ELICITATION AND ELICITATION SUPPORTED WITH THE PHENYLPROPANOIDS PATHWAY FEEDING FOR THE ELEVATION OF PHENOLICS CONTENT IN QUINOA SPROUTS

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ABSTRACT

The aim of this study was to evaluate the combined action of phenylpropanoids feeding (shikimic acid, phenylalanine, tyrosine) and elicitation as a strategy for the elevation of phenolic content in quinoa sprouts. The highest increase of flavonoids content was found for the sprouts treated with shikimic acid. All the studied modifications increased the antioxidant potential of sprouts. The highest reducing power was found for the sprouts treated with 200 mM H_2O_2 obtained by phenylalanine feeding (2.65 mg TE/g d.m.), and those treated with 50 mM H_2O_2 and fed with phenylalanine (2.54 mg TE/g d.m.). Therefore, elicitation and elicitation supported can be considered as a promising approach to improve the content of phenolics and allows to increase the nutraceutical potential of quinoa sprouts.

BACKGROUND

In recent years, quinoa sprouts have emerged as one of the most nutrient-packed, beneficial, and easy-to-make health food. Sprouts have a lot of nutrients, minerals, vitamins and antioxidants. The production of sprouts is easy and fast. Sprouting begins with soaking, where the endogenous enzymatic systems are activated in seeds. As a result, amylolytic, proteolytic and lipolytic enzymes are produced. During germination, reserve substances (proteins, fats and carbohydrates) are broken down to the simple compounds. The content of proteins in grain decreases, while their availability in sprouts increases. Proteins present in the sprouts have greater digestibility and nutritional value than the protein found in plant seeds. Similarly, carbohydrates are broken down from complex forms (polysaccharides) to simple compounds (disaccharides and simple sugars) [1].

Quinoa is one of the most nutritive grains. Quinoa is gluten-free, high in protein and contains all nine essential amino acids. The content of lysine, methionine and cysteine in quinoa is higher than in common cereals and legumes, making it complementary to these crops. It has a glycemic index of 53, which is considered low. Quinoa is rich in oil, containing beneficial fatty acids and a high content of tocopherols. In addition, it contains a large range of vitamins (ascorbic acid and tocopherols) and microelements (i.e., phosphorus, copper, manganese, iron, zinc, calcium, magnesium, sodium, and potassium) [2, 3].

The human body is unable to synthesize aromatic compounds, such as polyphenols, so they should be supplied with food. Rich in polyphenol compounds are fruits and vegetables in raw as well as processed form. Phenolics are also found in coffee, tea, wine and chocolate. Polyphenols have a significant impact on the health of our body. They have the ability to reduce the risk of diet-related diseases. They can minimize the effects of stress, inhibit the oxidation of lipids and nucleic acids, neutralize free radicals and the chelate metal ions. Flavonoids have also the ability to strengthen capillary walls and more over inhibits free radical oxidation reactions [4]. Previous studies have shown that phytochemicals can be increased after germination. The enhancement of the antioxidant activity during germination due to an increased content of polyphenols has been reported by several authors [5, 6].

Polyphenols in plants play diverse functions and are immensely important (act as agents in plant defense, hormones). Phenolics are mainly produced through the pentose phosphate, the shikimate, and the phenylpropanoid pathways. Phenylalanine and tyrosine ammonia-lyases, playing a fundamental role in the phenylpropanoids metabolism, transform aromatic amino acids into trans-cinnamic and p-coumaric acids, respectively. The function of these enzymes is increased under stress conditions, which results in an accumulation of “pathogen-related compounds,” including phenolics. This occurrence may be used for improving phenolics overproduction in plant systems [7]. Several authors have applied exogenous elicitors during germination of seeds to stimulate induction of phenylpropanoids metabolism for the production of buckwheat [8] broccoli [9], lentil [10], wheat [11] or mung bean sprouts [12].

In this study, the effects of hydrogen peroxide treatment and the phenylpropanoid pathway feeding on changes in the phenolics and antioxidant capacity of quinoa sprouts were studied.

MATERIAL AND METHODS

Chemicals

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), ammonium thiocyanate, and polyvinylpyrrolidone were purchased from Sigma-Aldrich Company (Poznan, Poland). All other chemicals were of analytical grade.

Sprouting conditions

Quinoa (*Chenopodium quinoa*) seeds were bought in local organic shop. Seeds were sterilized in 1% (v/v) sodium hypochloride for 3 min. and washed with distilled water. Before sprouting, quinoa seeds were soaked for 4 h in distilled water (control; C, C1, and C2) or phenolic precursors (0.1 mM shikimic acid, S, S1, and S2; 0.1 mM L-phenylalanine, F, F1, and F2; 0.1 mM L-tyrosine, Y, Y1, and Y2). Seeds were sprouted on petri dishes (Ø 125 mm) in darkness at 25 °C and 75% of humidity. Seeds were watered daily with water (control experiment) or with elicitor – hydrogen peroxide. For the treatment 1-day-old sprouts were sprayed with 5 mL of 50 mM (C1, F1, and Y1) or 200 mM (C2, F2, and Y2) hydrogen peroxide. After 3 days, sprouts were collected, freeze-dried, milled and stored in vacuum bags at −20 °C until further analysis.

Analysis of phenolics and antioxidant capacity

Extraction Procedure

500 mg of quinoa flour was extracted three times with 5 ml of 80% ethanol. The samples were shacked for 30 minutes at 40ºC at 50 rpm/min. After this time, the samples were centrifuged for 5 min, 6900 ×g. All fractions were collected, combined, and used for further analysis.

Total phenolics content

The amount of total phenolics was determined using Folin-Ciocalteau reagent [13]. To 100 µl of the extract 100 µl of H_2O and 400 µl of Folin–Ciocalteau reagent (1:5 H_2O) were added. After 3 min 2.5 mL of 10% Na_2CO_3 was added and samples were allowed to stand for 30 min at room temperature. After that time absorbance was measured at 725 nm. The amount of total phenolics was expressed as gallic acid equivalents (GAE) in mg per g of sprouts dry mass (d.m.).

Total flavonoid content

Total flavonoid content was determined according to the method described by Lamaison and Carnat [14]. 1 mL of the extract was mixed with 1 mL of 2% $AlCl_3$ x $6H_2O$ solution. Mixture was incubated at room temperature for 20 min. Following, absorbance was measured at 430 nm. Total flavonoids content was expressed as quercetin equivalents (QE) in mg per g of sprouts dry mass (d.m.).

Reducing power

Reducing power was determined by the method of Pulio et al. (2000). The extract (0.5 mL) was mixed with

phosphate buffer (0.5 mL, 200 mM, pH 6.6) and potassium ferricyanide $K_3[Fe(CN_6)]$ (0.5 mL, 1%). The mixture was incubated at 50 ºC for 20 min. Reactions were stopped with 0.5 mL 10% TCA and centrifuging for 10 min at 6500 × g. The upper layer of solution (2.5 mL) was mixed with distilled water (1.5 mL) and 300 µl of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Reducing power was expressed as Trolox equivalents in mg per g of sprouts dry mass (d.m.).

Antiradical activity (ABTS)

The experiments were carried out using the ABTS decolourization assay (Re et al. 1999). The ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting 7 mM stock solution of ABTS 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 $ABTS^{+\bullet}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Lambda 40 UV-Vis spectrophotometer, Perkin Elmer Inc. Waltham, USA). Then, 40 mL of the extract obtained after digestion *in vitro* were added to 1.8 mL of $ABTS^{+\bullet}$ solution and the absorbance was measured at the end time of 5 min. The affinity of test material to quench 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 the extract obtained after digestion *in vitro*

Free radical scavenging ability was expresses as Trolox equivalents in mg per g of sprouts dry mass (d.m.).

Statistical Analyses

All experimental results were mean ± SD of three parallel experiments (n= 9). The obtained data was subjected to a statistical analysis and the consequent evaluations were analysed for a variance analysis. The statistical differences ($p < 0.05$.) were estimated through Tukey's test using the Statistica 6.0 software (StatSoft, Inc., Tulsa, USA).

RESULTS

The phenylpropanoids pathway was stimulated by elicitation with hydrogen peroxide as well as feeding with shikimic acid, phenylalanine, tyrosine. Research results showed that elicitation enhanced significantly ($p \leq 0.05$) the phenolic content of quinoa sprouts (Tab. 1). Compared to the control, total phenolics content was significantly increased by treatment of control sprouts with 50 mM (2.43 mg/g d.m.) and 200 mM H_2O_2 (2.52 mg/g d.m.). A significant increase was also determined in the sprouts fed with shimic acid (2.34 mg/g d.m.) and those fed with shikimic acid treated with 50 mM H_2O_2 (2.06 mg/g d.m.). However, the lowest content of polyphenols was determined in the sprouts fed with tyrosine and treated with 50 mM H_2O_2. It should be emphasized that the enrichment of seeds with phenylalanine and tyrosine and their subsequent elicitation with 200 mM H_2O_2 allowed to increase the polyphenols content by 16% and 23%, respectively.

The highest increase of flavonoids content was found in the sprouts fed with shikimic acid (2.08 mg/g d.m.). Among the analysed sprouts, the lowest content of flavonoids was determined in the sprouts from seeds supplemented with shikimic acid and treated with 200 mM H_2O_2.

Fig. 1 presents the effect of elicitation with hydrogen peroxide and elicitation supported by precursors of the phenylpropanoid pathway on the antiradical potential of the received sprouts. The germination process increased the ability to neutralize free radicals in relation to seeds. It should be noted that in all the sprouts, the elicitation caused an increase in the ability to neutralize free radicals. The highest ability to neutralize free radicals was demonstrated by the sprouts fed with tyrosine and treated with 200 mM H_2O_2.

The highest reducing power was found for the sprouts treated with 200 mM H_2O_2 obtained by phenylalanine feeding (2.65 mg TE/g d.m.) and those obtained from the seeds fed with shikimic acid (2.45 mg TE/g DW) (Fig. 2). Germination caused more than twice increase in the reduction power as compared to seeds. Elicitation of the sprouts with hydrogen peroxide slightly changed the value of the reduction power. The lowest value was recorded for sprouts fed tyrosine and treated with 200 mM H_2O_2.

DISCUSSION

Recently many authors paid a huge attention on phenolic compounds because of their antioxidant properties and different protective roles against diseases related with oxidative stress, such as cancer or cardiovascular either neurodegenerative diseases [17]. The application of elicitation for improving the nutraceutical potential of sprouts is described previously in many publications [18-20]. It is well known that sprouts are an excellent source of substances that enhance food and improve its functionality [21]. Through germination, the capacity and bioactivity of compounds with nutraceutical potential change dynamically, and most importantly, may be strongly affected by the elicitation [22, 23]. Sprouting is a very complex process; however in quinoa sprouts phenolics level is significantly lower than in seeds.

In our study, all the studied modifications caused the overproduction of polyphenolics. An elevation of plant polyphenolics in response to elicitors was also observed by Świeca et al. [24]. Quinoa sprouts have the highest content of polyphenols (Y200: 2.43±0.29 mg/g d.m.) The results showed that the content of polyphenols was most effectively increased by the elicitation of sprouts obtained from seeds soaked with phenylpropanoids pathway precursors with hydrogen peroxide. This result is was lower than that presented in the work Paśko et al. [25] where the content of polyphenols was 3.75±0.05 mg GAE g^{-1}. The difference may be due to the origin of grain and the use of other methods of grain preparation, germination conditions or research methods. Moreover, different research studies reported that germination is an efficient process to increase the total phenolics in soybean, black bean [26], broccoli radish and sunflower [27, 28]. After analysing the content of flavonoids in sprouts, it was unexpectedly noticed a decrease in their amount compared to the quinoa grains. However, when the elicitor (hydrogen peroxide) was used the amount of

flavonoids increased. The highest increase of flavonoids content was found for the sprouts treated with shikimic acid.

Phnolics were proved to have a significant correlation with antioxidant activity [29, 30]. Changes in phenolic content were generally associated with the changes in antioxidant capacity of the studied sprouts. Based on the data in the present work, antioxidant activity of quinoa sprouts is much higher than the dry grains. Both hydrogen peroxide treatment and precursors feeding caused about a double increase of reducing ability. The highest activity was found for the sprouts obtained from seeds fed with tyrosine and treated with 200 mM H_2O_2 (2,17 mg TE/g DW). The reason for this are differences in the content of polyphenols. Elicitation with the precursors of the phenylpropanoids pathway additionally allows to increase the antioxidant potential [18, 22, 25]. Researchers in the cited study observed the effect the modification of germination on the antioxidant activity of sprout.

CONCLUSIONS

In conclusion, improvement of quinoa sprout quality by elicitation with H_2O_2 and elicitation supported by the addition the phenylpropanoids pathway precursors is a useful tool for designing some new product with an increased nutraceutical potential. It can also be used as an alternative to conventional techniques applied to improve the levels of health-promoting phytochemicals and bioactivity in the low-processed food. Elicitation may improve health-promoting potential of sprouts, although selection of elicitor is crucial to deliver marketplace ready-to-eat sprouts enriched in specific bioactive phytochemicals.

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ABBREVIATIONS

d.m – dry mass
GAE – Gallic Acid Equivalents
SD – Standard Deviation
TE – Trolox Equivalents
QE – Quercetin Equivalents

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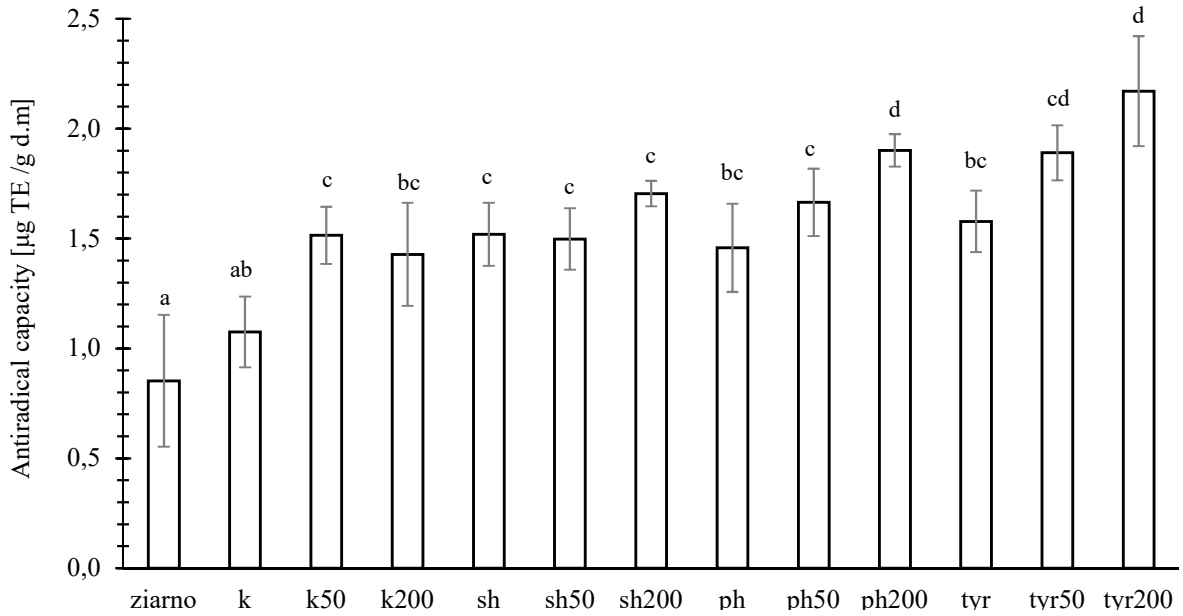
TAB. 1. THE EFFECT OF ELICITATION AND PRECURSOR FEEDING ON THE FLAVONOIDS AND PHENOLICS CONTENT.

		Total flavonoids [mg/g d.m.]	Total phenolics [mg/g d.m]
Sample	Seeds	1.60±0.17^{a}	1.10±0.08^{a}
	C	1.78±0.08^{ab}	2.31±0.25^{b}
	C50	1.78±0,07^{ab}	2.43±0,05^{b}
	C200	1.90±0.05^{b}	2.52±0,45^{b}
	S	2.08±0.09^{c}	2.34±0,23^{b}
	S50	1.92±0.08b^{c}	2.06±0,11^{bc}
	S200	1.62±0.03^{a}	2.19±0,32^{bc}
	F	1.92±0.03^{bc}	1.98±0,17^{c}
	F50	1.91±0.06^{b}	1.86±0,18^{c}
	F200	1.75±0.03^{a}	2.29±0,43^{bc}
	Y	1.84±0.03^{b}	1.85±0.08^{c}
	Y50	1.81±0.18^{abc}	1.89±0.07^{c}
	Y200	1.84±0.13^{ab}	2.27±0.29^{b}

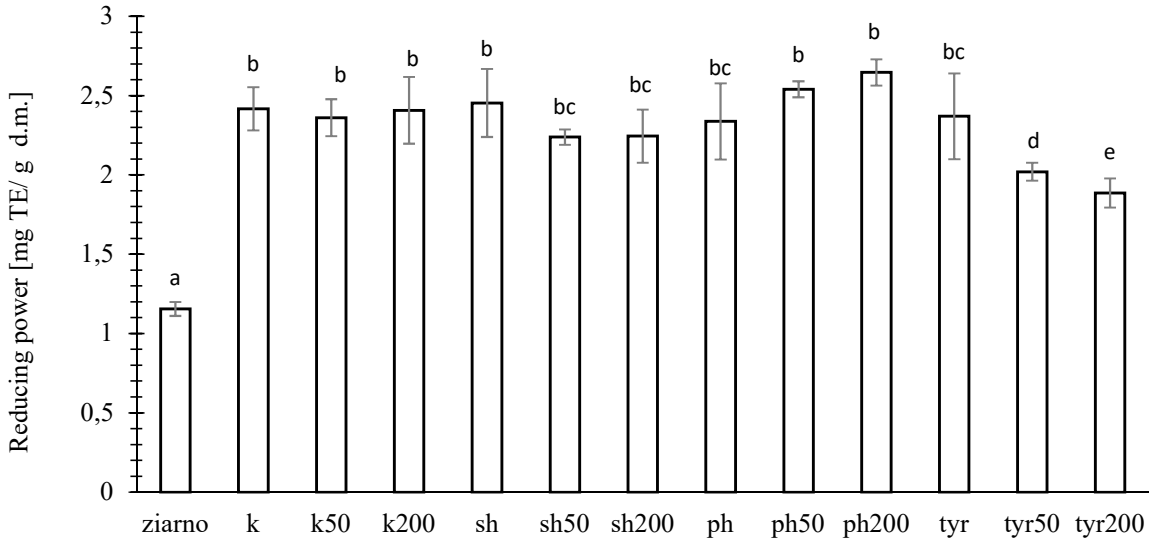
Value represents the mean of three independent experiments (±SD).
Means in columns followed by different letters are significantly different at $p \leq 0.05$.
C - control sprouts; C50- sprouts treated with 50 mM H_2O_2; S50- sprouts fed with shimic acid and treated with 50 mM H_2O_2; F50- sprouts fed with phenylalanine and treated with 50 mM H_2O_2; Y50- sprouts fed with tyrosine and treated with 50 mM H_2O_2; C200- sprouts treated with 200 mM H_2O_2; S200- sprouts fed with shimic acid and treated with 200 mM H_2O_2; F200- sprouts fed with phenylalanine and treated with 200 mM H_2O_2; Y200- sprouts fed tyrosine and treated with 200 mM H_2O_2.

FIG. 1. THE EFFECT OF ELICITATION AND PRECURSOR FEEDING ON THE ANTIRADICAL CAPACITY OF QUINOA SPROUTS.



Means followed by different small letters are significantly different ($p \leq 0.05$).
Each value represents the mean of three independent experiments (±SD).
C - control sprouts; C50- sprouts treated with 50mM H_2O_2; S50- sprouts fed with shimic acid and treated with 50mM H_2O_2; F50- sprouts fed with phenylalanine and treated with 50mM H_2O_2; Y50- sprouts fed with tyrosine and treated with 50mM H_2O_2; C200- sprouts terated with 200mM H_2O_2; S200- sprouts fed with shimic acid and treated with 200mM H_2O_2; F200- sprouts fed with phenylalanine and treated with 200mM H_2O_2; Y200- sprouts fed tyrosine and treated with 200mM H_2O_2.

FIG. 2. THE EFFECT OF ELICITATION AND PRECURSOR FEEDING ON THE REDUCING POWER OF QUINOA SPROUTS.



Note: means with different small letters are significantly different ($p \leq 0.05$). Each value represents the mean of three independent experiments (±SD). C - control sprouts; C50- sprouts treated with 50mM H_2O_2; S50- sprouts fed with shimic acid and treated with 50mM H_2O_2; F50- sprouts fed with phenylalanine and treated with 50mM H_2O_2; Y50- sprouts fed with tyrosine and treated with 50mM H_2O_2; C200- sprouts terated with 200mM H_2O_2; S200- sprouts fed with shimic acid and treated with 200mM H_2O_2; F200- sprouts fed with phenylalanine and treated with 200mM H_2O_2; Y200- sprouts fed tyrosine and treated with 200mM H_2O_2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