plants modify their metabolism, adjusting to existing conditions which allow the modification of the composition and activity of food of plant origin (Randhir, Lin, & Shetty, 2004; Zhao et al., 2005; Świeca et al., 2012). So far, induced overproduction of polyphenols, often supported by elicitation and/or precursor feeding, has been widely used in bioreactor systems (Matkowski, 2008); however, some attempts have also been undertaken to improve the phenolic composition and antioxidant potential of sprouts. An increase in the vitamin C content and antioxidative activity in *Vigna sinensis* sprouts has been obtained by seed induction under high pressure conditions (Doblado, 2007). An increase in the level of ascorbic acid, β-carotene, polyphenols and increased digestibility and solubility of proteins has been obtained by the application of variable lighting conditions (varicoloured lighting conditions, γ radiation) (Khattak et al., 2007). Elicitation of phenylpropanoid pathways with UV radiation and oregano extract, fish protein hydrolysate as well as lactoferrin in order to increase the antioxidative potential of the sprouts of mung beans and broad beans was also described by Randhir et al. (2004) and Shetty, Atallah, and Shetty (2002). It should, however, be emphasised that, despite analysis of the final effects (changes in bioactive component levels and bioactivity), the literature has cited the lack of data concerning mechanisms of feature enhancement. Thus, the major aim of the study was to broaden the current understanding of the use of UV stress and precursor feeding in the production of polyphenol-rich sprouts. Special emphasis was placed on the

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http://dx.doi.org/10.1016/j.foodchem.2014.04.012
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activity of enzymes involved in plant defense and phenolic synthesis and metabolism.

2. Material and methods

2.1. Chemicals

Ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenyl-sulphonic acid)-1,2,4-triazine), ABTS (2,2-diphenyl-1-picrylhydrazyl), α-amylase, pancreatin, pepsin, bile extract, linoleic acid, ammonium thiocyanate, thiobarbituric acid, polyvinylpyrrolidone and haemoglobin were purchased from Sigma–Aldrich company (Poznan, Poland). All others chemicals were of analytical grade.

2.2. Materials

Lentil seeds var. Tina were purchased from PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilised in 1% (v/v) sodium hypochloride for 10 min, then drained and washed with distiled water until they reached a neutral pH. The seeds were placed in distiled water (C, control and UV, UV light treatment) or a phenolic precursor solution (0.1 mM L-phenylalanine – Phe and Phe+UV; 0.1 mM L-tyrosine – Tyr and Tyr+UV) and soaked for 6 h at 25 °C. Seeds were germinated in the dark for 4 days (ready-to-eat sprouts) in a growth chamber on Petri dishes (ϕ 125 mm) lined with absorbent paper (approximately 150 seeds per dish). Seedlings were watered daily with 5 ml of Milli-Q water. For UV treatment (UV, Phe+UV and Tyr+UV), after soaking, seeds were irradiated with UV-B light (310 nm, Germicidal UV light, output 300 $\mu W/cm^2$) at a 20 cm distance for 5 h. After the exposure time, the plates were covered and seeds were germinated under the control conditions. The conditions of seed treatments were selected according to previous screening test (data not shown).

2.3. Growth analysis

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

2.4. 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 homogenised in 2 ml of 5% trichloroacetic acid (TCA) and centrifuged at 13 500 g for 15 min at room temperature. 1 ml of the supernatant was mixed with 1 ml of 0.5% (v/v) thiobarbituric acid (in 20% (v/v) TCA). The mixture was heated at 96 °C for 30 min, cooled in ice and centrifuged at 9500 g for 10 min. The content of MDA was expressed as nmol MDA per g of fresh mass (FW).

2.5. Protein content

A protein assay was performed according to the method of Bradford (1976). Bovine serum albumin was used as the protein standard.

2.6. Phenolic content and antioxidant activities

2.6.1. Extracts preparation

2.6.1.1. Solid–liquid extraction. Lyophilised sprouts (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, 6800g) the fractions were collected, combined and used for further analysis.

2.6.1.2. Digestion in vitro. For simulated mastication and gastrointestinal digestion, the germinated lentil sprouts (150 mg of lyophilised sprouts) were homogenised in 3.5 ml of simulated salivary fluid (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl, 200U α-amylase (E.C. 3.2.1.1 in 1 l H_2O, pH 6.75) and shaken for 10 min at 37 °C. Next, the samples were adjusted to pH = 1.2 with HCl (5 mM), suspended in 1.25 ml of simulated gastric fluid (300 U/ml of pepsin A, EC 3.4.23.1 in 0.03 M HCl, pH = 1.2) and shaken for 120 min at 37 °C. After simulated gastric digestion, samples were adjusted to pH = 6 with 0.1 M $NaHCO_3$ and suspended in simulated intestinal juice (0.05 g of pancreatin (activity equivalent 4× USP) and 0.3 g of bile extract in 2.0 ml 0.1 M $NaHCO_3$; adjusted to pH = 7 with 1 M NaOH, and finally 1.25 ml of 120 mM NaCl and 5 mM KCl were added to the sample. The prepared samples underwent *in vitro* intestinal digestion for 120 min (Świeca, Baraniak, & Gawlik-Dziki (2013)).

2.6.2. Phenolics analysis

The amount of total phenolics were determined using Folin–Ciocalteau reagent (Singleton, Orthofer, & Lamuela-Raventos, 1974). To 0.5 ml of the sample, 0.5 ml H_2O and 2 ml Folin–Ciocalteau reagent (1:5 H2O) were added, and after 3 min, 10 ml of 10% Na_2CO_3. The contents were mixed and allowed to stand for 30 min. The absorbance at 725 nm was measured. The amount of total phenolics was calculated as a gallic acid equivalent (GAE) in mg per g of fresh mass (FM).

The total flavonoid content was determined according to the method described by Lamaison and Carnet (1990). 1 ml of extract was mixed with 1 ml of 2% $AlCl_3 \times 6H_2O$ solution and incubated at room temperature for 10 min. Thereafter, the nabsorbance at 430 nm was measured. The total flavonoids content was calculated as a quercetin equivalent (QE) in mg per g of fresh mass (FM).

2.6.3. Antioxidant activities

2.6.3.1. Antiradical activity (ABTS). The experiments were carried out using an improved ABTS decolourisation assay (Re et al., 1999). The ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM of ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature prior to use. The $ABTS^{+\cdot}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Lambda 40 UV–Vis spectrophotometer, Perkin Elmer). The affinity of the test material to quench the ABTS free radical was evaluated according to the following equation:

$$\text{Scavenging } \% = [(A_C - A_A)/A_C)] \times 100, \text{where :}$$

A_C – absorbance of control, A_A – absorbance of sample.

The free radical scavenging ability was expressed as the Trolox equivalent in mg per g of fresh mass (FM).

2.6.3.2. Reducing power (RP). The reducing power was determined by the method of Oyaizu (1986). The analysed sample (2.5 ml) was mixed with phosphate buffer (2.5 ml, 200 mM, pH 6.6) and potassium ferricyanide $K_3[Fe(CN_6)]$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. Reactions were stopped with 0.5 ml 10% TCA and centrifuging for 10 min at 6500 g. The upper layer of the solution (2.5 ml) was mixed with distiled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. The reducing power was expressed as the Trolox equivalent in mg per g of fresh mass (FM).

2.6.3.3. Metal chelating activity (CHP). The Chelating power was determined by the method of Decker and Welch (1990). The

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