Food Chemistry 111 (2008) 622–630

Contents lists available at ScienceDirect

Food Chemistry

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

Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit*

Rebeca Fernandez-Orozco [a], Juana Frias [a], Henryk Zielinski [b], Mariusz K. Piskula [b], Halina Kozlowska [b], Concepción Vidal-Valverde [a,*]

[a] Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain
[b] Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland

ARTICLE INFO

Article history:
Received 21 January 2008
Received in revised form 15 February 2008
Accepted 10 April 2008

Keywords:
Mung bean
Soybean
Germination
Antioxidant capacity
Vitamin C
Vitamin E
Phenolic compounds
Reduced glutathione
Lipid peroxidation

ABSTRACT

The purpose of this study was to determine the antioxidant capacity and the content of antioxidant compounds in raw mung bean seeds and sprouts (*Vigna radiata* cv. *emmerald*) germinated for 2, 3, 4, 5 and 7 days and of soybean seeds of *Glycine max* cv. *jutro* germinated for 2, 3 and 4 days and of *Glycine max* cv. *merit* germinated for 2, 3, 4, 5 and 6 days. Antioxidant compounds, such as vitamin C and E, total phenolic compounds and reduced glutathione (GSH) were studied. Antioxidant capacity was measured by superoxide dismutase-like activity (SOD-like activity), peroxyl radical-trapping capacity (PRTC), trolox equivalent antioxidant capacity (TEAC) and inhibition of lipid peroxidation in unilamellar liposomes of egg yolk phosphatidylcholine (PC). The results indicated that changes in the contents of vitamin C, vitamin E and GSH depended on the type of legume and germination conditions. Sprouts of mung bean and soybeans provided more total phenolic compounds than did raw seeds. The SOD-like activity increased after germination of mung bean seeds for 7 days, by 308%, while no change was observed in sprouts of *Glycine max* cv. *jutro* and an increase was observed after 5 and 6 days of germination (~20%) in *Glycine max* cv. *merit*. PRTC and TEAC increased during the germination process and retentions of 28–70% and 11–14%, respectively, for soybean, and 248% and 61%, respectively, for mung bean were observed at the end of germination. The inhibition of lipid peroxidation increased by 389% in 5–7 days' germination of *Vigna radiata* cv. *emmerald* sprouts, and 66% in *Glycine max* cv. *merit* sprouts whilst, in *Glycine max* cv. *jutro*, germination did not cause changes in lipid peroxidation inhibition. According to the results obtained in this study, germination of mung bean and soybean seeds is a good process for obtaining functional flours with greater antioxidant capacity and more antioxidant compounds than the raw legumes.

1. Introduction

Antioxidants play an important role in food because they have beneficial effects on the human body. In legumes, the most important antioxidants are vitamin C, vitamin E, phenolic compounds and glutathione. The antioxidant activity of vitamin C is involved in the protection of lipids from *in vitro* and *in vivo* oxidation, mainly in people subjected to oxidative stress. Moreover, it has beneficial effects on vasodilatation in patients with cardiovascular diseases and coronary risk, and there is epidemiological evidence for its efficacy against cancer of lung, cervix, colon, rectum, pancreas and breast (Block, 1992; Cadenas & Packer, 2002). Foods rich in vitamin E may have positive effects on human health since these bioactive compounds can protect polyunsaturated fatty acids against oxidative damage in cell membranes. Vitamin E may also have anticarcinogenic properties because of its ability to destroy nitrite, a component in the food chain associated with the production of some types of cancers (Litescu & Radu, 2000). Phenolic compounds are located in the lipid–water interface of the membrane because of their lipophilic–hydrophilic structure. This location allows them to scavenge free radicals inside and outside the cell. Protective effects of GSH are widely known, and include protection against oxidative destruction in systems in which scavenging of free radicals, elimination of products of lipidic peroxidation, preservation of thiol-disulfide status of proteins, and repair processes occur (Shan, Aw, & Jones, 1990). Moreover, the consumption of foods high in glutathione may be associated with about a 50% reduction in the risk of oral and pharyngeal cancer (Valencia, Marin, & Hardy, 2001).

In this research we have explored different methods for studying antioxidant capacity, such as determination of SOD-like

* Corresponding author. Tel.: +34 915622900x241; fax: +34 915644853.
E-mail address: ificv12@ifi.csic.es (C. Vidal-Valverde).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.04.028

activity, since superoxide dismutase (SOD) is known to be an important enzyme which acts as a cellular antioxidant, accelerating dismutation of the toxic superoxide radical ($O_2^{\cdot-}$) produced during oxidative processes (Zielinski & Kozlowska, 2000). Peroxyl radical trapping capacity (PRTC) is a specific method for determining peroxyl radicals, which are implicated in fatty acid oxidation, since they are responsible for the chain propagation stage of the lipid oxidation process. Determination of the inhibition of lipid peroxidation in unilamellar liposomes shows cellular damage and can indicate premature aging diseases (Chatterjee & Agarwal, 1998; Terao, Piskula, & Yao, 1994). Furthermore, trolox equivalent antioxidant capacity (TEAC) gives a global measure of antioxidant capacity in foods (Re et al., 1999).

Legumes play an important role in the traditional diets of many regions throughout the world, including the Mediterranean diet. They are excellent sources of protein, carbohydrates, dietary fibre, lipids, a variety of micronutrients and phytochemicals (Anderson, Smith, & Washnock, 1999; Messina, 1999). However, they must be processed before consumption due to their content of non-nutritive factors, such as trypsin inhibitors, phytic acid and α-galactosides (Agustin, Klein, & Matthews, 1989; Vidal-Valverde et al., 2002). Because of the presence of many bioactive compounds in legumes with antioxidant activity and the relationship between antioxidants and disease risk, there is a wide interest in the effects of processing on the antioxidant compounds and antioxidant capacity of legumes.

Germination is an economical and effective technology which involves physiological changes, synthesis and breakdown of macromolecules, improving the digestibility and nutritive value of legumes. Numerous studies have reported higher levels of nutrients, such as amino acids, digestive protein, available carbohydrates, and other compounds, and lower levels of non-nutritive factors in legume sprouts as compared to ungerminated seeds (Urbano et al., 1995; Vidal-Valverde et al., 2002, 2003). Mung bean sprouts (germinated *Vigna radiata* seeds, and commonly called soy sprouts) are very popular in Asian cuisine, although, currently, their consumption is also increasing widely in western countries, not only for their better organoleptic characteristics compared with raw seeds but also for their health benefits. Soya bean seeds (*Glycine max* L.), however, are less frequently processed by germination, although their sprouts seem to be highly digestible and a good source of protein and minerals (Lee, Park, & Rhee, 1999). There is very little information on the effect of germination of these legumes or the antioxidant capacity of legumes, and a systematic study is required.

The objective of this research was to carry out a systematic germination process with one variety of mung bean and two varieties of soybean to obtain processed legumes with a high content in antioxidant compounds, such as vitamin C, vitamin E, phenolic compounds and glutathione, as well as with a high antioxidant capacity measured by SOD-like activity, PRTC and inhibition of lipid peroxidation of PC and TEAC. The optimized germination conditions will provide functional legume flours with added value to be used by the food industry.

2. Materials and methods

2.1. Seeds

Mung bean seeds (*Vigna radiata* cv. *emmerald*) and (*Glycine max* cv. *merit*) were obtained from Mang Fong Pacific Trading, S.A. Soybean seeds (*Glycine max* cv. *jutro*) were provided by the Institute of Animal Reproduction and Food Research of The Polish Academy of Science in Olsztyn (Poland). Seeds were cleaned and stored in darkness in polyethylene containers in a cool room at 4 °C prior to use.

2.2. Germination process

Mung bean seeds and soybean seeds (200 g) were soaked in 1000 ml of 0.07% sodium hypochlorite for 30 min. Then, the seeds were washed with distilled water until they reached neutral pH. Afterwards, seeds were soaked with 1000 ml of distilled water for 5 h and 30 min and shaken every 30 min. The hydrated seeds were placed in germination trays where a wet laboratory paper was extended, and were then covered. The trays were introduced into the germination machine, G-120 model (ASL Snijders International S.L., Holland) and the paper was in contact with the circulating water of the germinator; therefore seeds were always wet by capillarity. Germination time for all seeds was chosen to get sprouts of full freshness. *Vigna radiata* cv. *emmerald* seeds were germinated for 2, 3, 4, 5 and 7 days, *Glycine max* cv. *jutro* seeds for 2, 3 and 4 days and *Glycine max* cv. *merit* for 2, 3, 4, 5 and 6 days. Germination processes were carried out at 20 °C, at 99% relative humidity and in darkness. The germination process was evaluated by the percentage of germinated seeds and the sprouts seeds were separated, freeze-dried, milled and passed through a sieve of 0.5 mm. The flour obtained was stored in plastic bags, in darkness under vacuum conditions, in a desiccator at 4 °C.

2.3. Chemical analysis

2.3.1. Determination of vitamin C

Vitamin C in raw and germinated seeds was determined by the micellar electrokinetic capillary electrophoresis (MECC) method, as described by Frias, Miranda, Doblado, and Vidal-Valverde (2005), using a P/ACE system 2050 (Beckman Instruments, Fullerton, CA, USA) and UV detection at 254 nm.

2.3.2. Determination of tocopherols

The tocopherol isomers (α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol) determination in raw or germinated seeds was carried out by HPLC according to the method of Frias et al. (2005), using a Waters 470 scanning fluorescence detector (Waters Associates, Mildford, CT, USA) at λ_{exc} = 295 nm and λ_{em} = 330 nm. The content of tocopherol isomers are expressed in mg/100 g dry matter. Vitamin E activity was calculated as α-TE/100 g of dry matter according to Eitenmiller and Landen (1999): mg α-TE/100 g = [α-tocopherol (mg) × 1.0 + β-tocopherol (mg) × 0.5 + γ-tocopherol (mg) × 0.1 + δ-tocopherol (mg) × 0.03].

2.3.3. Determination of total phenolic compounds (TPC)

The content of total phenolic compounds was determined in 80% methanolic extracts according to Naczk and Shahidi (1989), using spectrophotometric measurement at 725 nm and room temperature.

2.3.4. Determination of reduced (GSH) and oxidized (GSSG) glutathione

Reduced (GSH) and oxidized (GSSG) glutathione were extracted from the samples according to Smith, Vierheller, and Thorne (1988); samples were submitted to a previous extraction with HPLC-grade *n*-heptane until lipid was removed (extract should remain clear and not opaque), and determined according to the spectrofluorometric method of Hissin and Hilf (1976). This method was based on the reaction of *o*-phthalaldehyde (OPT) as a fluorescent reagent with GSH at pH 8.0 and GSSG at pH 12.0. GSH was complexed to *N*-ethylmaleimide (NEM) to prevent interference of GSH with measurement of GSSG. The fluorescence was measured at λ_{em} = 420 nm and λ_{exc} = 420 nm. The assays were performed using a Perkin-Elmer LS 50B Luminescence Spectrometer (Perkin-Elmer Corp., Norwalk, CT). The GSH/GSSG ratio was also calculated.

2.3.5. Determination of SOD-like activity

The superoxide-scavenging activity of the phosphate-buffered saline extracts followed by a HPLC-grade *n*-heptane extraction, was done according to Fernandez-Orozco, Zielinski, Frias, Vidal-Valverde, and Piskula (2002), using the superoxide dismutase kit (RANSOD, Cat. No. SD 125, Randox Laboratories Ltd., Antrim, UK). The assays were performed in a spectrophotometer set at 37 °C (UV-160 1PC with CPS-Controller, Shimadzu, Japan). The superoxide dismutase activity was measured by the degree of inhibition of this reaction at 505 nm.

2.3.6. Determination of peroxyl radical-trapping capacity (PRTC)

Samples were extracted with 80% aqueous methanol (1/10 w/v) on an electromagnetic stirrer with continuous mixing for 2 h at ambient temperature in the dark. The assay was carried out according to the spectrophotometric method of Bartosz, Janaszewska, Ertel, and Bartosz (1998) and Fernandez-Orozco et al. (2006). Spectrophotometric measurements were obtained at 37 °C and 414 nm.

2.3.7. Determination of the inhibition of lipid peroxidation in unilamellar liposomes

The antioxidant activities of 80% methanolic extracts of raw and germinated seeds were evaluated according to the method described by Terao et al. (1994), using a model system consisting of unilamellar liposomes of egg yolk phosphatidylcholine (PC) and the water-soluble azo compound AAPH as a free radical generator.

The antioxidant capacity in this system was calculated according to the equation of Azuma, Ippoushi, Ito, Higashio, and Terao (1999) as follows:

$$\left(\frac{[\text{PC-OOH}_p] - [\text{PC-OOH}_{inh}]}{[\text{PC-OOH}_p]}\right) \times 100$$

where [PC-OOH$_p$] is the concentration of phosphatidyl choline peroxides (PC-OOH) for 80% methanol after 2 h of incubation and [PC-OOH$_{inh}$] is the concentration of PC-OOH for seed extracts after 2 h of incubation.

2.3.8. Determination of trolox equivalent antioxidant capacity (TEAC)

The determination of TEAC for phosphate-buffered saline (phosphate buffer 0.1 M pH 7.4 and NaCl 0.15 M) extracts of raw and germinated seeds was carried out according to the methods of Doblado et al. (2005) and Re et al. (1999). These methods use potassium persulfate to generate free radicals and spectrophotometric measurement at 734 nm for 10 min at 37 °C.

2.4. 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) and multiple correlation using the Statistica 5.1 Program (Statsoft, Tulsa, Okla 74104 USA) for Windows using a PC.

3. Results and discussion

Fig. 1 shows the percentages of germination of *Vigna radiata* cv. *emmerald* for 2, 3, 4, 5 and 7 days, *Glycine max* cv. *jutro* for 2, 3 and 4 days and *Glycine max* cv. *merit* for 2, 3, 4, 5 and 6 days. The percentage of germination reached 100% after the third day of germination for mung bean and after the fourth day of germination for soybean *Glycine max* cv. *jutro* while, for *Glycine max* cv. *merit*, the process started with a high efficiency during the first four days (99%) and after 5 and 6 days the percentage of germination decreased to values of about 88%.

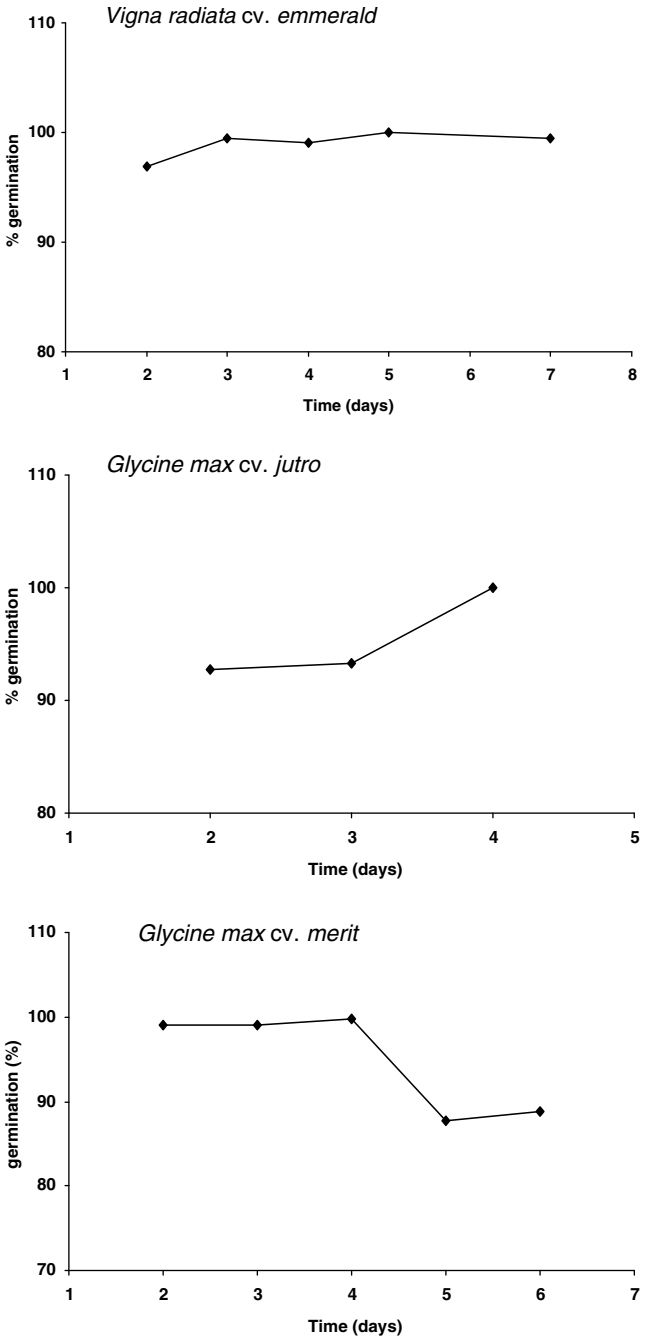

Fig. 1. Germination of seeds expressed as percentage.

The contents of vitamin C and tocopherols and vitamin E activity in raw and germinated mung bean seeds (*Vigna radiata* cv. *emmerald*) are shown in Table 1. The content of vitamin C in raw mung bean was 1.9 mg/100 g d.m. Germination brought about a sharp rise in the content of this vitamin and the highest value was obtained after 7 days of germination (9.1 mg/100 g d.m.). In raw seeds, γ-tocopherol was the isomer with the highest concentration (9.2 mg/100 g d.m.), then δ-tocopherol (0.6 mg/100 g d.m.) and finally α-tocopherol (0.1 mg/100 g d.m.). β-Tocopherol was not detected. The vitamin E activity of raw mung beans was 1.0 mg α-TE mg/100 g d.m. Germination caused a rise in the content of α-tocopherol, which was very noticeable after 7 days of germination (309%). However, γ-tocopherol and δ-tocopherol contents decreased gradually. Germination did not affect vitamin E activity

Table 1
Effect of germination on the vitamin C, tocopherols and vitamin E of *Vigna radiata* cv. *emmerald*[*]

Vigna radiata cv. *emmerald*	Vitamin C (mg/100 g d.m.)	Tocopherols (mg/100 g d.m.)			Vitamin E activity[**] (α-TE mg/100 g d.m.)
		α-Tocopherol	γ-Tocopherol	δ-Tocopherol	
Raw	1.86 ± 0.06[a]	0.11 ± 0.00[a]	9.16 ± 0.17[c]	0.60 ± 0.04[c]	1.05 ± 0.02[c]
Germination time (days)					
2	1.88 ± 0.15[a]	0.32 ± 0.01[b]	7.32 ± 0.18[b]	0.52 ± 0.07[b]	1.06 ± 0.01[c]
3	2.82 ± 0.09[b]	0.33 ± 0.02[c]	7.45 ± 0.12[b]	0.50 ± 0.06[b]	1.09 ± 0.03[c]
4	7.02 ± 0.07[c]	0.31 ± 0.01[b]	7.26 ± 0.40[b]	0.47 ± 0.08[b]	1.05 ± 0.03[c]
5	7.61 ± 0.07[d]	0.30 ± 0.01[b]	2.55 ± 0.27[a]	0.25 ± 0.03[a]	0.56 ± 0.03[a]
7	9.07 ± 0.02[e]	0.45 ± 0.02[d]	2.57 ± 0.23[a]	0.26 ± 0.02[a]	0.72 ± 0.04[b]

[*] Mean value ± standard deviation. Different letters in the same column mean significant differences ($P \leqslant 0.05$).
[**] Vitamin E activity as αTE (mg/100 g d.m.) = (mg α-tocopherol × 1.0) + (mg β-tocopherol × 0.5) + (mg γ-tocopherol × 0.1) + (mg δ-tocopherol × 0.03).

during the first 4 days, while a significant ($P \leqslant 0.05$) decrease was observed after the fifth and seventh day of the process (47% and 30%, respectively).

The content of vitamin C and tocopherols and vitamin E activity in raw and germinated soybean seeds (*Glycine max* cv. *jutro*) are shown in Table 2. Vitamin C was not detected, either in raw or in germinated seeds. In raw seeds, γ-tocopherol was also the most abundant isomer (12.9 mg/100 g d.m.), followed by α-tocopherol (2.9 mg/100 g d.m.), δ-tocopherol (2.9 mg/100 g d.m.) and β-tocopherol (0.2 mg/100 g d.m.). Vitamin E activity of this seed was 4.4 α-TE mg/100 g d.m. Germination of *Glycine max* cv. *jutro* induced an increase in the tocopherol isomer content after the third day of the process and, in consequence, vitamin E activity also rose (175% and 99% after 3 and 4 days of germination, respectively).

The contents of vitamin C and tocopherols and vitamin E activity in raw and germinated soybeans (*Glycine max* cv. *merit*) are shown in Table 3. Vitamin C was not detected in this seed, either raw or germinated. In raw *merit* soybean, γ-tocopherol presented the highest content (4.1 mg/100 g d.m.), followed by δ-tocopherol (1.6 mg/100 g d.m.), α-tocopherol (1.0 mg/100 g d.m.), β-tocopherol (0.2 mg/100 g d.m.) and vitamin E activity was 1.6 mg of α-TE/100 g d.m. Sprouting caused increases of α-, β-, γ- and δ-tocopherol contents and vitamin E activity. The highest values were observed after 5 days (93%, 60%, 83%, 413% and 97%, respectively) (Table 3).

No information has been found about the vitamin C content in raw *Vigna radiata*. The data provided in our work for raw soybeans cv. *jutro* and cv. *merit* are in agreement with those published by Bau, Villaume, Nicolas, and Méjean (1997) and Xu, Dong, and Zhu (2005) who did not detect vitamin C in different varieties of *Glycine max* seeds. However, Plaza, De Ancos, and Cano (2003) and Zielinski (2003) found 0.6 and 10 mg/100 g d.m. of vitamin C in soybean seeds.

Our data about the effect of germination on the vitamin C content of *Vigna radiata* cv. *emmerald* agree with those reported by other authors (Abdullah & Baldwin, 1984; Plaza et al., 2003; Sattar, Durrani, Mahmood, Ahman, & Khan, 1989; Zielinski, 2003). Xu et al. (2005) observed that germination of *Glycine max* seeds for 4 days produced an increase of vitamin C whilst subsequent germination up to 9 days caused a decrease. In other legumes, increases in vitamin C have been found after germination (Fernandez-Orozco et al., 2006; Frias et al., 2002, 2005).

Soybean seeds are a source of vitamin E. This compound is a major biological antioxidant, quenches free radicals and acts as a terminator of lipid peroxidation, particularly in membranes with high concentrations of unsaturated fatty acids (Burton & Traber, 1990). The results obtained in the present work on tocopherol isomers and vitamin E activity for raw legumes are in agreement with those previously reported (Fernandez-Orozco et al., 2006; Frias et al., 2002, 2005). Zielinski (2003) studied tocopherol content in raw seeds of *Glycine max* cv. *mazovia* and he reported values for

Table 2
Effect of germination on the vitamin C, tocopherols and vitamin E of *Glycine max* cv. *jutro*[*]

Glycine max cv. *jutro*	Vitamin C (mg/100 g d.m.)	Tocopherols (mg/100 g d.m.)				Vitamin E activity[**] (α-TE mg/100 g d.m.)
		α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	
Raw	ND	2.94 ± 0.05[a]	0.21 ± 0.02[b]	12.93 ± 0.18[a]	2.88 ± 0.06[a]	4.44 ± 0.04[a]
Germination time (days)						
2	ND	2.94 ± 0.12[a]	0.19 ± 0.01[a]	13.07 ± 0.19[a]	2.85 ± 0.01[a]	4.34 ± 0.04[a]
3	ND	8.37 ± 0.23[c]	0.33 ± 0.02[c]	34.06 ± 0.28[c]	5.08 ± 0.16[b]	12.22 ± 0.16[c]
4	ND	5.79 ± 0.25[b]	0.34 ± 0.01[c]	25.64 ± 0.26[b]	5.96 ± 0.19[c]	8.82 ± 0.14[b]

ND = not detected.
[*] Mean value ± standard deviation. Different letters in the same column mean significant differences ($P \leqslant 0.05$).
[**] Vitamin E activity α-TE (mg/100 g d.m) = (mg α-tocopherol × 1.0) + (mg β-tocopherol × 0.5) + (mg γ-tocopherol × 0.1) + (mg δ-tocopherol × 0.03).

Table 3
Effect of germination on the vitamin C, tocopherols and vitamin E of *Glycine max* cv. *merit*[*]

Glycine max cv. *merit*	Vitamin C (mg/100 g d.m.)	Tocopherols (mg/100 g d.m.)				Vitamin E activity[**] (α-TE mg/100 g d.m.)
		α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	
Raw	ND	1.00 ± 0.05[a]	0.25 ± 0.02[a]	4.10 ± 0.02[a]	1.65 ± 0.05[a]	1.59 ± 0.02[a]
Germination time (days)						
2	ND	1.13 ± 0.03[b]	0.33 ± 0.02[b]	5.23 ± 0.08[b]	2.90 ± 0.04[b]	1.89 ± 0.01[b]
3	ND	1.12 ± 0.03[b]	0.34 ± 0.02[b]	5.90 ± 0.09[d]	3.04 ± 0.06[c]	1.98 ± 0.04[c]
4	ND	1.57 ± 0.09[d]	0.37 ± 0.02[c]	7.33 ± 0.07[e]	5.83 ± 0.11[e]	2.69 ± 0.09[e]
5	ND	1.93 ± 0.03[e]	0.40 ± 0.03[d]	7.51 ± 0.08[f]	8.46 ± 0.09[f]	3.13 ± 0.04[f]
6	ND	1.32 ± 0.08[c]	0.38 ± 0.02[cd]	5.32 ± 0.08[c]	4.29 ± 0.07[d]	2.20 ± 0.06[d]

ND= not detected.
[*] Mean value ± standard deviation. Different letters in the same column mean significant differences ($P \leqslant 0.05$).
[**] Vitamin E activity α-TE (mg/100 g d.m.) = (mg α-tocopherol × 1.0) + (mg β-tocopherol × 0.5) + (mg γ-tocopherol × 0.1) + (mg δ-tocopherol × 0.03).

α-, β-, γ- and δ-tocopherol of 0.01, 0.01, 0.28 and 0.43 mg/100 g d.m., respectively.

The effect of germination on the content of vitamin E, obtained in this work, coincides with the information obtained from the literature: α-tocopherol increases during the first days of germination and the other isomers depend on the type and variety of legume (Fernandez-Orozco et al., 2006; Frias et al., 2002, 2005; Zielinski, 2003).

The content of total phenolic content compounds (TPC) in raw and germinated seeds of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit* is shown in Table 4. During germination of *Vigna radiata* cv. *emmerald*, significant ($P \leqslant 0.05$) increases in TPC content were observed (217%) after 7 days of germination although, in *Glycine max* cv. *jutro* (2.8 mg catechin/g d.m. in raw seed) TPC did not significantly ($P \leqslant 0.05$) change. TPC in raw *Glycine max* cv. *merit* was 3.0 mg catechin/g d.m. and, after 4 days of germination, its content increased significantly ($P \leqslant 0.05$), reaching 17% by the sixth day of germination.

Amarowicz, Troszynska, Barylko-Pikielna, and Shahidi (2004), Fernandez-Orozco, Zielinski, and Piskula (2003), Madhujith, Naczk, and Shahidi (2004), and Zielinski (2003) reported similar TPC values, ranging from 1.9 to 5.7 mg/g d.m., in different raw legumes. Zielinski (2003) reported that germination of *Glycine max* cv. *mazovia* caused an increase in TPC from 2.6 mg catechin/g d.m. in raw seeds to 3.1 mg catechin/g d.m. after 7 days of germination. Lin and Lai (2006) showed that, in most *Glycine max* cultivars studied, flavonoid content increased after 4 days of germination. In contrast, Randhir, Lin, and Shetty (2004) reported that germination caused a decrease of TPC in *Vigna radiata* seeds.

The changes in reduced (GSH) and oxidized (GSSG) glutathione and the ratio GSH/GSSG in raw and germinated seeds of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit* are shown in Table 5. GSH contents of these raw seeds were very similar (4.5, 5.9 and 5.7, respectively). During germination of *Vigna radiata* cv. *emmerald*, GSH content increased up to 4 days of germination and after that time, gradually decreased. GSSG content showed no significant ($P \leqslant 0.05$) difference during the first 3 days of germination, but after 4 days an increase was found that was maintained up to day 7. The germination process did not significantly alter ($P \leqslant 0.05$) the GSH/GSSG ratio during the first 3 days although, from the fourth day, it decreased, and this decrease was constant up to the seventh day of the process. GSH values in *Glycine max* cv. *jutro* were not significantly ($P \leqslant 0.05$) modified by consequence of germination, although GSSG content increased, reaching a 49% rise after 4 days of germination. The GSH/GSSG ratio decreased during germination. The germination process in *Glycine max* cv. *merit* significantly ($P \leqslant 0.05$) increased the GSH content during the first 4 days (17–26%); after that time GSH decreased up to the sixth day of germination, reaching a value similar to raw seeds. A small increase in GSSG was noted during germination, reaching the highest value at the fifth and sixth days of germination. The GSH/GSSG ratio showed a pronounced decrease after 5 days.

Different contents of GSH and GSSG in raw legumes have been found in the literature (Doblado et al., 2005; Fernandez-Orozco et al., 2003, 2006; Mills, Stinson, Liu, & Lang, 1997; Zielinski, 2003) and they show that the effect of germination on the glutathione content depends on the kind of legume studied. Zielinski (2003) reported an increase of 25% after 7 days of germination of *Glycine max* cv. *mazovia* and a 47% reduction in the GSH/GSSG ratio, due to the GSSG rise produced. We found an increase in GSSG after germination. Changes in the GSH content could be due, not only to the oxidation process, but also to the proteolitic action of enzymes occurring during germination (Galleschi et al., 2002; Hemalatha & Siva-Prasad, 2003; Neves & Lourenco, 2001; Ogbonna, Obi, & Okolo, 2004).

The SOD-like activities of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit* are shown in Tables 6–8. The SOD-like activity values of raw *Vigna radiata* cv. *emmerald* seeds (314 U SOD/g d.m.) increased after 3 days of germination, achieving an increase of 307% by the seventh day of germination (Table 6). The SOD-like activity of raw *Glycine max* cv. *jutro* (566 U SOD/g d.m.) did not change significantly ($P \leqslant 0.05$) with the germination process (Table 7). The SOD-like activity of raw *Glycine max* cv. *merit* seeds was 557 U SOD/g d.m.; during the first 4 days of germination a slight decrease in SOD-like activity was noted while an increase was observed after 5 and 6 days of germination (~20%) (Table 8).

Table 4
Effect of germination on the total phenolic compound contents of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit*[*]

Legumes	Total phenolic compounds (mg catechin/g d.m.)
Vigna radiata cv. *emmerald*	
Raw	1.09 ± 0.11^{a}
Germination (days)	
2	1.43 ± 0.08^{c}
3	1.30 ± 0.02^{b}
4	2.24 ± 0.02^{d}
5	3.30 ± 0.08^{e}
7	3.46 ± 0.07^{f}
Glycine max cv. *jutro*	
Raw	2.85 ± 0.06^{bc}
Germination time (days)	
2	2.71 ± 0.03^{ab}
3	2.65 ± 0.15^{a}
4	3.00 ± 0.09^{c}
Glycine max cv. *merit*	
Raw	2.98 ± 0.06^{a}
Germination time (days)	
2	3.06 ± 0.05^{ab}
3	3.10 ± 0.06^{ab}
4	3.18 ± 0.06^{bc}
5	3.24 ± 0.08^{c}
6	3.49 ± 0.08^{d}

[*] Mean value ± standard deviation. Different letters in the same column for each legume mean significant differences ($P \leqslant 0.05$).

Table 5
Effect of germination on the reduced and oxidized glutathione content of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit*[*]

Legumes	GSH (μmol/g d.m.)	GSSG (μmol/g d.m.)	GSH/GSSG
Vigna radiata cv. *emmerald*			
Raw	3.45 ± 0.23^{a}	0.77 ± 0.03^{a}	4.50 ± 0.14^{b}
Germination (days)			
2	3.98 ± 0.25^{b}	0.80 ± 0.07^{a}	4.99 ± 0.72^{b}
3	4.04 ± 0.01^{b}	0.85 ± 0.01^{a}	4.75 ± 0.04^{b}
4	4.40 ± 0.08^{c}	1.34 ± 0.03^{b}	3.28 ± 0.01^{a}
5	4.09 ± 0.20^{b}	1.39 ± 0.03^{b}	2.94 ± 0.20^{a}
7	3.64 ± 0.10^{a}	1.38 ± 0.04^{b}	2.64 ± 0.15^{a}
Glycine max cv. *jutro*			
Raw	3.55 ± 0.10^{a}	0.61 ± 0.02^{a}	5.86 ± 0.04^{d}
Germination (days)			
2	3.51 ± 0.13^{a}	0.73 ± 0.01^{b}	4.84 ± 0.13^{b}
3	3.69 ± 0.05^{a}	0.69 ± 0.01^{b}	5.34 ± 0.07^{c}
4	3.60 ± 0.02^{a}	0.91 ± 0.01^{c}	3.95 ± 0.02^{a}
Glycine max cv. *merit*			
Raw	3.51 ± 0.09^{a}	0.61 ± 0.01^{a}	5.71 ± 0.26^{d}
Germination (days)			
2	4.09 ± 0.10^{b}	0.88 ± 0.01^{b}	4.65 ± 0.27^{c}
3	4.36 ± 0.07^{c}	0.87 ± 0.06^{b}	5.06 ± 0.62^{cd}
4	4.43 ± 0.02^{c}	0.89 ± 0.03^{b}	4.96 ± 0.18^{cd}
5	4.06 ± 0.05^{b}	1.08 ± 0.03^{c}	3.77 ± 0.15^{b}
6	3.52 ± 0.11^{a}	1.25 ± 0.06^{d}	2.83 ± 0.23^{a}

[*] Mean value ± standard deviation. Different letters in the same column for each legume mean significant differences ($P \leqslant 0.05$).

Table 6
Effect of germination on the SOD-like activity, peroxyl radical trapping capacity, and antioxidant activity in liposomal PC suspension system and trolox equivalent antioxidant capacity of *Vigna radiata* cv. *emmerald*[*]

Vigna radiata cv. *emmerald*	SOD-like activity (U SOD/ g d.m.)	Peroxyl radical trapping capacity (μmol trolox/ g d.m.)	Antioxidant activity in liposomal PC suspension system (% inhibition)	TEAC (μmol trolox/ g d.m.)
Raw	314 ± 12.3[a]	2.65 ± 0.33[a]	17.8 ± 1.82[a]	27.0 ± 0.37[a]
2 days	363 ± 27.5[a]	3.08 ± 0.18[ab]	26.2 ± 6.42[a]	30.0 ± 0.26[b]
3 days	670 ± 13.1[b]	3.30 ± 0.45[b]	20.1 ± 5.85[a]	27.9 ± 0.85[a]
4 days	716 ± 7.0[b]	4.89 ± 0.26[c]	22.6 ± 4.93[a]	32.1 ± 0.89[c]
5 days	878 ± 47.2[c]	8.80 ± 0.12[d]	65.6 ± 1.35[b]	40.2 ± 0.54[d]
7 days	1283 ± 59.6[d]	9.21 ± 0.30[d]	87.1 ± 0.25[c]	43.5 ± 1.18[e]

[*] Mean value ± standard deviation. Different letters in the same column mean significant differences ($P \leqslant 0.05$).

Table 7
Effect of germination on the on the SOD-like activity, peroxyl radical trapping capacity, and antioxidant activity in liposomal PC suspension system and trolox equivalent antioxidant capacity of *Glycine max* cv. *jutro*[*]

Glycine max cv. *jutro*	SOD-like activity (U SOD/ g d.m.)	Peroxyl radical trapping capacity (μmol trolox/ g d.m.)	Antioxidant activity in liposomal PC suspension system (% inhibition)	TEAC (μmol trolox/ g d.m.)
Raw	566 ± 2.7[a]	3.25 ± 0.17[a]	58.8 ± 3.07[a]	37.3 ± 0.50[a]
Germination (days)				
2	552 ± 9.2[a]	3.32 ± 0.28[a]	49.1 ± 0.85[a]	40.2 ± 0.16[b]
3	506 ± 30.2[a]	3.65 ± 0.34[a]	45.6 ± 7.68[a]	41.9 ± 0.25[c]
4	562 ± 38.9[a]	4.15 ± 0.01[b]	51.5 ± 6.82[a]	41.3 ± 0.06[c]

[*] Mean value ± standard deviation. Different letters in the same column mean significant differences ($P \leqslant 0.05$).

Table 8
Effect of germination on the on the SOD-like activity, peroxyl radical trapping capacity, and antioxidant activity in liposomal PC suspension system and trolox equivalent antioxidant capacity of *Glycine max* cv. *merit*[*]

Glycine max cv. *merit*	SOD-like activity (U SOD/ g d.m.)	Peroxyl radical trapping capacity (μmol trolox/ g d.m.)	Antioxidant activity in liposomal PC suspension system (% inhibition)	TEAC (μmol trolox/ g d.m.)
Raw	557 ± 5.7[c]	2.90 ± 0.07[ab]	47.5 ± 3.35[a]	63.0 ± 0.50[a]
Germination (days)				
2	523 ± 6.6[a]	2.69 ± 0.00[a]	52.4 ± 5.71[a]	69.2 ± 1.31[b]
3	529 ± 0.0[b]	2.71 ± 0.00[ab]	55.0 ± 3.20[a]	68.4 ± 0.02[b]
4	523 ± 6.6[ab]	2.72 ± 0.11[a]	52.5 ± 2.05[a]	71.5 ± 0.59[c]
5	673 ± 6.8[d]	2.95 ± 0.15[b]	50.4 ± 6.06[a]	71.6 ± 0.06[c]
6	687 ± 0.0[e]	4.93 ± 0.18[c]	65.7 ± 2.59[b]	71.8 ± 0.03[c]

[*] Mean value ± standard deviation. Different letters in the same column mean significant differences ($P \leqslant 0.05$).

Scarce information has been found about SOD-like activity in raw and germinated legumes. According to the bibliography, the SOD-like activity depends on the type of seeds and time of germination (Fernandez-Orozco et al., 2002, 2006; Zielinski, Frias, Piskula, Kozlowska, & Vidal-Valverde, 2006).

Table 6 shows the peroxyl radical-trapping capacity (PRTC) of raw and germinated mung bean *Vigna radiata* cv. *emmerald* seeds. Germination caused increases in the PRTC of 16%, 25%, 85%, 232% and 248% after 2, 3, 4, 5 and 7 days, respectively. PRTC in raw *Glycine max* cv. *jutro* seeds presented values of 3.2 μmol trolox/g d.m. and germination did not change this value significantly ($P \leqslant 0.05$) during the first 3 days of the process but, after 4 days, a significant ($P \leqslant 0.05$) increase of 28% was noticed (Table 7). In *Glycine max* cv. *merit*, the PRTC of raw seeds was 2.9 μmol trolox/g d.m. (Table 8) and, during the first 5 days of germination, PRTC did not show significant ($P \leqslant 0.05$) modifications compared with raw seeds although, after 6 days of germination, a sharp increase in PRTC (70%) was found (Table 8).

PRTC values of raw seeds studied are in the range found in the literature for different legumes (Doblado et al., 2005; Fernandez-Orozco et al., 2003; Zielinski, 2002). During germination of soybean seeds, Zielinski (2002) found an increase in PRTC. In other legumes, however, the changes in PRTC seem to depent on the type of seed (Fernandez-Orozco et al.; 2003, 2006).

The antioxidant activities of germinated *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit* extracts, in phospholipid bilayers obtained by measuring the inhibition of lipid peroxidation in large unilamellar vesicles of egg yolk PC, are compiled in Figs. 2–4, respectively. Tables 6–8 show the percentages of peroxidation inhibition after 2 h of incubation for raw and germinated seeds of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit*, calculated according to the Azuma et al. (1999) formula. The 5- and 7 day-germinated *Vigna radiata* cv. *emmerald* extracts presented a higher inhibition than did the raw seeds extract (268% and 389%, respectively) (Fig. 2, Table 6). In *Glycine max* cv. *jutro*, raw seeds showed an inhibition of lipid peroxidation of 58.8%, and germination did not produce any significant change ($P \leqslant 0.05$) (Fig. 3, Table 7). In *Glycine max* cv. *merit*, the raw seeds showed a percentage of peroxidation inhibition of 47% after 2 h and percentages of peroxidation inhibition were not significantly ($P \leqslant 0.05$) modified up to 5 days of germination; however, after 6 days, a higher percentage of peroxidation inhibition was found (65.7%) (Fig. 4, Table 8).

Most of the studies found in the literature on the inhibition of PC peroxidation are based on model systems for evaluating antioxidant capacity using pure compounds, such as epicatechin, epicatechin gallate, quercetin, or vitamin E (Koga & Terao, 1996; Terao et al., 1994). Nevertheless, few references have been found on peroxidation inhibition using a food matrix, such as raw and processed legume seeds and, in general, germination brings about an increment of this parameter (Doblado et al., 2005; Fernandez-Orozco et al., 2006; Frias et al., 2005; Troszynska & Ciska, 2002).

TEAC values of raw and germinated *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit* are shown in Tables 6–8. Raw seeds of *Vigna radiata* cv. *emmerald* presented a TEAC of 27.0 μtrolox/g d.m. and germination caused a large increase after 4 days of germination, reaching an increment of 61% after 7 days (Table 6). Raw seeds of *Glycine max* cv. *jutro* presented a TEAC of 37.3 μmol trolox/g d.m. and germination brought about an increment of 8–11% (Table 7). TEAC content of raw *Glycine max* cv. *merit* seeds was 63.0 μmol trolox/g d.m. and germinated seeds presented an increase of 10–14% after 6 days (Table 8).

Values of the TEAC obtained in this work are in agreement with the range found in the literature for different legumes (Doblado et al., 2005; Fernandez-Orozco et al., 2003, 2006; Frias et al., 2005; Torres, Frias, Granito, & Vidal-Valverde, 2006). During germination, Zielinski (2003) reported a sharp decrease in TEAC values after germination for 7 days of *Glycine max* cv. *mazovia*. However, our results show an increase of TEAC after germination, which is in agreement with those obtained by different authors in other legumes (Fernandez-Orozco et al., 2006; Frias et al., 2005; Torres, Frias, Granito, & Vidal-Valverde, 2007).

On the basis of the data presented in this work, it was possible to calculate the relative contribution of antioxidant compounds to the antioxidant capacity of raw and germinated seeds. In order to carry out calculations, the following TEAC values of individual compounds were taken: 2.4 μmol/g d.m. for catechin (Rice-Evans & Miller, 1994), 0.97 μmol/g d.m. for vitamin E, 0.90 μmol/g d.m. for GSH and 0.99 μmol/g d.m. for vitamin C (Rice-Evans, Miller, & Paganda, 1996).

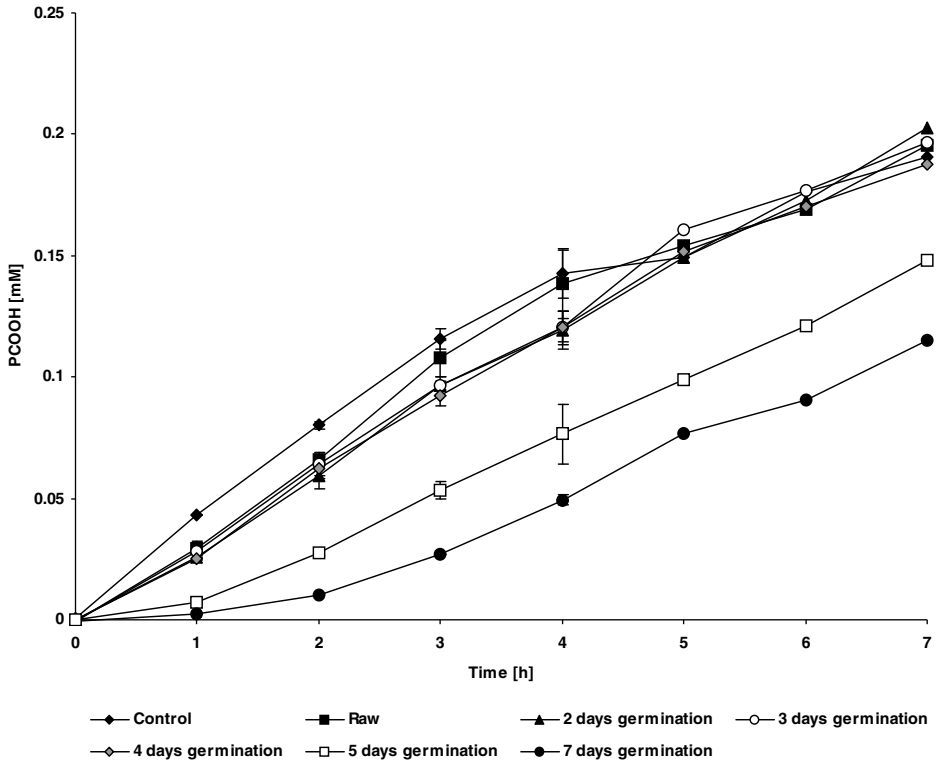

Fig. 2. Inhibition by raw and germinated *Vigna radiata* cv. *emmerald* extracts of AAPH-initiated peroxidation of PC liposomes.

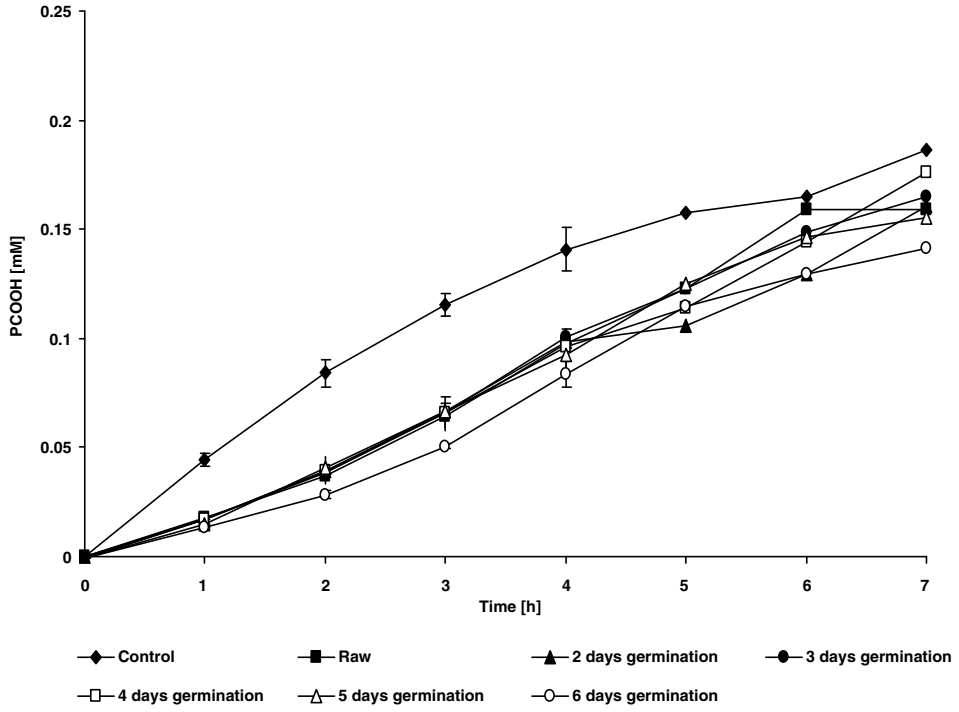

Fig. 3. Inhibition by raw and germinated *Glycine max* cv. *jutro* extracts of AAPH-initiated peroxidation of PC liposomes.

In raw *Vigna radiata* cv. *emmerald* seeds, it was found that TPC were the compounds which contributed the most (74%) to total antioxidant capacity, followed by GSH (25%), vitamin C (0.9%) and vitamin E (0.2%). The germination process increased the contribution of TPC (76–88%) and vitamin C (0.7–1.7%) to the total antioxidant capacity, whilst it reduced the GSH (23–10%) and vitamin E (0.2–0.04%) contribution. In raw seeds of *Glycine max* cv. *jutro* it was again observed that TPC were the compounds which contributed most to the total antioxidant activity (88%), followed by GSH (12%) and, finally, vitamin E (0.4%). In this legume,

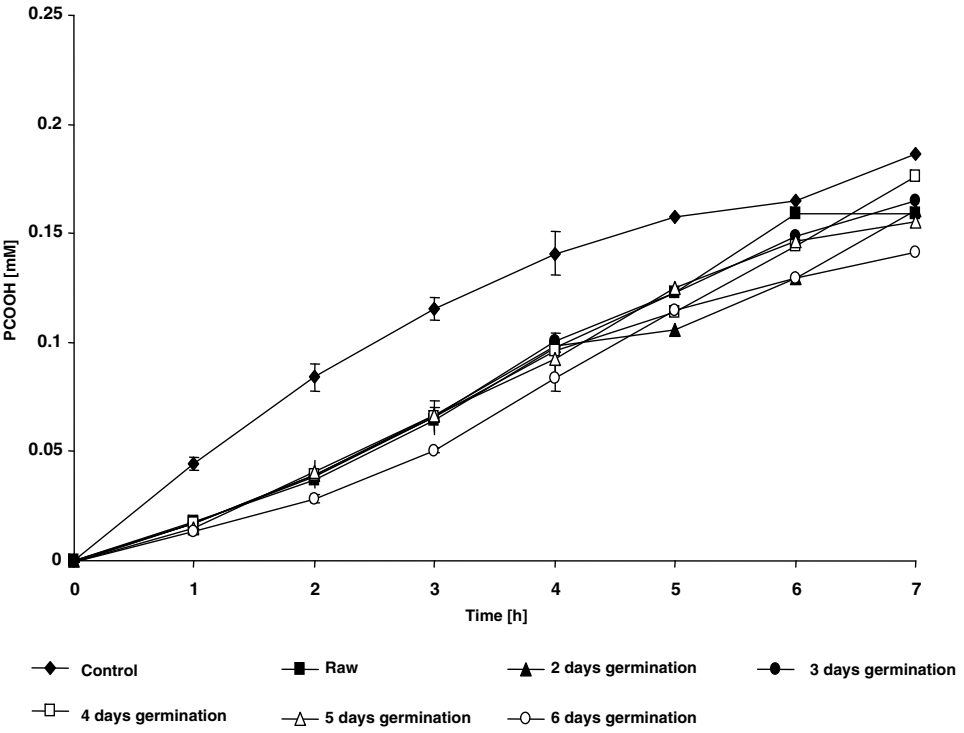

Fig. 4. Inhibition by raw and germinated *Glycine max* cv. *merit* extracts of AAPH-initiated peroxidation of PC liposomes.

germination caused slight changes in the contribution of TPC (86–88%), GSH (11–13%) and vitamin E (0.4–1.1%) to the total antioxidant capacity. In *Glycine max* cv. *merit*, a markedly higher contribution percentage of total phenolic compounds was observed in raw seeds (89%), whilst GSH and vitamin E activity delivered about 11% and 0.1%, respectively. Germination produced slight changes in these contributions: TPC 87–90%, GSH 10–13% and vitamin E activity ~0.2%.

4. Conclusions

The optimal conditions for obtaining legume sprouts with high antioxidant capacity are the following: for *Vigna radiata* cv. *Emmerald*, 7 days of germination increased the contents of vitamin C, total phenolic compounds and glutathione by 388%, 217% and 6%, respectively, and SOD-like activity, PRTC, inhibition of PC peroxidation and TEAC increased by 308%, 248%, 389% and 61%, respectively. For *Glycine max* cv. *jutro*, germination for 4 days brought about an increase in vitamin E activity (99%), total phenolic compounds (5%), PRTC (28%) and TEAC (11%). For *Glycine max* cv. *merit*, germination for 6 days increased vitamin E activity and TPC by 38 and 17%, respectively. Also, SOD-like activity, inhibition of PC peroxidation, PRTC and TEAC increased by 23%, 38%, 70% and 14%, respectively.

Acknowledgements

The authors are indebted to the Spanish Comision Interministerial de Ciencia y Tecnologia (CICYT) for the financial support of projects AGL2002-02905/ALI and AGL2004-0886/ALI. This article is a part of the Ph.D. project of R. Fernandez-Orozco.

References

Abdullah, A., & Baldwin, R. E. (1984). Mineral and vitamin contents of seeds and sprouts of newly available small-seeded soybeans and market samples of mung beans. *Journal of Food Science, 49*, 656–657.

Agustin, J., Klein, B. P., & Matthews, R. H. (1989). Nutrient composition of raw cooked canned and sprouted legumes. In J. Agustin, B. P. Klein, & R. H. Matthews (Eds.), *Legumes chemistry technology and human nutrition*. New York: Marcel Dekker.

Amarowicz, R., Troszynska, A., Barylko-Pikielna, N., & Shahidi, F. (2004). Polyphenolics extracts from legume seeds: Correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of Food Lipids, 11*, 278–286.

Anderson, J. W., Smith, B. M., & Washnock, C. S. (1999). Cardiovascular and renal benefits of dry bean and soybean intake. *American Journal of Clinical Nutrition, 70*(Suppl.), 464S–474S.

Azuma, K., Ippoushi, K., Ito, H., Higashio, H., & Terao, J. (1999). Evaluation of antioxidative activity of vegetable extracts in linoleic acid emulsion and phospholipid bilayers. *Journal of the Science of Food and Agriculture, 79*, 2010–2016.

Bartosz, G., Janaszewska, A., Ertel, D., & Bartosz, M. (1998). Simple determination of peroxyl radical-trapping capacity. *Biochemistry and Molecular Biology International, 46*, 519–528.

Bau, H. M., Villaume, C., Nicolas, J., & Méjean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soybean (*Glycine max*) seeds. *Journal of the Science of Food and Agriculture, 73*, 1–9.

Block, G. (1992). Vitamin C status and cancer. Epidemiologic evidence of reduced risk. *Annals of the New York Academy of Sciences, 669*, 280–286.

Burton, G. W., & Traber, M. G. (1990). Vitamin E antioxidant activity, biokinetics and bioavailability. *Annual Review of Nutrition, 10*, 357–382.

Cadenas, E., & Packer, L. (2002). *Handbook of antioxidants*. New York: Marcel Dekker, Inc.

Chatterjee, S. N., & Agarwal, S. (1998). Liposomes as membrane model for study of lipid peroxidation. *Free Radical Biology and Medicine, 4*, 51–72.

Doblado, R., Zielinski, H., Piskula, M., Kozlowska, H., Muñoz, R., Frias, J., et al. (2005). Effect of processing on the antioxidant capacity of *Vigna sinensis* var. *carilla*. *Journal of Agricultural and Food Chemistry, 53*, 1215–1222.

Eitenmiller, R. R., & Landen, W. O. (1999). *Vitamin analysis for the health and food sciences*. New York: CRC Press.

Fernandez-Orozco, R., Piskula, M., Zielinski, H., Kozlowska, H., Frias, J., & Vidal-Valverde, C. (2006). Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. *zapaton*. *European Food Research and Technology, 223*, 495–502.

Fernandez-Orozco, R., Zielinski, H., Frias, J., Vidal-Valverde, C., & Piskula, M. (2002). Superoxide dismutase-like activity of raw, cooked and germinated lentils. *Polish Journal of Food and Nutrition Sciences, 11(52)*(SI 2), 39–44.

Fernandez-Orozco, R., Zielinski, H., & Piskula, M. (2003). Contribution of low molecular weight antioxidants to the antioxidant capacity of raw and processed lentil seeds. *Nahrung/Food, 47*(5), 291–299.

Frias, J., Fernandez-Orozco, R., Zielinski, H., Piskula, M., Kozlowska, H., & Vidal-Valverde, C. (2002). Effect of germination on the content of vitamins C and E of lentils. *Polish Journal of Food and Nutrition Sciences, 11/52*, 39–44.

Frias, J., Miranda, L. M., Doblado, R., & Vidal-Valverde, C. (2005). Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. *multolupa*. *Food Chemistry, 92*, 211–220.

Galleschi, L., Capocchi, A., Ghiringhelli, S., Saviozzi, F., Calucci, L., Pinzio, C., et al. (2002). Antioxidants, free radicals, storage proteins, and proteolytic activities in wheat (*Triticum durum*) seeds during accelerated aging. *Journal of Agricultural and Food Chemistry, 50*, 5450–5457.

Hemalatha, K. P. J., & Siva-Prasad, D. (2003). Changes in the metabolism of protein during germination of sesame (*Sesamum indicum* L.) seeds. *Plant Foods for Human Nutrition, 58*, 1–10.

Hissin, J., & Hilf, R. (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Analytical Biochemistry, 74*, 214–226.

Koga, T., & Terao, J. (1996). Antioxidant behaviours of vitamin E analogues in unilamellar vesicles. *Bioscience Biotechnology and Biochemistry, 60*, 1043–1045.

Lee, Y. S., Park, R. D., & Rhee, C. O. (1999). Effect of chitosan treatment on growing characteristics of soybean sprouts. *Journal of Food Science and Technology, 31*, 153–157.

Lin, P., & Lai, H. (2006). Bioactive compounds in legumes and their germinated products. *Journal of Agricultural and Food Chemistry, 54*, 3807–3814.

Litescu, S. C., & Radu, G. L. (2000). Estimation of antioxidative properties of tocopherols––An electrochemical approach. *European Food Research and Technology, 211*, 218–221.

Madhujith, T., Naczk, M., & Shahidi, F. (2004). Antioxidant activity of common beans (*Phaseolus vulgaris* L.). *Journal of Food Lipids, 11*, 220–233.

Messina, M. J. (1999). Legumes and soybeans. Overview of their nutritional profits and health effects. *American Journal Clinical Nutrition, 70*(Suppl.), 439S–450S.

Mills, B. J., Stinson, C. T., Liu, M. C., & Lang, C. A. (1997). Glutathione and cyst(e)ine profiles of vegetables using high performance liquid chromatography with dual electrochemical detection. *Journal of Food Composition and Analysis, 10*, 90–101.

Naczk, M., & Shahidi, F. (1989). The effect of methanol–ammonia treatment on the content of phenolic acids of canola. *Food Chemistry, 31*, 159–164.

Neves, V. A., & Lourenco, E. J. (2001). Changes in protein fractions, trypsin inhibitor and proteolytic activity in the cotyledons of germinating chickpea. *Archivos Latinoamericanos de Nutrición, 51*, 269–275.

Ogbonna, A. C., Obi, S. K. C., & Okolo, B. N. (2004). Optimization of proteolytic activities in malting sorghum. *Process Biochemistry, 39*, 711–716.

Plaza, L., De Ancos, B., & Cano, P. (2003). Nutritional and health-related compounds in sprouts and seeds of soybean (*Glycine max*), wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa*) treated by a new drying method. *European Food Research and Technology, 216*, 138–144.

Randhir, R., Lin, Y., & Shetty, K. (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry, 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 Radical Biology and Medicine, 26*, 1231–1237.

Rice-Evans, C., & Miller, N. J. (1994). Total antioxidant status in plasma body fluids. *Methods in Enzymology, 234*, 279–293.

Rice-Evans, C., Miller, N. J., & Paganda, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933–956.

Sattar, A., Durrani, S. K., Mahmood, F., Ahman, A., & Khan, I. (1989). Effect of soaking and germination temperatures on selected nutrients and antinutrients of mung bean. *Food Chemistry, 34*, 111–120.

Shan, X. Q., Aw, T. Y., & Jones, D. P. (1990). Glutathione-dependent protection against oxidative injury. *Pharmacology and Therapeutics, 47*, 61–71.

Smith, I. K., Vierheller, T. L., & Thorne, C. A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Analytical Biochemistry, 175*, 408.

Terao, J., Piskula, M., & Yao, Q. (1994). Protective effect of epicatechin, epicatechin galate and quercetin on lipid peroxidation in phospholipid bilayers. *Archives of Biochemistry and Biophysics, 308*, 278–284.

Torres, A., Frias, J., Granito, M., & Vidal-Valverde, C. (2006). Fermented pigeon pea (*Cajanus cajan*) ingredients in pasta products. *Journal of Agricultural and Food Chemistry, 54*, 6685–6691.

Torres, A., Frias, J., Granito, M., & Vidal-Valverde, C. (2007). Germinated Cajanus cajan seeds as ingredients in pasta products: chemical, biological and sensory evaluation. *Food Chemistry, 101*, 202–211.

Troszynska, A., & Ciska, E. (2002). Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. *Czech Journal of Food Science, 20*(1), 15–22.

Urbano, G., López-Jurado, M., Hernández, J., Fernández, M., Moreu, M. C., Frias, J., et al. (1995). Nutritional assessment of raw, heated and germination lentils. *Journal of Agricultural and Food Chemistry, 43*, 1871–1877.

Valencia, E., Marin, A., & Hardy, G. (2001). Glutathione-nutritional and pharmacological viewpoints: Part IV. *Nutrition, 17*, 783–784.

Vidal-Valverde, C., Frias, J., Hernández, A., Martín-Álvarez, P. J., Sierra, I., Rodríguez, C., et al. (2003). Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) seeds. *Journal of the Science of Food and Agriculture, 83*, 298–306.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., & Kuo, Y. (2002). New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology, 215*, 472–477.

Xu, M., Dong, J., & Zhu, M. (2005). Effects of germination conditions on ascorbic acid level and yield of soybeans sprouts. *Journal of the Science of Food and Agriculture, 85*, 943–947.

Zielinski, H. (2002). Peroxyl radical-trapping capacity of germinated legume seeds. *Nahrung/Food, 46*, 100–104.

Zielinski, H. (2003). Contribution of low molecular weight antioxidants to the antioxidant screen of germinated soybean seeds. *Plant Foods for Human Nutrition, 58*, 1–20.

Zielinski, H., Frias, J., Piskula, M., Kozlowska, H., & Vidal-Valverde, C. (2006). The effect of germination process on the superoxide dismutase-like activity and thiamine, riboflavin and mineral contents of rapeseeds. *Food Chemistry, 99*, 516–520.

Zielinski, H., & Kozlowska, H. (2000). Superoxide scavenging activity of cereal grains before and after hydrothermal processing. *Polish Journal of Food Nutrition Science, 9/50*(3S), 85–90.