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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

centrifugation (10 min, 8000*g*) fractions were collected, combined and used for further analysis.

2.2.2. Phenolic analysis

The amount of total phenolics was determined using Folin–Ciocalteu phenol reagent (Singleton et al., 1974). The amount of total phenolics was calculated as a gallic acid equivalent (GAE) in mg/g of flour.

Total flavonoid content was determined according to the method described by Lamaison and Carnet, 1990. Total flavonoid content was calculated as a quercetin equivalent (QE) in mg/g of flour.

Condensed tannin content was determined according to the method described by Sun et al. (1998). Condensed tannin content was calculated as a (+)-catechin equivalent (CE) in mg/g of flour.

Qualitative–quantitative analysis of phenolics was performed using a Varian ProStar HPLC System separation module (Varian, Palo Alto, CA) equipped with Varian ChromSpher C18 reverse phase column (250 mm × 4.6 mm) and ProStar DAD detector (Swieca and Baraniak, 2014c).

2.2.3. Antioxidant activities

2.2.3.1. Antiradical activity (ABTS). The experiments were carried out using an improved ABTS decolorization assay (Re et al., 1999). Free radical scavenging ability was expressed as IC_{50} in mg flour per ml.

2.2.3.2. Reducing power (RP). Reducing power was determined by the method of Oyaizu, 1986. Reducing power was expressed as quercetin equivalent (Q) in μg/g of flour.

2.2.3.3. Metal chelating activity (CHP). Chelating power was determined by the method of Decker and Welch (1990). Chelating power was expressed as EDTA equivalent (EDTA) in μg/100 mg of flour.

2.2.3.4. Inhibition of linoleic acid peroxidation (LPI). The antioxidant activity was determined as the degree of inhibition of the hemoglobin-catalyzed peroxidation of linoleic acid (Groupy et al., 2007). Inhibition of linoleic acid peroxidation was expressed as quercetin equivalent (Q) in μg/mg of flour.

2.2.3.5. Total antioxidant activity index (AI). Four complementary antioxidant methods were intergraded to obtain the total antioxidant activity index (AI) (Świeca et al., 2014b). The index may be useful for evaluation of the total antioxidant potential of sprouts from different germination conditions with respect to the control.

2.3. Nutritional quality

2.3.1. Digestion in vitro

Simulated mastication and gastrointestinal digestion were performed according to Swieca et al. (2013a). Lentil sprouts (150 mg of flour) were homogenized in 3.5 ml of simulated salivary fluid (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl, 200 U 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^{-1} 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 of 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 was added to the sample. The samples thus prepared underwent *in vitro* intestinal digestion for 120 min.

2.3.2. Protein content and digestibility

A 500 mg of sample was twice extracted with 5 ml of 0.5 M NaCl solution in 0.05 M Tris–HCl buffer pH 7.0 at 4 °C for 2 h. After centrifugation (10 min, 8000*g*) fractions were collected, combined and used for further analysis. The total protein contents were determined with the Lowry method (1951), using bovine serum albumin as the standard protein.

The *in vitro* protein digestibility was evaluated on the basis of total soluble protein content and the content of protein determined after digestion *in vitro* (Świeca et al., 2013b).

2.3.3. Non-protein nitrogen

A 100 mg of sample was twice extracted with 5 ml of 10% ethanol (v/v) at 4 °C for 2 h. After centrifugation (10 min,

Table 1 Phenolic composition of lentil sprouts elicited by different abiotic stresses.

Cultivation conditions	Total phenolics [mg/g flour]	Total flavonoids [mg/g flour]	Condensed tannins [mg/g flour]
C	4.40 ± 0.03^{b}	0.38 ± 0.02^{b}	0.91 ± 0.03^{d}
Ox1	4.74 ± 0.14^{bc}	0.59 ± 0.01^{d}	0.86 ± 0.03^{cd}
Ox2	3.96 ± 0.13^{a}	0.50 ± 0.00^{c}	0.63 ± 0.01^{a}
Os1	5.13 ± 0.22^{c}	0.50 ± 0.02^{c}	1.19 ± 0.04^{e}
Os2	6.52 ± 0.13^{d}	0.70 ± 0.11^{e}	1.73 ± 0.05^{f}
S-O1	4.19 ± 0.15^{a}	0.43 ± 0.04^{b}	0.76 ± 0.05^{b}
S-O2	6.56 ± 0.33^{d}	0.79 ± 0.02^{e}	1.63 ± 0.11^{f}
TC	4.02 ± 0.14^{a}	0.28 ± 0.01^{a}	0.88 ± 0.02^{c}
TH	5.10 ± 0.34^{b}	0.50 ± 0.04^{c}	0.88 ± 0.12^{bc}

Means in columns followed by different letters are significantly different at $p = 0.05$.
Each value represents the mean of 3 independent experiments (± SD).
C, control; Ox1, induction with 20 mM H_2O_2; Ox2, induction with 20 mM H_2O_2; Os1, induction with 200 mM mannitol; Os2, induction with 600 mM mannitol; S-O1, induction with 100 mM NaCl; S-O2, induction with 300 mM NaCl; TC, induction at 4 °C; TH, induction at 40 °C.

8000*g*) fractions were collected, combined and used for further analysis. Non-protein nitrogen was determined with 2,4,6-trinitrobenzene sulfonic acid (TNBS) according to the methods described by Habeeb (1966), using L-leucine as the standard.

2.3.4. Starch content and digestibility

The total starch (TS) content was determined after dispersion of the starch granules in 2 M KOH (50 mg sample, 6 ml KOH) at room temperature (30 min, constant shaking) and hydrolysis of the solubilized starch with 80 µl (1 mg/ml) amyloglucosidase (14 U mg^{-1}; EC 3.2.1.3) at 60 °C for 45 min (Goni et al., 19967). The glucose content was determined by using the standard dinitrosalicylic acid (DNSA) method (Miller, 1959). Total starch was calculated as glucose ×0.9. The free reducing sugar content of the samples was determined in order to correct the obtained total starch values. The sucrose content of the samples was also determined in order to correct the obtained total starch values. The samples dispersed in sodium acetate buffer, pH 5.0, were treated with 200 µl of (10 mg in 1 ml of 0.4 M sodium acetate buffer, pH 5.0) invertase (EC 3.2.1.26; 300 U/mg) for 30 min at 37 °C. After centrifugation, reducing sugars were analyzed in the supernatants using the DNS reagent. The resistant (RS) and potentially bioavailable (AS) starch content was analyzed on the basis of results obtained after digestion *in vitro*. After centrifugation (8000*g*, 15 min) and removal of supernatant, the pellet was dispersed with 2 M KOH, hydrolyzed with amyloglucosidase, and then liberated glucose was quantified, as described above, for total starch (TS). Resistant starch (RS) was calculated as glucose ×0.9. The potentially bioavailable starch (AS) content was calculated as the difference between TS and RS.

The *in vitro* digestibility of starch was evaluated on the basis of total starch content (TS) and resistant starch (RS) determined after digestion *in vitro* according to Świeca et al. (2013a).

2.4. Statistical analysis

All experimental results were mean ± S.D. of three parallel experiments ($n = 9$). One-way analysis of variance (ANOVA and Tukey test) was used to compare groups within different elicitors. P values < 0.05 were regarded significant.

3. Results and discussion

Currently, one of the main areas of research is the search for new food products that, in addition to nutritional quality, also possessed natural ingredients with biological activity (e.g., antioxidant, antiviral, antihypertensive, etc). There are some reports describing the application of elicitation in order to improve the nutraceutical potential of sprouts (Świeca et al., 2014a,b, 2013a; Pérez-Balibrea et al., 2011; Gawlik-Dziki et al., 2013; Oh et al., 2009).

In this study, elicitation of sprout metabolism by abiotic elicitors significantly changed the qualitative and quantitative profile of phenolics. The levels of total phenolics and flavonoids determined in sprouts treated with 200 mM and 600 mM mannitol and at 40 °C were significantly higher in comparison with those from the control (Table 1). Among

Table 2 Phenolic composition of lentil sprouts cultivated under abiotic stress conditions.

Germination conditions	Compounds [µg/g flour]										
	Gallic acid	(+) catechin	*p*-coumaric acid	Benzoic acid	Salicylic acid	Chlorogenic acid	Caffeic acid	Ferulic acid	Naringenin	Quercetin	Kemferol	Daidzein
C	0.48 ± 0.08^{a}	48.56 ± 1.9^{e}	33.08 ± 1.0^{bc}	18.68 ± 2.9^{de}	28.30 ± 1.2^{a}	3.05 ± 0.8^{a}	8.64 ± 0.4^{c}	1.08 ± 0.1^{a}	0.05 ± 0.10^{a}	4.16 ± 0.2^{d}	2.69 ± 0.11^{d}	0.17 ± 0.01^{a}
Ox1	1.05 ± 0.61^{bc}	110.14 ± 3.0^{f}	51.43 ± 2.7^{e}	22.82 ± 2.6^{e}	37.25 ± 4.6^{b}	5.89 ± 0.3^{b}	7.22 ± 0.7^{bc}	2.94 ± 0.9^{cd}	2.67 ± 0.62^{e}	3.87 ± 0.1^{d}	4.32 ± 0.20^{e}	0.27 ± 0.01^{b}
Ox2	0.66 ± 0.42^{ab}	121.28 ± 2.1^{g}	42.12 ± 2.9^{d}	13.50 ± 1.9^{c}	30.45 ± 2.3^{ab}	8.65 ± 0.2^{c}	7.13 ± 0.3^{b}	2.09 ± 0.9^{bcd}	1.74 ± 0.64^{de}	1.40 ± 0.2^{a}	0.09 ± 0.04^{a}	0.72 ± 0.12^{d}
Os1	0.58 ± 0.03^{a}	26.77 ± 2.1^{c}	37.52 ± 1.0^{b}	1.57 ± 1.4^{a}	42.88 ± 1.7^{c}	6.91 ± 0.0^{b}	7.83 ± 0.2^{b}	1.86 ± 0.1^{b}	0.53 ± 0.06^{c}	9.65 ± 1.5^{d}	0.02 ± 0.00^{a}	1.56 ± 0.14^{f}
Os2	0.78 ± 0.02^{b}	39.86 ± 6.8^{de}	34.11 ± 2.4^{c}	17.50 ± 2.3^{cd}	39.54 ± 2.2^{bc}	14.20 ± 1.0^{e}	11.48 ± 0.8^{d}	2.30 ± 0.2^{bc}	1.71 ± 0.17^{d}	11.17 ± 0.1^{e}	0.01 ± 0.00^{a}	1.06 ± 0.12^{e}
S-O1	3.79 ± 0.14^{d}	6.56 ± 0.1^{a}	30.95 ± 1.5^{b}	6.07 ± 1.0^{b}	27.50 ± 0.6^{a}	3.27 ± 0.2^{a}	4.06 ± 0.2^{a}	0.98 ± 0.1^{a}	0.23 ± 0.14^{ab}	4.33 ± 0.4^{d}	6.80 ± 0.27^{f}	0.14 ± 0.00^{a}
S-O2	1.59 ± 0.00^{c}	39.24 ± 0.8^{d}	29.21 ± 2.6^{b}	28.34 ± 1.0^{f}	33.86 ± 2.6^{b}	11.94 ± 0.1^{d}	6.65 ± 0.8^{b}	2.86 ± 0.1^{d}	0.74 ± 0.06^{c}	2.80 ± 0.2^{b}	0.02 ± 0.00^{a}	0.10 ± 0.01^{a}
TC	0.96 ± 0.16^{b}	21.10 ± 0.9^{b}	24.85 ± 0.9^{a}	6.22 ± 1.6^{b}	42.50 ± 2.5^{c}	9.96 ± 0.5^{c}	4.49 ± 0.3^{a}	1.16 ± 0.0^{a}	1.49 ± 0.03^{d}	1.24 ± 0.0^{a}	0.93 ± 0.04^{b}	0.12 ± 0.01^{a}
TH	1.42 ± 0.02^{c}	28.95 ± 1.0^{e}	35.02 ± 6.3^{abd}	15.68 ± 0.2^{c}	43.47 ± 2.5^{c}	11.42 ± 0.5^{cd}	8.38 ± 0.6^{bc}	1.41 ± 0.2^{b}	0.60 ± 0.22^{bc}	3.54 ± 0.6^{bc}	1.30 ± 0.05^{c}	0.39 ± 0.02^{c}

*Means in columns followed by different letters are significantly different at $p = 0.05$. Each value represents the mean of 3 independent experiments (±SD).
C, control; Ox1, induction with 20 mM H_2O_2; Ox2, induction with 200 mM H_2O_2; Os1, induction with 200 mM mannitol; Os2, induction with 600 mM mannitol; S-O1, induction with 100 mM NaCl; S-O2, induction with 300 mM NaCl; TC, induction at 4 °C; TH, induction at 40 °C.

the sprouts studied, those obtained by elicitation by osmotic (Os2) and ion-osmotic (S-O2) shocks had the highest total phenolic content levels: 6.52 and 6.56 mg/g flour, respectively, (Table 1). In comparison with control total flavonoid and condensed tannin contents were also elevated; an increase of about 90% for osmotic stress and 79% for salt-osmotic stress (Table 1). In response to shock treatments lentil sprouts accumulated a number of phenolic compounds. The qualitative–quantitative analysis of phenolics indicated the presence of eleven hydroxycinnamic and hydroxybenzoic acids and six flavonoids. It was found that in 8-day-old sprouts *p*-coumaric, benzoic acids and (+)-catechin were the dominant phenolic components (Table 2). Among the studied elicitors, the highest amounts of polyphenolics were determined for sprouts treated with H_2O_2 and mannitol (Table 2). The stimulated influence of these factors was clearly visible in the case of chlorogenic, ferulic, *o*-coumaric and salicylic acids. It should be noted that among the studied modifications of sprouting a direct oxidative stress (Ox1, Ox2) produced with H_2O_2 significantly enhanced the levels of (+)-catechin and *p*-coumaric acid. A marked elevation of chlorogenic and gallic acid contents was determined for sprouts incubated at 4 °C and 40 °C, about the threefold and twofold increase in respect to control, respectively. The dominant flavonoid in the lentil sprouts was (+)-catechin. Daidzein and quercetin levels in sprouts were increased about twofold in sprouts obtained by elicitation with mannitol. An extreme reduction in benzoic acid levels, linked with elevation of salicylic acid content, was observed in sprouts treated with 200 mM mannitol, 100 mM NaCl and incubated at 4 °C (Table 2).

Under normal conditions, during germination lentil produces various phenolics (Świeca et al., 2012). Data concerning polyphenolic profiles of lentil sprouts are in agreement with those obtained by Cevallos-Casals et al. (2010) and Ghavidel et al. (2007). Studies on the stress signaling pathway suggest that ROS signaling plays a key role in the cross-talk between biotic and abiotic signaling (Fujita et al., 2006). There is much evidence that generation of ROS during stress in plant induces overproduction of phenolics (Shulaev et al., 2008). These compounds are mainly produced to protect plants from biotic/abiotic stresses such as photooxidation stress, reactive oxygen species, wounds, disease and herbivores (Shetty, 2004). Application of different cultivation conditions (biotic and abiotic stresses) can result in an enhanced production of these antioxidants. These findings were attributed to the biochemical changes in sprout metabolism, which might influence the production of compounds, such as anthocyanins and flavonoids, or release aglycones from conjugated glycosides due to enzymatic activation (Shetty, 2004). Although, all the studied modifications caused the overproduction of polyphenolic the qualitative analysis suggests that response differs significantly. An elevation of plant polyphenolics in response to elicitors was also observed by Swieca et al. (2014a) and Randhir et al. (2004). The cited investigators reported an induction of the pentose phosphate pathway, the shikimate and the phenylpropanoid pathways during plant growth under stress condition. Plants increase the level of salicylic acid (signaling compound) and other phenolics acting as antioxidants and/or precursors for the synthesis of lignin, suberin and phenolic barriers. High levels of *p*-coumaric acids might be involved in the overproduction of coumarylCoA, a key compound in flavonoid biosynthesis (Shetty, 2004). High amounts of *p*-coumaric, ferulic and caffeic acids (potential precursors of barriers) in lentil sprouts treated with mannitol may suggest that plant response in this case is mainly bound with sealing up the cells. These observations seem to confirm results obtained by Bandeoğlu et al. (2004). It should be noted that, similar to studies performed by Dixon (2001), the stress conditions applied caused a significant increase in protecting compounds e.g. chlorogenic acid. Also, Feng et al. (2010) reported the accumulation of flavonoids in soybean seedlings under stress. Similar results were also reported in this work for osmotic stress conditions.

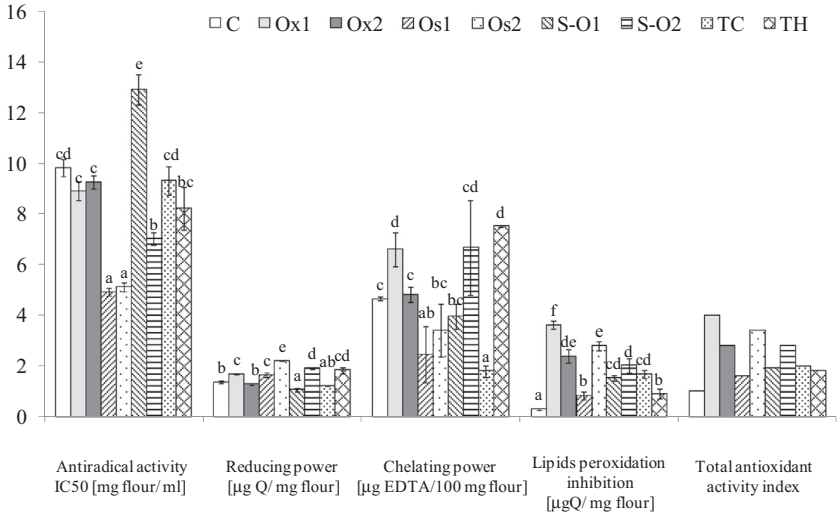

Figure 1 Antioxidant activities of sprouts cultivated under abiotic stress conditions C, control; Ox1, induction with 20 mM H_2O_2; Ox2, induction with 20 mM H_2O_2; Os1, induction with 200 mM mannitol; Os2, induction with 600 mM mannitol; S-O1, induction with 100 mM NaCl; S-O2, induction with 300 mM NaCl; TC, induction at 4 °C; TH, induction at 40 °C. Means followed by different letters are significantly different at $p < 0.05$. Each value represents the mean of 3 experiments (±SE).

Table 3 Influence of elicitation on growth rate and nutritional quality of sprouts.

	Cultivation conditions								
	C	Ox1	Ox2	Os1	Os2	S-O1	S-O2	TC	TH
Growth ratio	4.56 ± 0.01^{d}	4.14 ± 0.01^{c}	4.68 ± 0.14^{d}	3.74 ± 0.09^{b}	2.91 ± 0.00^{a}	4.83 ± 0.09^{d}	2.96 ± 0.15^{a}	5.07 ± 0.08^{d}	4.75 ± 0.31^{cd}
Proteins[mg/g flour]	227.99 ± 5.83^{c}	189.83 ± 1.51^{a}	181.49 ± 6.37^{a}	207.83 ± 2.97^{b}	251.80 ± 3.68^{d}	250.49 ± 8.56^{d}	280.39 ± 10.05^{e}	226.96 ± 3.80^{c}	226.21 ± 4.84^{c}
Non-protein nitrogen [mg/g flour]	96.00 ± 1.48^{c}	99.12 ± 0.82^{cd}	104.05 ± 1.22^{e}	96.72 ± 2.22^{c}	70.23 ± 2.68^{b}	74.89 ± 2.06^{b}	57.99 ± 1.37^{a}	108.19 ± 1.13^{f}	102.86 ± 2.01^{de}
Protein digestibility [%]	54.25 ± 3.25^{cb}	67.94 ± 4.08^{ef}	70.94 ± 4.26^{f}	60.56 ± 3.63^{de}	52.06 ± 3.12^{b}	42.65 ± 2.56^{a}	47.19 ± 2.83^{ab}	54.87 ± 3.29^{cb}	56.22 ± 3.37^{cd}
Total starch[mg/g flour]	255.28 ± 7.89^{a}	244.30 ± 17.82^{a}	245.92 ± 11.96^{a}	264.82 ± 10.18^{a}	288.40 ± 3.82^{c}	276.70 ± 4.58^{b}	296.86 ± 2.51^{d}	286.60 ± 2.80^{e}	258.52 ± 5.25^{a}
Resistant starch [mg/g flour]	33.59 ± 6.01^{a}	93.96 ± 24.18	70.70 ± 11.39^{cd}	92.25 ± 7.00^{e}	90.90 ± 4.58^{ef}	78.44 ± 10.88^{de}	56.57 ± 5.79^{c}	46.26 ± 1.40^{b}	33.44 ± 3.63^{a}
Potentially bioavailable starch [mg/g flour]	221.69 ± 11.08^{cd}	150.34 ± 7.52^{a}	175.22 ± 8.76^{b}	172.57 ± 8.63^{ab}	197.50 ± 9.87^{c}	198.26 ± 9.91^{c}	244.29 ± 10.01^{d}	240.34 ± 12.02^{d}	225.08 ± 11.25^{cd}
Starch digestibility [%]	86.84 ± 4.34^{c}	61.54 ± 3.08^{a}	71.25 ± 3.56^{b}	65.16 ± 3.26^{ab}	68.48 ± 3.42^{ab}	71.65 ± 3.58^{b}	80.95 ± 4.05^{c}	83.86 ± 4.19^{c}	87.07 ± 4.35^{c}

C, control; Ox1, induction with 20 mM H_2O_2; Ox2, induction with 20 mM H_2O_2; Os1, induction with 200 mM mannitol; Os2, induction with 600 mM; mannitol; S-O1, induction with 100 mM NaCl; S-O2, induction with 300 mM NaCl; TC, induction at 4 °C; TH, induction at 40 °C.

*Means in rows followed by different letters are significantly different at $P < 0.05$.

Each value represents the mean of 3 independent experiments (±SD).

Changes in phenolic content were generally associated with the changes in antioxidant capacity of the studied sprouts. The antioxidant potential of sprouts was evaluated on the basis the four complementary methods. Radical scavengers acted in a dosage-dependent manner (data not shown), thus calculation of the 50% inhibitory concentration (IC_{50}) was possible. The highest abilities to quench free radicals were observed in sprouts induced with osmotic stress – Os1 and Os2 (IC_{50}- 4.91 and 5.12 mg flour per ml, respectively), whereas the lowest was in salt-ionic case (100 mM NaCl- IC_{50}- 12.88 mg flour per ml) (Fig. 1). A significant decrease of reducing abilities was observed in sprouts treated with 100 mM NaCl and incubated at 4 °C (1.07 and 1.22 μg Q/g flour, respectively), while the highest abilities were recorded for seedlings treated with 600 mM mannitol and 300 mM NaCl (2.21 and 1.92 μg Q/g flour, respectively), (Fig. 1). The highest chelating power was determined for sprouts from Ox1, S-O2 and TH treatments (7.36, 5.21 and 5.40 μg EDTA/g flour, respectively), (Fig. 1). Among the studied activities the ability to protect lipids against peroxidation was affected by elicitation at the highest degree. A marked elevation of this ability was determined for sprouts treated with 20 mM, 200 mM H_2O_2 and 600 mM mannitol, about a 12-fold, 8-fold and 9.5-fold increase above that for the sprouts cultivated at control conditions, respectively (Fig. 1). Due to the fact that antioxidant capacity of complex food systems is created by the activities that differ in the mode of action, there is no simple and universal method by which ''total antioxidant capacity'' can be measured accurately and quantitatively. Thus, for better evaluation of the total antioxidant potential the total antioxidant activity index (AI) was proposed. It should be noted that all studied modifications of sprouting caused an elevation of the antioxidant potential of sprouts. The highest values of AI were determined for sprouts elicited with 20 mM H_2O_2 and 600 mM mannitol: 4.0 and 3.4, respectively (Fig. 1).

Sprouting improves nutritional value of lentil, however negatively affects phenolics content and antioxidant activity, thus looking forward for an effective, safe and acceptable method of elevation of antioxidant activities is very important. Generally, in these studies the elevated concentrations of phenolics (especially flavonoids) were linked with a subsequent increase of antioxidant potential of sprouts, which may suggest that these compounds act as effective antioxidants (Table 4). The level of phenolics was most effectively increased by application of H_2O_2 and mannitol as elicitors. Data concerning antioxidant activity are in agreement with the previous reports by Shetty (2004) and Randhir et al. (2004). The cited investigators reported that elicitation of bean seeds with fish protein hydrolysates, lactoferrin and oregano extract enhanced production of phenolic antioxidants and ability to quench free radicals. The nutraceutical value of edible sprouts was also effectively enhanced by Gawlik-Dziki et al. (2013). Elicitations of sprouts with salix bark extract and yeast extract significantly increased their reductive potential and their abilities to prevent lipids against oxidation and to scavenge free radicals. It should be noted that sprouts obtained in this studies had a very strong ability to prevent lipids. It may be speculated that similarly to the studies performed by Gawlik-Dziki et al. (2013), an increase of this ability was linked with increased concentrations of flavonoids. Additionally, the high reducing potential of seedlings may be due to the high content of hydroxycinnamic acids, which are able to reduce iron ions (Table 4).

Table 4 Relationships between phenolics and antioxidant activities in lentil sprout (Pearson correlation coefficient).

	Total phenolics	Total flavonoids	Condensed tannins	Hydroxycinnamic acids	Hydroxybenzoic acids
Antiradical activity	0.71	0.56	0.73	0.79	0.59
Reducing power	0.91	0.81	0.81	0.89	0.66
Chelating power	−0.01	0.25	−0.16	0.37	0.36
Lipid peroxidation inhibition	0.21	0.55	0.17	0.34	0.46

As it was presented elicitation is an effective method for improving nutraceutical quality of sprout but sometimes it may negatively influence growth and nutritional quality of sprouts. Thus, the influence of studied factors on biomass accumulation, protein and starch content and digestibility was studied. Water deficient conditions, caused by mannitol and NaCl treatments, significantly reduced the growth of lentil sprouts. Cultivations watered with high osmotic potential solutions (about – 1.4 MPa; 600 mM mannitol (Os2) and 300 mM NaCl (S-O2)) were characterized by the largest biomass reduction – about 35% when compared to control sprouts. In contrast, apart from Os1 and Ox1, the treatments applied did not cause any significant biomass reduction in 8-day-old sprouts (Table 3). In spite of the fact that lentils are known to be salt sensitive in this work only higher concentration of NaCl negatively influenced plant growth. This finding is partially in opposition to results obtained by Bandeoglu et al. (2004). The citied investigators determined a statistically significant reduction of biomass in 9-day-old lentil seedlings after treatments with 100 mM and 200 mM NaCl (62% and 43%, respectively).

Total protein content was significantly reduced in sprouts obtained at Ox1 and Ox2 conditions; in comparison with control conditions their levels were lower about 16.7 and 20.4%, respectively. In respect to control seedlings sprouts from these cultures were also characterized by elevated protein digestibility. Protein digestibility and non-nitrogen contents determined for sprouts elicited with 100 mM and 300 mM NaCl and 600 mM mannitol were significantly lower than those determined for control sprouts. Changes in total starch content caused by elicitation did not exceed 15%. The highest total starch content was determined for S-O2 conditions, whereas the lowest was for Ox1 and Ox2 conditions. 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 (Table 3). In the recent literature there is a lack of information concerning the influence of elicitation on the nutritional quality of sprouts. A decrease in protein content and subsequent elevation of non-protein nitrogen fraction may be explained by induction of proteolytic systems in response to stress conditions what was already observed by Świeca et al. (2014). Data on starch content and digestibility are in agreement with previous data reported by Ghavidel et al. (2007) and Hoover and Zhou (2003). There are no literature data concerning starch content and bioaccessibility in the light of metabolism inductions by elicitors. Changes in total starch content and an increase of resistant starch level in elicited sprouts may be due to modification of seedling metabolism in response to stress conditions (Ghavidel et al., 2007). An increase in starch mobilization is often observed during stress and is linked with the increased demand for energy needed to produce secondary metabolites (Feng et al., 2010). It should be noted that in our studies 2-day–old sprouts were elicited and undesirable effects of abiotic elicitors (environmental shock) were reduced. Additionally, elicitation is based on the natural mechanisms involving in plant response to pathogen, thus microbial contaminations are probably reduced (Peñas et al., 2009). According to the literature data it is also known that in genus *Lens* there is no overproduction of "dangerous" metabolites such as alkaloids, toxins (Lin and Lai, 2006).

4. Conclusion

The results of this study show that application of abiotic elicitors (environmental shocks) was an effective method for improvement of sprout pro-health potential *via* an increase of phenolic contents and subsequent elevation of antioxidant potential. Innovative application of elicitors on 2-day-old sprouts (not seed) allowed the elimination of the unfavorable influence of the factors employed on germination yield and biomass production. Assuming that the optimal germination conditions are those which most effectively increase the antioxidant potential without any negative influence on biomass accumulation and nutritional quality the elicitation with 20 mM H_2O_2 for the future applications is recommended. As the use of abiotic elicitors is cheap and relatively easy to adopt in the edible sprout production industry, this biotechnology seems to be an alternative to conventional techniques applied to improve the health promoting phytochemical levels and bioactivity of low-processed food.

References

Ahmed, S.A. and Baig, M.M.V., 2014. Biotic elicitor enhanced production of psoralen in suspension cultures of *Psoralea corylifolia* L. Saudi J. Biol. Sci. 21 (5), 499–504.

Bandeoğlu, E., Eyidoğan, F., Yücel, M., Öktem, H.A., 2004. Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regul. 42, 69–77.

Baenas, N., García-Viguera, C., Moreno, D.A., 2014. Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19 (9), 13541–13563, Biotechnology.

Cevallos-Casals, B.A., Cisneros-Zevallos, L., 2010. Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chem. 119 (4), 1485–1490.

Decker, E.A., Welch, B., 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem. 38, 674–677.

Dixon, R.A., 2001. Natural products and plant disease resistance. Nature 411, 843–847.

Feng, S., Song, L., Lee, Y.K., Huang, D., 2010. The effects of fungal stress on the antioxidant contents of black soybeans under germination. J. Agric. Food Chem. 58, 12491–12496.

Fernandez-Panchon, M.S., Villano, D., Troncoso, A.M., Garcia-Parrilla, M.C., 2008. Antioxidant activity of phenolic compounds:

from *in vitro* results to *in vivo* evidence. Crit. Rev. Food Sci. Nutr. 48, 649–671.
Francis, G.A., Gallone, A., Nychas, G.J., Sofos, J.N., Colelli, G., Amodio, M.L., Spano, G., 2012. Factors affecting quality and safety of fresh-cut produce. Crit. Rev. Food Sci. Nutr. 52, 595–610.
Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr. Opin. Plant Biol. 9 (4), 436–442.
Gawlik-Dziki, U., Jeżyna, M., Świeca, M., Dziki, D., Baraniak, B., Czyż, J., 2012. Effect of bioaccessibility of phenolic compounds on in vitro anticancer activity of broccoli sprouts. Food Res. Int. 49, 469–476.
Gawlik-Dziki, U., Świeca, M., Dziki, D., Sugier, D., 2013. Improvement of nutraceutical value of broccoli sprouts by natural elicitors. Acta Sci. Pol., Hortoru. 12 (1), 129–140.
Ghavidel, R.A., Prakash, J., 2007. The impact of germination and dehulling on nutrients antinutrients *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seed. Food Sci. Technol. 40, 1292–1299.
Goni, I., Garcia-Alonso, A., Saura-Calixto, F., 1997. A starch hydrolysis procedure to estimate glycemic index. Nutr. Res. 17, 427–437.
Groupy, P., Vulcain, E., Caris-Veyrat, C., Dangles, O., 2007. Dietary antioxidants as inhibitors of the heme-induced peroxidation of linoleic acid: Mechanism of action and synergism. Free Radic. Biol. Med. 43, 933–943.
Habeeb, A., 1966. Determination of free amino groups in proteins by trinitrobenzeno sulfonic acid. Anal. Biochem. 14, 328–336.
Hoover, R., Zhou, T., 2003. In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes – a review. Carbohydr. Polym. 54, 401–417.
Lamaison, J.L.C., Carnet, A., 1990. Teneurs en principaux flavonoids des fleurs de Crataegeus monogyna Jacq et de Crataegeus laevigata (Poiret D. C) en fonction de la vegetation. Pharm. Acta Helv. 65, 315–320.
Lin, P.-Y., Lai, H.-M., 2006. Bioactive compounds in legumes and their germinated products. J. Agric. Food Chem. 54 (11), 3807–3814.
Lowry, O.H., Rosebrough, N.J., Farr, L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.
Oh, M.-M., Rajeshekar, C.B., 2009. Antioxidant content of edible sprouts: effect of environmental shocks. J. Sci. Food Agric. 89, 2221–2227.
Oyaizu, M., 1986. Studies on products of browning reaction – Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315.
Pająk, P., Socha, R., Gakowska, D., Rożnowski, J., Fortuna, T., 2014. Phenolic profile and antioxidant activity in selected seeds and sprouts. Food Chem. 143, 300–306.
Peñas, E., Gómez, R., Frías, J., Vidal-Valverde, C., 2009. Efficacy of combinations of high pressure treatment, temperature and antimicrobial compounds to improve the microbiological quality of alfalfa seeds for sprout production. Food Control 20, 31–39.
Pérez-Balibrea, S., Moreno, D.A., García-Viguera, C., 2011. Improving the phytochemical composition of broccoli sprouts by elicitation. Food Chem. 129 (1), 35–44.
Randhir, R., Lin, Y.-T., Shetty, K., 2004. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochem. 39, 637–646.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.
Shetty, K., 2004. Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. Process Biochem. 39, 789–804.
Shulaev, V., Cortes, D., Miller, G., Mittler, R., 2008. Metabolomics for plant stress response. Plant Physiol. 132 (2), 199–208.
Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1974. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299, 152–178.
Sun, B., Ricardo-Da-Silva, J.M., Spranger, I., 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46 (10), 4267–4274.
Świeca, M., Baraniak, B., Gawlik-Dziki, U., 2014. Effect of selected divalent cations on protein mobilization in lentil (*Lens* culinaris) sprouts. J. Elem. 19 (2), 577–585.
Świeca, M., Baraniak, B., 2014b. Influence of elicitation with H_2O_2 on phenolics content, antioxidant potential and nutritional quality of *Lens* culinaris sprouts. J. Sci. Food Agric. 94 (3), 489–496.
Świeca, M., Baraniak, B., 2014c. Nutritional and antioxidant potential of lentil sprouts affected by elicitation with temperature stress. J. Agric. Food Chem. 62 (14), 3306–3313.
Świeca, M., Baraniak, B., Gawlik-Dziki, U., 2013a. *In vitro* digestibility and starch content, predicted glycemic index and potential *in vitro* antidiabetic effect of lentil sprouts obtained by different germination techniques. Food Chem. 138 (2–3), 1414–1420.
Świeca, M., Gawlik-Dziki, U., Dziki, D., Baraniak, B., Czyz, J., 2013b. The influence of protein–flavonoid interactions on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. Food Chem. 141 (1), 451–458.
Świeca, M., Gawlik-Dziki, U., Kowalczyk, D., Zotek, U., 2012. Impact of germination time and type of illumination on the antioxidant compounds and antioxidant capacity of *Lens* culinaris sprouts. Sci. Hortic. (Amsterdam) 140, 87–95.
Świeca, M., Sęczyk, ., Gawlik-Dziki, U., 2014. Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts. Food Chem. 161, 288–295.
Tsurunaga, Y., Takahashi, T., Katsube, T., Kudo, A., Kuramitsu, O., Ishiwata, M., Matsumoto, S., 2013. Effects of UV-B irradiation on the levels of anthocyanin, rutin and radical scavenging activity of buckwheat sprouts. Food Chem. 141 (1), 552–556.
Zhao, J., 2007. Nutraceuticals nutritional therapy phytonutrients and phytotherapy for improvement of human health: a perspective on plant biotechnology application. Recent Pat. Biotechnol. 1, 75–97.