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Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. *carilla*) seeds induced by high pressure treatment

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Abstract

The effect of high pressure treatment on the vitamin C content and antioxidant capacity of raw and germinated cowpea seeds (*Vigna sinensis* var. *carilla*) at 300, 400 and 500 MPa for 15 min at room temperature has been investigated. A considerable amount of vitamin C was detected in germinated cowpeas, but the vitamin was not detected in raw seeds. An increase on the antioxidant capacity (TEAC) in cowpea sprouts was also observed (58–67%). High pressure treatment (HP) slightly modified vitamin C content and TEAC and, after pressurization at 500 MPa, the decrease was more pronounced, although the germinated seeds submitted to this HP treatment still provided a high amount of vitamin C (15–17 mg/100 g d.m.) and the antioxidant capacity was 26–59% higher than that of the raw cowpeas. The HP process can provide minimally processed fresh-like sprouts of high quality.

Keywords: Cowpea; Germination; High pressure; Vitamin C; Antioxidant capacity

1. Introduction

Dry cowpeas (*Vigna sinensis*) seeds are a good source of protein, carbohydrates, fibre, water-soluble vitamins and microelements, and they are consumed widely, satisfying the requirements of population living in developing countries (Chavan, Kadam, & Salunkhe, 1989). Dry cowpeas also contain antinutritional factors, which can be removed or diminished by processing before consumption. Furthermore, legumes have been linked with beneficial health properties that may be due to their contents of bioactive compounds with antioxidant properties.

Dietary antioxidants may play an important role in protecting the cell against damage caused by free radicals, e.g. as radical scavengers, reducing agents, potential complexes of pro-oxidant metals and quenchers of singlet oxygen formation (Hochstein & Atallah, 1988). There is an increasing interest in knowing the antioxidant capacity of food and its evolution during industrial processing. Consumption of foods containing antioxidants may prevent some diseases and, therefore, it is very important to determine their antioxidant capacity in order to estimate the repercussion on oxidative stress in living beings. Vitamin C is an important water-soluble antioxidant in biological fluids, protecting other substrates from oxidative damage and regenerates, by reduction, other antioxidants, such as α-tocopherol, glutathione and β-carotene (Carr & Frei, 2002).

It is well known that germination improves nutritional quality of seeds (Deshpande et al., 2002; Ghorpade & Kadam, 1989; Michaelsen, Med, & Friis, 1998; Vidal-Valverde et al., 2002). Legume sprouts are widely consumed in some parts of Asia as traditional foods and recently their consumption has been increased in western Europe and America as fresh and healthy vegetables (Mwikya, Camp, Rodríguez, & Huyghebaert, 2001). Germination has often been proposed as a useful and easy process for improving nutritional quality of legume seeds. Complex macromolecules, such as

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starch and proteins, are broken down to smaller and more digestible nutrients (Jyoti & Reddy, 1981; Subbulakshmi, Ganeshkumar, & Venkataraman, 1976; Vidal-Valverde & Frías, 1992). Furthermore, as consequence of germination, some antinutritional factors decrease or even disappear (Frías, Díaz-Pollán, Hedley, & Vidal-Valverde, 1995; Honke, Kozlowska, Vidal-Valverde, Frías, & Gorescki, 1998; Vidal-Valverde et al., 2002; Vidal-Valverde et al., 1998), and some compounds with antioxidant effect increase (Chen, Wells, & Fordham, 1975; Frías et al., 2002; Frías, Miranda, Doblado, & Vidal-Valverde, 2005; Plaza, De Ancos, & Cano, 2003; Prodanov, Sierra, & Vidal-Valverde, 1997; Vidal-Valverde et al., 2002). However, the shelf life of sprouts is very short (3–4 days) and the application of minimal processing methods, such as high pressure, may preserve them fresh-like, stable and safe for longer time.

The high pressure process is a novel alternative to conventional heat treatments for the preservation of foods. High pressure produces high quality and safe foods with longer shelf lives. This treatment is able to reduce or remove spoiling food microorganisms and inactivates most detrimental enzymes, without affecting the sensory and nutritional quality (Gould, 2001; Krebbers, Matser, Koets, & van der Berg, 2002; Ludikhuyze, Van Loey, Indrawati, Smout, & Hendrickx, 2003; Sancho et al., 1999; San Martín, Balbosa-Cánovas, & Swanson, 2002).

There is scarce information about antioxidant activity and vitamin C content of legumes (Frías et al., 2002; Frías et al., 2005; Lin, Wu, Wang, Yang, & Chang, 2001; Tsuda, Makino, Kato, Osawa, & Kawakishi, 1993) and no information has been found about the effect of the high pressure process on these parameters.

The aim of this paper was to obtain high-pressured germinated cowpea seeds and to study the effect of germination and subsequent high pressure treatment on vitamin C content and antioxidant capacity, in order to use these sprouts as a fresh food with high quality, greater safety and enhanced shelf life.

2. Materials and methods

2.1. Material

Cowpea seeds (*Vigna sinensis* var. *carilla*) were purchased in a market and used in the experiment.

2.2. Germination

Seeds were immersed for 30 min in sodium hipochlorite solution (0.07%, v/v) in the dark in order to remove surface microflora of seeds. Cowpeas were then washed with distilled water to neutral pH. The liquid was removed and seeds were kept for 5 h and 30 min in distilled water (1:5, w/v) in the dark, and they were shaken every 30 min. Water was eliminated and seeds were placed in a plastic disc with the bottom covered with filter paper. Germination was carried out in an incubator (Climabic Cabinet, model Economic Deluxe EC00-065, Snijders Scientific b.v, Netherlands) with controlled temperature at 20 °C in the dark. Seeds were sprinkled with distilled water every 12 h. Germinated cowpea samples were taken after 4 and 6 days. The sprouts seeds were analyzed and also submitted to high-pressure treatment.

2.3. High-pressure treatment

Germinated seeds (50 g) were packed in plastic bags under vacuum. Seeds germinated for 4 and 6 days were submitted to 300, 400 and 500 MPa for 15 min at room temperature in a prototype device of the Institute of High Pressure of the Polish Academy of Sciences in Warsaw. After processing, high-pressured germinated cowpeas were freeze-dried and kept in the dark at 4 °C under vacuum in a desiccator prior to analysis. Each sample (after 4 and 6 days of germination) was treated in seven replications. Raw cowpeas were subjected to high pressure as above and they were taken as control. Samples were ground in a ball mill (Glen Creston Ltd., Stanmore, UK), sieved and the 0.050–0.250 mm fraction was collected immediately before analysis.

2.4. Determination of trolox equivalent antioxidant capacity (TEAC)

The extracts of raw and processed cowpeas were obtained with phosphate buffered saline (PBS) (0.1 M pH 7.4) and the determination of TEAC for the extracts was carried out using potassium persulfate as free radical generator, following the procedure described by Re et al. (1999) and modified by Frías et al. (2005).

2.5. Determination of vitamin C

The vitamin C quantification in raw or processed cowpea flours was carried out by micellar electrokinetic capillary electrophoresis (MECC) according to the procedure described by Thompson and Trenerry (1995) and modified by Frías et al. (2005).

2.6. Moisture

Moisture of samples was determined in a vacuum heater at 35 °C to constant weight.

2.7. Statistical methods

Data were subjected to multifactor ANOVA using the least-squared difference test with the Statgraphic 5.0 Program (Statistical Graphics Corporation, Rockville, MD, USA).

3. Results and discussion

Table 1 shows the vitamin C content and antioxidant capacity of raw and processed cowpeas.

Table 1
Effect of high-pressure process on vitamin C content and antioxidant capacity of raw and germinated cowpeas (*Vigna sinensis* var. *carilla*)

Sample	Vitamin C (mg/100 g d.m.)	TEAC (μmol trolox/g d.m.)
Raw cowpeas	ND	27.4 ± 0.04
High-pressured raw cowpeas		
Raw cowpeas and 300 MPa	ND	23.2 ± 0.23^{a}
Raw cowpeas and 400 MPa	ND	24.7 ± 0.95^{a}
Raw cowpeas and 500 MPa	ND	24.9 ± 0.03^{a}
Germinated for 4 days	23.3 ± 0.42^{a}	43.3 ± 2.09^{c}
High-pressured germinated cowpeas		
Germinated for 4 days and 300 MPa	21.0 ± 0.94	42.0 ± 1.05^{c}
Germinated for 4 days and 400 MPa	18.4 ± 0.52	38.1 ± 0.28^{b}
Germinated for 4 days and 500 MPa	16.7 ± 0.69	34.5 ± 0.16
Germinated for 6 days	25.2 ± 0.78	45.7 ± 1.17
High-pressured germinated cowpeas		
Germinated for 6 days and 300 MPa	22.9 ± 1.05^{a}	43.7 ± 0.67^{c}
Germinated for 6 days and 400 MPa	22.5 ± 0.81^{a}	39.4 ± 0.37
Germinated for 6 days and 500 MPa	14.9 ± 1.24^{b}	36.5 ± 0.74^{b}

Mean value ± standard deviation of three determinations. The same superscript in the same column means no significant difference ($P \leqslant 0.05$).

The presence of vitamin C was not detected in raw cowpea seeds. No information about the content of vitamin C in cowpea seeds has been found. In other legumes, different authors give data of vitamin C from negligible amounts (lentils and beans) to 10 mg/100 g in soya bean and lupins (Frías et al., 2005; Plaza et al., 2003).

As a result of germination, vitamin C appeared in sprouted cowpeas and the content reached up to 23.3 mg/100 g d.m. and 25.2 mg/100 g d.m. after 4 and 6 days of germination, respectively (Table 1). Different authors have reported that germination causes an increment of vitamin C content in legumes (Abdullah & Baldwin, 1984; Ahmad & Pathak, 2000; Chen et al., 1975; Fordham, Wells, & Chen, 1975; Yang, Basu, & Ooraikul, 2001). Vanderstoep (1981) observed that ascorbic acid content of soya beans, mungbeans and peas increased from 4- to 20-fold during germination. Frías et al. (2005) reported that germination brought about a sharp rise in the vitamin C content in sprouts of *Lupinus albus* seeds. Similarly, Plaza et al. (2003) found that vitamin C content of wheat, soybean and alfalfa increased 54%, 218% and 919%, respectively, after germination for 96 h at 28 °C in the dark, and Sood and Malhotra (2001) observed that the ascorbic acid content of chickpea increased between four and seven times as a consequence of germination.

The effects of high pressure treatment on vitamin C content of raw and germinated cowpeas (*Vigna sinensis* var. *carilla*) are shown in Table 1. As expected, vitamin C was not detected in raw cowpea seeds after submission to high pressure at 300, 400 and 500 MPa for 15 min at room temperature. The amount of vitamin C decreased significantly ($P \leqslant 0.05$) in cowpeas germinated for 4 and 6 days after high pressure treatment (10–28% and 9–41%, respectively) and this decrease was higher when the intensity of hydrostatic pressure increased (Table 1).

No information has been found about the influence of high pressure treatment on vitamin C content of legumes. Most authors have studied the effect of high pressure in juices, fruits and vegetable products. In green beans, Krebbers et al. (2002) observed that ascorbic acid retention was 92% after high pressure treatment at room temperature for 1 min at 500 MPa. Sancho et al. (1999) reported that ultra-high hydrostatic pressure played only a minor role in degradation kinetics of vitamin C when the pressures applied to a multivitamin model system were 200, 400 and 600 MPa for 30 min at room temperature. Butz et al. (2003) evaluated the influence of applying 600–800 MPa of pressure on vitamin C contents of different kinds of fruit juices, fruits and vegetables and they concluded that in most cases, high pressure did not induce loss of vitamin C content in the fruit and vegetable matrices. Fernández García, Butz, Bognár, and Tauscher (2001) found that orange and mixed juice (orange, lemon and carrot) processed at 500 and 800 MPa for 5 min showed none or only insignificant reductions of vitamin C compared to unprocessed juice. Others authors, e.g. Polydera, Stoforos, and Taoukis (2003) and Bull et al. (2004) reported that the ascorbic acid content was not significantly affected when orange juice was treated with high pressure of 500 MPa (35 °C, 5 min) or 600 MPa (20 °C, 1 min). All these authors observed little change of vitamin C content during high pressure treatment, taking into account the type of food matrices and the short application time. In our case, the observed loss of vitamin C content, as a consequence of high pressure treatment could be not only due to a greater time of pressure exposure (15 min) but also to modifications of the vegetative structure of cowpea seeds during germination (Blaszczak, Doblado, Frias, Vidal-Valverde, & Fornal, 2006). We have observed that vitamin C suffered the largest reduction when the highest pressure was applied (500 MPa), and this could be due to changes in the structure of the seed during germination, making vitamin C more accessible to degradation.

The antioxidant capacity, measured as trolox equivalents (TEAC), of raw cowpeas was 27.4 μmol trolox/g d.m (Table 1). Tsuda et al. (1993) reported that the total antioxidant capacities of methanolic extracts of some species of cowpeas were very low in comparison with other legumes, since different authors have reported an antioxidant capacity (TEAC) between 13 and 23 μmol trolox/g d.m in lentils and 71.4 μmol trolox/g d.m in lupins (Frías et al., 2002; Frías et al., 2005).

The antioxidant capacity (TEAC) of cowpeas increased by 58% and 67% after 4 and 6 days of germination, respectively (Table 1, Fig. 1). Frías et al. (2005) found that TEAC of PBS extracts of different germinated lupins increased from 8% to 46% and correlated positively with the increment observed in vitamin C. López-Amorós, Hernandez, and Estrella (2006) observed that the antioxidant activity of beans and peas increased after germination but decreased in germinated lentils. Doval, Romero, Sturla, and Judis (2001) found that soya buds presented antioxidant activity which inhibited lipid oxidation through suppression of hydroperoxides and conjugated dienes formation. Falcioni et al. (2002) observed that purified wheat sprout fractions had antioxidative activities due to the increased amount of antioxidant compounds during the germination process. The increase of TEAC in sprout seeds could be due to synthesis of antioxidant hydrosoluble vitamins (vitamin C) and/or other compounds, such as polyphenols, with antioxidative characteristics during the germination process (Frías et al., 2005; López-Amorós et al., 2006).

Table 1 and Fig. 1 show the effect of the high pressure process on antioxidant capacity (TEAC) of raw and germinated cowpeas (*Vigna sinensis* var. *carilla*). Raw seeds pressurized at 300, 400 and 500 MPa underwent a slight loss in TEAC (10–15%) and no significant differences ($P \leqslant 0.05$) among the three pressure intensities used were found. When 300 and 400 MPa were applied to 4- and 6-day germinated seeds, reductions of 3–4% and 12–14%, respectively, in TEAC were observed. However, when the intensity of treatment increased to 500 MPa, the TEAC values of 4-day and 6-day cowpea sprouts decreased by 20%.

No information about the effect of high pressure treatment on antioxidant capacity of cowpeas, or any other legume seed, has been found in the literature. Butz et al. (2002) evaluated the changes in the antioxidant capacity, against $ABTS^{\cdot+}$ and hydroxyl radicals, of pressure treated vegetables (tomato and carrot) at 500 and 800 MPa for 5 min at 25 °C. Results did not show a great difference in TEAC after pressurization to water-soluble fraction of tomato and carrot homogenates. High pressure caused a small decrease in antioxidant capacity of carrot (11%) and only a minimal loss in tomato. In another trial, Butz et al. (2003) observed that TEAC did not significantly change after the application of 600–800 MPa of pressure to several fruit and vegetable products. Prestamo and Penas (2004) studied the antioxidant capacity of soybean whey and its hydrolysates processed under high pressure. They found that the pressurization increased the antioxidant activity of soy whey protein, but the antioxidant activity of the hydrolysates decreased.

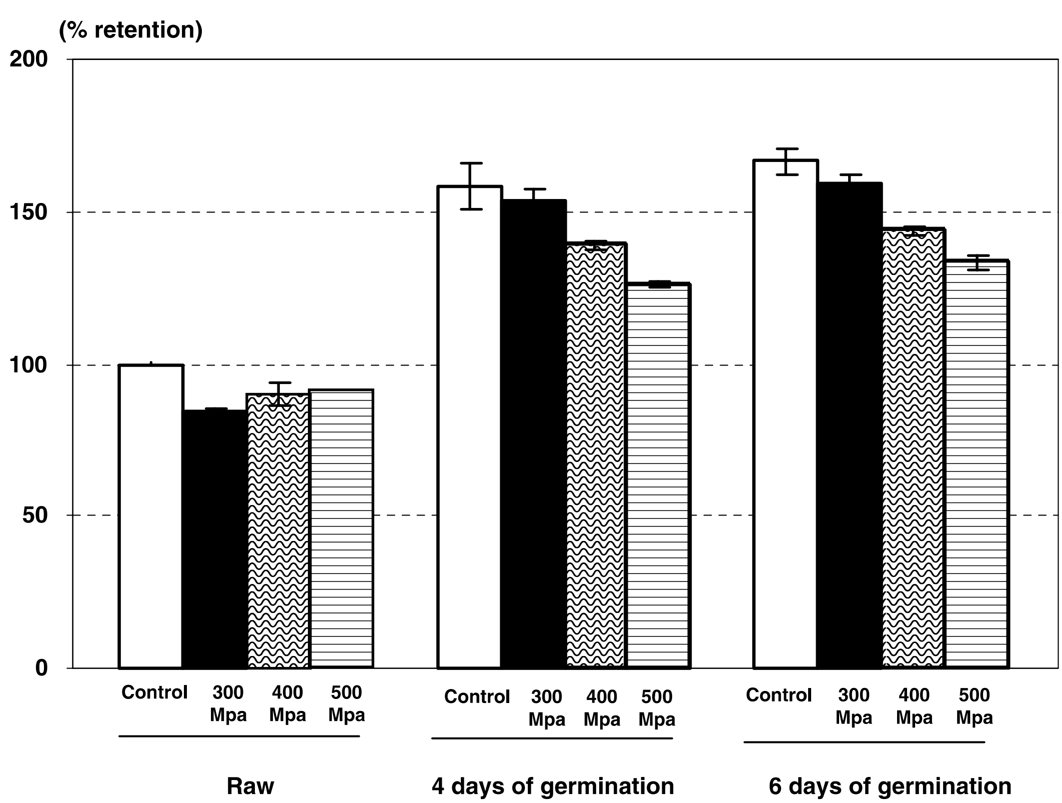

Fig. 1. Effect of high pressure process on the retention of antioxidant capacity in raw and germinated cowpeas (*Vigna sinensis* var. *carilla*).

4. Conclusions

According to the results obtained in this paper, germination of cowpeas (*Vigna sinensis* var. *carilla*) is a good procedure for improving vitamin C content and increasing antioxidant activity (TEAC). The application of high pressure treatment produced slight changes in the vitamin C and TEAC when the intensity of the process was less than 500 MPa for 15 min. Despite this, 4- and 6-day cowpea sprouts, after the HP process, had a higher amount of vitamin C and higher antioxidant capacity than the raw cowpeas. The HP process can provide minimally processed fresh-like sprouts of high quality, greater safety and prolonged shelf life, and could be used as an alternative to conventional heat treatments for fresh food preservation.

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