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Effect of Processing on the Antioxidant Vitamins and Antioxidant Capacity of *Vigna sinensis* Var. Carilla

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Cowpea (*Vigna sinensis* L. var. Carilla) flours obtained by fermentation with inoculum *Lactobacillus plantarum* (PF) or with the natural microorganisms present in the flour (NF) and subsequent heat treatment in an autoclave were prepared to study the effect of fermentation on the antioxidant vitamin content and on the antioxidant capacity. Bacterial counts and pH values, vitamins C and E, carotenoids, glutathione (GSH), superoxide dismutase-like activity (SOD-like activity), peroxyl radical-trapping capacity (PRTC), lipid peroxidation in unilamillar liposomes, and Trolox equivalent antioxidant capacity (TEAC) were evaluated in raw and processed cowpea flours. γ-Tocopherol and δ-tocopherol were found in raw cowpea, whereas vitamin C and carotenoids were not detected. An increase in the vitamin E activity was observed in PF, whereas vitamin C and carotenoids were not detected in fermented cowpea flours. Fermentation or heat treatment in an autoclave after fermentation produced processed cowpea flours with lower PRTC, glutathione content, and SOD-like activity than those of the raw seeds. However, those processes increased the capacity to inhibit the lipid peroxidation in unilamellar lipoposomes and TEAC. According to the results obtained in this study, the fermentation of cowpeas (naturally or with *L. plantarum*) and fermentation and subsequent heat treatment in an autoclave are good processes to obtain functional cowpea flours having higher antioxidant capacity than the raw legume.

KEYWORDS: Cowpeas; natural fermentation; *Lactobacillus plantarum* fermentation; antioxidant vitamins; antioxidant capacity

INTRODUCTION

During the past decade, it has been reported that the consumption of certain foods may have a positive effect on an individual's health. Foodstuffs supply not only energy, essential amino acids, fiber, vitamins, and minerals but also some active compounds such as antioxidants (tocopherols, carotenoids, vitamin C, phenolic compounds, etc.) that may have different beneficial functions in the body. Dietary components, which are capable of acting as antioxidants, are likely to be beneficial by augmenting cellular defenses and helping to protect components of the cell from oxidative damage (*1*, *2*). Dietary antioxidants may play an important role in protecting the cell against damage caused by free radicals, such as radical scavengers, reducing agents, potential complexes of prooxidant metals, and quenchers of singlet oxygen formation (*3*). Consumption of foods containing antioxidants may prevent those diseases and, therefore, it is very important to determine their antioxidant capacity in order to estimate repercussion in oxidative stress in living beings. The health-promoting capacity of plant foods depends on the biologically active compounds identified, and different methods are emerging to assign the antioxidant activity of compounds and foods in order to establish the potential effect on health and live status.

An antioxidant is defined as any substance that when present at low concentrations compared to oxidizable substrate delays significantly or prevents substrate oxidation (*4*). The features of an oxidation are a substrate, an oxidant, an initiator, intermediates, and final products, and measurement of any one of these can be used to assess antioxidant activity (*5*). Several attempts have been made to assess the antioxidant capacity of plant extracts, foods, or body fluids, reflecting the increasing interest in this area. These systems have in common that they are all inhibition assays: a free radical species is generated, there is an end point by which the presence of the radical is detected, and antioxidant activity of the added sample inhibits the end point by scavenging the free radical. Reaction progress can be observed and quantified (*6*).

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Total antioxidant capacity is a parameter that quantifies the ability of a complex biological sample to scavenge free radicals (*7*). Such methods have the advantage of regarding antioxidant capacity as a global characteristic of a product and can be used to characterize the raw material and its evolution during industrial processing or storage and, thus, take its place alongside other quality control parameters. Moreover, individual measurement of the antioxidant capacity of all components in a sample is possible, but this can be time-consuming and expensive. In addition, there may be synergism between antioxidants, and examining one in isolation may not accurately reflect their combined action. Hence, it is of interest to measure the total antioxidant capacity that can be quantified by defining the amount of a suitable standard needed to produce the same end point as the compound or material being analyzed (*8*, *9*). The ABTS assay is based on the inhibition by antioxidants of the absorbance of the radical 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate ($ABTS^{\bullet+}$). The original method was based on the activation of metmyoglobin, acting as peroxidase, with H_2O_2 via the formation of ferrylmyoglobin radical, which then oxidizes the phenothiazine compound ABTS, forming the $ABTS^{\bullet+}$ radical cation (*10*, *11*). Recently, a modification of this method has been introduced by a decoloration technique in which the radical is generated directly in a stable form using potassium persulfate (*12*). Afterward, the former radical is mixed with the antioxidant extract in the reaction medium and the percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of concentration of antioxidant. The usual use of Trolox as a standard allows the assay to be called Trolox equivalent antioxidant capacity (TEAC). Such methods are used to assess the antioxidant capacity of biological matrices, food components, and food extracts (*13*−*15*).

Glutathione (GSH) is a tripeptide composed of cysteine, glutamic acid, and glycine. It is an antioxidant ubiquitously present in most plant and animal tissues and plays a vital role in protecting cells against the toxic effect of reactive oxygen species. It is an antioxidant primarily of the enzyme system containing glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase, removing hydrogen peroxide and organic peroxides, although it also functions as an antioxidant independent of enzymes; for example, it donates hydrogen to repair damaged DNA (*16*). Finally, the oxidized form (GSSG) is readily converted to the reduced form (GSH) by the enzyme glutathione reductase. Several studies have shown that foods high in GSH enhance metabolic clearance and decrease net absorption of dietary peroxidized lipids (*17*, *18*) and may be associated with about a 50% reduction of oral and pharyngeal cancer risk (*19*). Reduced and oxidized glutathione (GSH and GSSG) can be determined by the reaction of *o*-phthalaldehyde (OPT) as a fluorescent reagent at pH 8.0 and 12, respectively (*20*, *21*).

Peroxyl radical-trapping capacity (PRTC) is also a method in which the decomposition of 2,2′-azobis(2-amidopropane) hydrochloride (ABAP) is the source of peroxyl radicals that oxidize ABTS to give the green cation radical $ABTS^{\bullet+}$. The antioxidants present in the sample inhibit this oxidation by reacting with peroxyl radicals (*22*, *23*).

The superoxide radical ($O_2^{\bullet-}$) is generated in vivo in epithelial cells, macrophages, neutrofils, and neurons during catalysis by xanthine oxidase oxidation of hypoxanthine and xanthine to uric acid and by the NADPH oxidase system. Superoxide radicals formed in vivo are largely coverted by superoxide dismutase (SOD) into hydrogen peroxide (H_2O_2). In the living cells as well as in plant cells SOD controls the concentration of the superoxide anion (*24*). Then the SOD is a defense enzyme that protects the cells against cellular damage caused by reactive oxygen species arising during plant development and cellular metabolism in biological systems (*25*). The efficiency of the antioxidant system in plants has an important effect on the status of the remaining constituents in food of plant origin and plant-based diets (mainly vegetables, fruits, cereals, and pulses) and can help to lower the risk of human diseases, in which the increased level of free radicals is implicated (*26*).

Peroxidation of membrane phospholipids has been suggested as causing physiological damage in living organisms (*27*). Polyunsaturated fatty acids (PUFA) constituting phospholipids are susceptible to oxidation and produce hydroperoxides as the primary oxidation products (*28*). The antioxidant activity of food extracts in phospholipid bilayers evaluated by measuring the inhibition of lipid peroxidation in large unilamellar vesicles composed of egg yolk phosphatidylcholine (PC) is often used as a model for studying the antioxidant activity in vitro because liposomes can be related to the lamellar structures of biological membranes found in vivo (*29*). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) is used to initiate lipid peroxidation and PC−OOH formation is measured by HPLC (*30*, *31*). This method was used to assess the antioxidant capacity of food components (*32*, *33*) and food extracts (*34*−*36*).

Legume seeds occupy an important place in the human diet all over the world. Besides them, dry cowpeas (*Vigna sinensis*) are a good source of protein and carbohydrates, fiber, water-soluble vitamins. and microelements, and they are consumed widely, satisfying the requirements of populations living in developing countries (*37*). Furthermore, legumes have been always linked to the Mediterranean diet, the beneficial health properties of which might be due to their content in bioactive composition linked to antioxidant properties. Recently there has been increasing interest in knowing the antioxidant capacity of food and its evolution during industrial processing, and there are a few papers about antioxidant activity in legume extracts (*38*, *39*). Vitamin C is an important water-soluble antioxidant in biological fluids, protecting other substrates from oxidative damage and regenerating by reduction other small molecule antioxidants such as α-tocopherol, glutathione, and *β*-carotene (*40*). Vitamin E is the major chain-breaking antioxidant in body tissues, and it is considered to be the first line of defense against lipid peroxidation, protecting cell membranes at an early stage of free radical attack (*41*). The antioxidant activity of carotenoids is largely due to an extended system of conjugated double bounds, a structural feature that allows carotenoids to quench or inactivate some excited molecules (*42*, *43*). Legume seeds contain appreciable amounts of tocopherols. Tsuda et al. (*38*) reported the tocopherol isomer content in several legumes and found *γ*- and *δ*-tocopherols as major components (from 11.5 mg/100 g to not detected), whereas α- and *β*-tocopherols were from nondetectable amounts to 0.2 mg/100 g of seed. The content of vitamin C in legumes seems to be very low, and different authors give data from negligible amounts (lentils and beans) to 10 mg/100 g in soybean and lupins (*36*, *44*). With regard to carotenoids, different publications show levels ranging from nondetection of *β*-carotene in different varieties of soybeans (*45*) to 142 *μ*g/100 g in several lupin species (*46*).

Fermentation is an ancient technology that remains one of the most practical methods for preserving foods and enhancing their nutritional and organoleptic qualities. It is a desirable method for processing and preserving of food because of its low cost, low energy requirements, and high yield with acceptable and diversified flavors for human consumption.

Products such as soy sauce, tempeh, miso, natto, or sofu, largely consumed in Eastern countries, are now being also consumed in the West (*47*).

It has been reported that lactic acid fermentation causes a general improvement in the nutritional value of legumes, desirable changes in taste and texture, and decreases of some antinutritional compounds, giving an improved food with enhanced nutritional properties, a so-called functional food (*47*, *48*). Several studies have been published about the antioxidant capacity in tempeh (*49*−*51*) and natto (*51*−*53*). However, there is no information about the modifications on antioxidant vitamins and the antioxidant capacity after fermentation of cowpeas and subsequent heating.

The aim of this paper was to obtain functional cowpea flours and to study the effect of fermentation (with *Lactobacillus plantarum* or natural) and subsequent heat treatment in an autoclave on the antioxidant vitamin content and antioxidant capacity of cowpeas (*Vigna sinensis* L. var. Carilla).

MATERIALS AND METHODS

Seeds. Cowpea seeds (*V. sinensis* L. var. Carilla) were purchased in a wholesale market.

Processed Cowpeas. Raw cowpeas have been submitted to two different fermentation conditions, with *L. plantarum* (PF) and by natural process (NF). The fermented flours have been autoclaved to reduce the thermal antinutritional factors.

Bean Flour Preparation for Fermentation (HB). Seeds were washed three times with sterile distilled water in aseptic conditions and dried at 55 °C for 24 h to reduce the competitive microflora of starter culture of the inoculated fermentations. After drying, cowpeas were ground in a ball mill (Glen Creston Ltd., Stanmore, U.K.). The fraction of 0.050−0.250 mm was collected.

Fermented Bean Flours. The HB flour was fermented with *L. plantarum* (PF) or by natural process (NF) in sterile distilled water as in Doblado et al. (*48*) in a proportion of 300 g/L (w/v) for 48 h at 37 °C in a 5 L stirred fermentor (Infors ISF-100, Infors AG).

Fermented Bean Flour Heated in an Autoclave. PF and NF flours were autoclaved for 20 min at 121 °C as in Doblado et al. (*48*), and PFH and NFH flours, respectively, were obtained.

Preparation of Cultures. L. plantarum CECT 748 (ATCC 14917) was obtained from the Spanish Type Culture Collection (CECT). Stock cultures were grown and maintained on MRS Agar (Difco). Cultures were transferred from slants to MRS broth (Difco), which was then incubated for 24 h at 37 °C. The cells were washed twice with sterile saline solution (8.5% NaCl) and used as inoculum.

Microbiological Analysis. Samples taken before and after fermentation were microbiologically analyzed. Serial decimal dilutions of the samples were prepared in sterile saline solution (8.5% NaCl). Lactic acid bacteria were counted on MRS agar (Oxoid) plates after incubation in an anaerobic jar at 35 °C for 72 h. Enumeration of aerobic mesophilic bacteria was done on standard plate-count agar (Difco) after incubation at 30 °C for 72 h. Enterobacteriaceae were counted on violet red bile glucose (VRBG) agar (Oxoid) plates incubated at 30 °C for 24 h, the purple-red colonies being considered Enterobacteriaceae. Enumeration of yeasts and molds was done on chloramphenicol/Bromophenol-Blue agar (CBB) after incubation at 25 °C for 5 days. Sulfite-reducing clostridia were determined in Sulfite Polymyxin Sulfadiazine (SPS) agar (Difco) by incubation under anaerobic conditions at 37 °C for 48 h.

Chemical Analysis. *Determination of Vitamin C.* The vitamin C quantification in raw or processed cowpea flours by micellar electrokinetic capillary electrophoresis (MECC) was based on the procedure described by Thomson and Trenerry (*54*) as modified by Frías et al. (*36*).

Determination of Tocopherols. The tocopherol isomers determination in raw or processed cowpea flours was carried out by HPLC according to the method of Sierra et al. (*55*) as modified by Frías et al. (*36*). Vitamin E activity was calculated as α-TEs/100 g of dry matter (dm) according to the method of Eitenmiller and Landen (*56*), and the content on tocopherols was expressed as milligrams of each tocopherol/100 g of dm: α-TEs/100 g = [α-tocopherol (mg) × 1.0 + β-tocopherol (mg) × 0.5 + γ-tocopherol (mg) × 0.1 + δ-tocopherol (mg) × 0.03].

Determination of Carotenoids. The carotenoids determination in raw or processed cowpea flours was carried out by HPLC according to the method of Pérez-Gálvez and Mínguez-Mosquera (*57*).

Determination of Reduced (GSH) and Oxidized Glutathione (GSSG). The GSH and GSSG in raw or processed cowpea flours were determined according to the spectrofluorometric method of Hissin and Hilf (*20*). This method is 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 is complexed to *N*-ethylmaleimide (NEM) to prevent interference of GSH with measurement of GSSG. The extraction was conducted according to the method of Smith et al. (*58*).

Determination of SOD-like Activity. The SOD-like activity was measured using the superoxide dismutase kit (Ransod, catalog no. SD 125, Randox Laboratories Ltd., Antrim, U.K.) according to the method of Zielinski and Kozlowska (*24*). This method employs the system xanthine−xanthine oxidase to generate superoxide radicals ($O_2^{\bullet -}$), which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is measured by the degree of inhibition of this reaction.

Determination of Peroxyl Radical-Trapping Capacity (PRTC). The PRTC measurements were carried out according to the method of Bartosz et al. (*22*).

Determination of Lipid Peroxidation in Unilamillar Liposomes. The antioxidant activity of PBS extracts from the samples was evaluated according to the methods of Terao et al. (*32*) and Koga et al. (*30*) using a model system consisting of unilamellar liposomes and water-soluble azo compound AAPH as a free radical generator.

The antioxidative capacity in this system was calculated according to the equation of Azuma et al. (*31*) as

$$\frac{[\mathrm{PC{-}OOH_p}] - [\mathrm{PC{-}OOH_{inh}}] \times 100}{[\mathrm{PC{-}OOH_p}]}$$

where [PC−OOH_p] is the concentration of PC−OOH after the reaction for 2 h without a cowpea extract and [PC−OOH_{inh}] is the concentration of PC−OOH after the reaction for 2 h with a cowpea extract.

Determination of Total Equivalent Antioxidant Capacity (TEAC). Two methods were used to evaluated TEAC.

Method A. The hydrophilic extracts of raw and processed cowpeas were obtained with phosphate-buffered saline (PBS) following the procedure described by Re et al. (*12*) and the lipophilic extracts with dichloromethane (DCM) following the procedure described by Pellegrini et al. (*8*). The determination of TEAC for hydrophilic and lipophilic extracts was carried out according to the method of Re et al. (*12*) using potassium persulfate as free radical generator.

Method B. The raw and processed cowpeas were extracted with methanol, and TEAC was determined according to the method of Miller and Rice-Evans (*11*) with metmyoglobin as free radical generator using the Total Antioxidant Status Kit (catalog no. NX2332, Randox Laboratories Ltd.).

Statistical Methods. Data were subjected to multifactor ANOVA using the least-squares difference test with the Statgraphic 5.0 program (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

The raw cowpeas (*V. sinensis* var. Carilla) have been fermented with inoculum of *L. plantarum* or by the natural microorganisms present in the flour. The fermented flours have been submitted to autoclave heating to reduce the thermolabile antinutritional factors. The effect of processing in the content of antioxidant vitamins and antioxidant capacity in cowpeas, measured by different methods, has been studied.

Figure 1 shows the total bacteria count for NF and PF after inoculation and after 48 h of fermentation. As deduced from

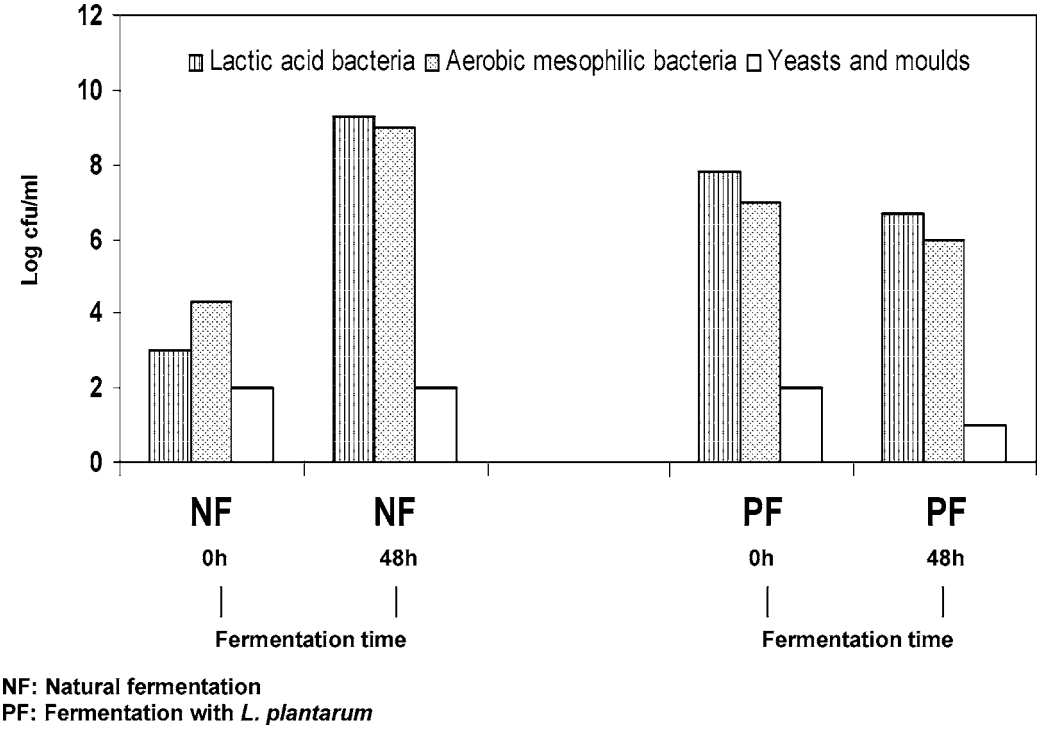

Figure 1. Microbiological study of fermented *V. sinensis* var. Carilla flours.

Table 1. Effect of Processing on the Antioxidant Vitamin Content of *V. sinensis* Var. Carilla[a]

V. sinensis var. Carilla	vitamin C (mg/100 g of dm)	β-tocopherol (mg/100 g of dm)	γ-tocopherol (mg/100 g of dm)	δ-tocopherol (mg/100 g of dm)	activity α-TEs/ 100 g of dm	carotenoids (μg/100 g of dm)
raw seeds	NDa	NDa	0.43 ± 0.02e	1.83 ± 0.11e	0.10 ± 0.01b	NDa
preparation of flour (HB)	NDa	NDa	0.31 ± 0.02d	1.35 ± 0.10d	0.07 ± 0.01a	NDa
fermented with *L. plantarum* (PF)	NDa	0.28 ± 0.05c	0.10 ± 0.01a	0.61 ± 0.05b	0.16 ± 0.02c	NDa
fermented with *L. plantarum* and autoclaved (PFH)	NDa	0.28 ± 0.06c	0.09 ± 0.01a	0.50 ± 0.09a	0.17 ± 0.01c	NDa
naturally fermented (NF)	NDa	0.11 ± 0.00b	0.17 ± 0.02c	1.00 ± 0.10c	0.10 ± 0.00b	NDa
naturally fermented and autoclaved (NFH)	NDa	0.04 ± 0.02ab	0.14 ± 0.01b	0.61 ± 0.01b	0.05 ± 0.01a	NDa

[a] Mean value ± standard deviation of three determinations. The same letter in the same column indicates no significant difference ($P \leq 0.05$); ND, not detected.

Figure 1, yeast and mold growth was inhibited in the fermentation inoculated with *L. plantarum*. It was demonstrated that *L. plantarum* ATCC 14917 was able to dominate the cowpea fermentation because the aerobic mesophilic bacteria and the lactic acid bacteria grown from the PF sample were always the inoculated bacteria. However, the strains isolated in natural fermentation after 48 h showed a varied bacterial population, including different genera of *Lactobacillus* spp. and *Leuconostoc* spp. The adequate sanitary quality of the product was indicated by the count of Enterobacteriaceae and sulfite-reducing clostridia that were lower than 1 cfu/mL in the final fermented cowpea flours.

The tocopherol isomer content and total vitamin E activity in raw and processed cowpeas are collected in **Table 1**. In raw seeds the γ- and δ-tocopherol contents and vitamin E activity were 0.43 mg/100 g of dm, 1.83 mg/100 g of dm, and 0.10 mg of α-TE/100 g of dm, respectively. The preparation of the flours for the fermentation process caused decreases of γ- and δ-tocopherol contents and vitamin E activity (28, 26, and 30%, respectively). Fermentation brought about sharp decreases in γ- and δ-tocopherol contents (from 77 to 67% and from 61 to 45% in PF and NF, respectively) and in the presence of β-tocopherol (0.28 and 0.11 mg/100 g of dm in PF and NF, respectively) (**Table 1**). The vitamin E activity increased notably in PF (60%), mainly due to the contribution of β-tocopherol. Autoclaving PF produced only a slight decrease in δ-tocopherol content but a sharp reduction in all of the isomers and vitamin E activity was observed in NF.

No information has been found about the content of tocopherols in cowpea seeds. In other legumes, Wyatt et al. (*59*) report vitamin E activities for lentils and chickpeas of 3.54 α-TEs mg/100 g of dm, whereas for kidney beans it was 0.09 α-TEs mg/100 g of dm, γ-tocopherol being the major isomer present in these seeds. Fordham et al. (*60*) and Plaza et al. (*44*) observe values of α-tocopherol of 0.1−0.3 and 0.09 mg/100 g in peas and soybeans, respectively. Frías et al. (*36*) obtained in *Lupinus albus* L. var. Multolupa contents for α-tocopherol, γ-tocopherol, and δ-tocopherol and total vitamin E activity of 0.19 mg/100 g of dm, 20.11 mg/100 g of dm, 0.25 mg/100 g of dm, and 2.21 α-TEs mg/100 g of dm, respectively.

Vitamin C and carotenoids were not detected in raw and processed cowpeas (**Table 1**). No information was found about the content of these antioxidant vitamins in cowpea seeds. In soybeans and lentils Plaza et al. (*44*) and Frías et al. (*36*) found levels of vitamin C of 10 and 6 mg/100 g, respectively. The content of carotenoids in beans ranges between 0.23 and 68 μg/100 g (*61, 62*) and in lupins from 23 to 142 μg/100 g (*46*), whereas it is not detected in soybeans (*45*).

Table 2. Effect of Processing on the Content of Glutathione and SOD-like Activity of *V. sinensis* Var. Carilla[a]

V. sinensis var. Carilla	glutathione			SOD-like activity (units of SOD/mg of protein)
	GSH[b] (μmol/g of dm)	GSSG[c] (μmol/g of dm)	GSH/GSSG	
raw seeds	3.25 ± 0.01e	0.58 ± 0.03a	5.61 ± 0.26d	4.64 ± 0.31c
preparation of flour (HB)	3.32 ± 0.06e	0.65 ± 0.03a	5.13 ± 0.37c	3.24 ± 0.13b
fermented with *L. plantarum* (PF)	2.57 ± 0.01d	1.03 ± 0.02b	2.51 ± 0.06b	2.14 ± 0.00b
fermented with *L. plantarum* and autoclaved (PFH)	2.19 ± 0.03c	1.02 ± 0.02b	2.15 ± 0.02b	2.15 ± 0.10b
naturally fermented (NF)	1.52 ± 0.07b	0.94 ± 0.01b	1.62 ± 0.09a	0.15 ± 0.01a
naturally fermented and autoclaved (NFH)	1.15 ± 0.05a	0.99 ± 0.07b	1.17 ± 0.09a	0.18 ± 0.01a

[a] The same letter in the same column indicates no significant difference ($P \leq 0.05$). [b] Reduced glutathione. [c] Oxidized glutathione.

No information has been found about the effect of fermentation on the antioxidant vitamins in cowpeas (*V. sinensis*). In okara koji, a fermented product from soybeans with *Aspergillus oryzae*, Matsou (*63*) detected the presence of γ- and δ-tocopherol but not α-tocopherol. Esaki et al. (*51*) report that in soybean fermented with *A. oryzae* (miso) the tocopherol content decreases, whereas it is not modified with *Bacillus natto* (natto) or with *Rhizopus oligosporus* (tempeh). However, Denter et al. (*64*) observed that in tempeh the tocopherol content decreases, whereas the vitamin E activity is kept almost constant. Frías et al. (*36*) reported an increment in α-tocopherol and reductions in γ- and δ-tocopherol when lupin seeds were fermented naturally but considerable reductions in all of the tocopherol isomers when lupins were fermented with *L. plantarum*. These authors also report sharp reductions in vitamin E activity and vitamin C content after both fermentations. Denter et al. (*64*) observed a significant increment in β-carotene content during the fermentation of tempeh.

GSH and GSSG contents in raw and processed cowpeas are shown in **Table 2**. After bean fermentation, the GSH of raw beans (3.2 μmol/g of dm) decreased to 2.6 μmol/g of dm in PF and to 1.5 μmol/g of dm in NF, whereas the GSSG increased from 0.6 μmol/g of dm in raw seeds to 1.0 and 0.9 μmol/g of dm in PF and NF, respectively ($P \leq 0.05$). The ratio GSH/GSSG of raw cowpeas (5.6 μmol/g of dm) decreased as a consequence of the processing, and this effect was more pronounced after natural fermentation (2.3 μmol/g of dm in PF and 1.6 μmol/g of dm in NF). Autoclaving did not significantly change ($P \leq 0.05$) the ratio GSH of to GSSG of raw fermented flours (**Table 2**).

There is a lack of references about the glutathione content in *V. sinensis* seeds. In other legumes, Burkey et al. (*65*) found in *Phaseolus vulgaris* glutathione values that ranged from 0.11 to 0.22 μmol/g of dm. Fernández-Orozco et al. (*21*) reported GSH values from 0.97 to 1.5 μmol/g of dm in different Spanish lentils. In other seeds such as cereals and cruciferae values between 0.3 and 4.37 μmol of GSH/g of dm were reported (*66*, *67*). No information has been found on the effect of either fermentation or heat treatments on the GSH or GSSG in cowpeas. In other processed legumes, Fernández-Orozco et al. (*21*) observed a reduction of GSH in lentils after cooking. Valencia et al. (*16*) reported values of 0.6 mg of GSH/100 g in canned and heated pinto beans.

SOD-like activity (**Table 2**) decreased after fermentation, the reduction being more acute in NF (4.6, 2.1, and 0.15 units of SOD/mg of protein for raw, PF, and NF, respectively). Autoclaving did not change ($P \leq 0.05$) the SOD-like activity of PF and NF (**Table 3**). The values obtained for raw cowpeas are similar to those reported in lentils by Fernández-Orozco et al. (*21*). No information has been found about the fermentation and heating on the SOD-like activity of legumes. Fernández-Orozco et al. (*21*) reported a sharp reduction in SOD-like activity in cooked lentils, whereas in cereals, Zielinski and Kozlowska (*24*) observed a large decrease in SOD-like activity after extrusion. Lin et al. (*39*) showed that for some varieties of *Phaseolus* and soybean there is an inhibition of superoxide anion formation and remarkable inhibition of $FeCl_2$−ascorbic acid induced lipid peroxidation of mouse liver homogenate.

Table 3. Effect of Processing on the Peroxyl Radical Trapping Capacity of *V. sinensis* Var. Carilla[a]

V. sinensis var. Carilla	PRTC/g of dm	
	PBS extract	MeOH extract
raw seeds	8.77 ± 0.38d2	6.63 ± 0.64d1
preparation of flour (HB)	7.40 ± 0.32c2	5.31 ± 0.28bc1
fermented with *L. plantarum* (PF)	4.24 ± 0.11a1	4.72 ± 0.55ab1
fermented with *L. plantarum* and autoclaved (PFH)	5.72 ± 0.23b1	5.89 ± 0.37cd1
naturally fermented (NF)	4.46 ± 0.12a2	3.93 ± 0.07a1
naturally fermented and autoclaved (NFH)	5.77 ± 0.50b1	5.31 ± 0.84bc1

[a] Mean value ± standard deviation of three determinations. The same letter in the same column indicates no significant difference ($P \leq 0.05$). The same number in the same row indicates no significant difference ($P \leq 0.05$).

Table 4. Antioxidative Activities of Fermented *V. sinensis* Var. Carilla in Liposomal PC Suspension System[a]

V. sinensis var. Carilla	antioxidative activity
raw seeds	48 ± 2a
preparation of flour (HB)	46 ± 2a
fermented with *L. plantarum* (PF)	53 ± 11ab
fermented with *L. plantarum* and autoclaved (PFH)	61 ± 1bc
naturally fermented (NF)	61 ± 9c
naturally fermented and autoclaved (NFH)	78 ± 8d

[a] Against control (extract without sample). Mean value ± standard deviation of two determinations. The same letter in the same column indicates no significant difference ($P \leq 0.05$).

The PRTC values of PBS and methanol extracts of raw and processed cowpeas are collected in **Table 3**. The PRTC of raw seeds (8.8 and 6.6 μmol of Trolox/g of dm in PBS and methanol extracts, respectively) decreased after the preparation of fermentation sample (HB) (7.4 and 5.3 μmol of Trolox/g of dm, respectively) (**Table 4**). Fermentation caused a noticeable decrease in PRTC (52 and 49% for PBS extracts and 29 and 41% for methanolic extracts from PF and NF, respectively). Autoclave treatment brought about a significant increment ($P \leq 0.05$) in PRTC compared with the fermented cowpeas (**Table 3**). These results are in agreement with those of Fernández-Orozco et al. (*21*) and Zielinski (*68*), who report different PRTC values in lentils, soybean, and alfalfa depending on the solvent used for the extraction and the type of legume. No information has been found about the effect of fermentation on the PRTC in cowpeas. Concerning the heat treatment, in cooked lentils

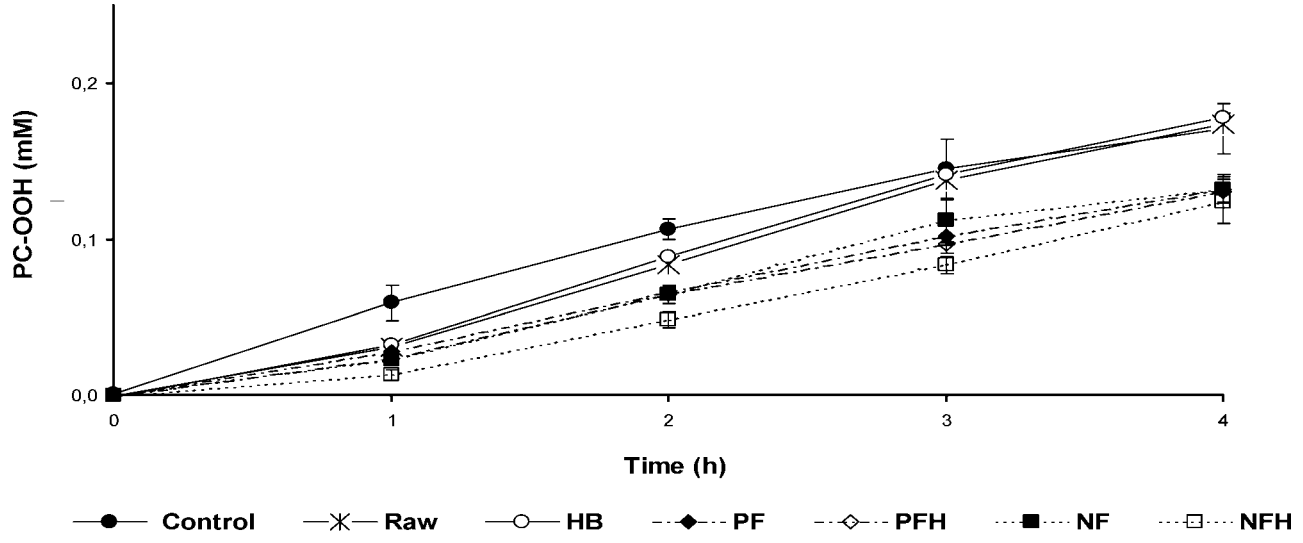

Figure 2. Inhibition by fermented *V. sinensis* var. Carilla extracts of AAPH-initiated peroxidation of PC liposomes.

Table 5. Effect of Processing on the Trolox Equivalent Antioxidant Capacity of *V. sinensis* Var. Carilla[a]

	hydrophilic extract (μmol of Trolox/g of dm)		lipophilic extract (μmol of Trolox/g of dm)	total TEAC (μmol of Trolox/g of dm)
V. sinensis var. *Carilla*	PBS extract[b]	MeOH extract[c]	DCM extract[b]	PBS + DCM extract[c]
raw seeds	27.39 ± 0.04a2	12.73 ± 0.51b1	0.57 ± 0.03a	27.95 ± 0.05b
preparation of flour (HB)	26.31 ± 1.73a2	9.37 ± 1.61a1	0.68 ± 0.01b	26.99 ± 1.33a
fermented with *L. plantarum* (PF)	59.40 ± 0.49c2	18.78 ± 0.79c1	0.87 ± 0.03c	60.28 ± 0.41e
fermented with *L. plantarum* and autoclaved (PFH)	56.83 ± 1.03b2	20.06 ± 0.56c1	0.91 ± 0.04cd	57.38 ± 0.83d
naturally fermented (NF)	55.24 ± 0.85b2	21.90 ± 0.66d1	0.98 ± 0.06d	55.85 ± 0.73c
naturally fermented and autoclaved (NFH)	59.01 ± 0.00c2	22.63 ± 0.26d1	0.70 ± 0.00b	59.71 ± 0.00e

[a] Mean value ± standard deviation of three determinations. The same letter in the same column indicates no significant difference ($P \leq 0.05$). The same number in the same row for each hydrophilic extract indicates no significant difference ($P \leq 0.05$). [b] Method A. [c] Method B.

Fernández-Orozco et al. (*21*) showed different PRTC values depending on the solvent used for the extraction and the lentil variety.

The antioxidative activities of PBS extracts in phospholipid bilayers obtained by measuring the inhibition of lipid peroxidation in large unilamellar vesicles composed of egg yolk PC are collected in **Figure 2**. **Table 4** shows the percentages of peroxidation inhibition after 2 h of incubation. The oxidation of PC was inhibited by all fermented cowpea extracts in comparison with the control assay (**Figure 2**). The fermented flour (PF and NF) extracts provided higher inhibition than the raw seed extract (**Figure 2**; **Table 4**). The inhibition of PC oxidation by extracts from naturally fermented flours (NF) was better than that by extracts from PF (**Figure 2**; **Table 4**). No differences were observed in the inhibition of PC oxidation between the raw beans and HB. Autoclaving increased the ability to inhibit of PC oxidation, but it was significant ($P \leq 0.05$) for only NFH (**Figure 2**; **Table 5**).

Most of the studies found in the literature about the inhibition of PC peroxidation are based on model systems to evaluate antioxidant capacity using pure compounds such as epicatechin, epicatechin gallate, quercetin, or vitamin E (*32*, *33*). In food extracts Troszynska and Ciska (*34*) evaluated the inhibition of PC oxidation in acetone and methanol extracts of two varieties of peas and found that extracts of colored pea variety showed stronger antioxidant capacity, which was related to the polymerization degree of tannins, whereas in white-coated pea it was related with cinnamic acids (ferulic, caffeic, and coumaric). Azuma et al. (*31*) studied methanolic extracts of 25 vegetables in PC liposomal suspensions and observed inhibition percentages of PC peroxidation between 0 and 99%.

Table 5 shows the TEAC (μmol of Trolox/g of dm) of hydrophilic (in PBS and methanol) and lipophilic extracts of raw and processed cowpea flours. The hydrophilic TEAC values in PBS extracts were always significantly higher ($P \leq 0.05$) than those of methanolic extracts. Because it is not clear whether the antioxidants in the methanol extracts are polar or nonpolar substances (*38*), the total TEAC was calculated as the sum of PBS extract TEAC and lipophilic extract TEAC (*69*). Total TEAC did not change after the preparation of the fermented flours (HB). Fermentation caused a significant increase ($P \leq 0.05$) in the total TEAC of bean flours (116 and 112% in PF and NF, respectively). Autoclaving PF and NF flours produced a reduction (5%) and an increase (7%) of total TEAC, respectively (**Table 5**). It has been observed that cowpeas' hydrophilic compounds contribute more than the lipophilic antioxidants to the total TEAC (**Table 5**). In the same samples studied in this work, Dueñas et al. (*70*) observed that fermentation of cowpeas modified the content of phenolic compounds. They report that some compounds that were not present in the raw seeds, such as tyrosol and quercetin, appear after fermentation in quite high amounts (7 and 89 μg/g for tyrosol and 22 and 11 μg/g for quercetin in PF and NF, respectively). It is known that these phenolic compounds show a high antioxidant capacity, which can contribute a great deal to the total TEAC of fermented cowpeas. Despite the increment in TEAC, other

antioxidant parameters measured in the raw cowpea seeds (PRTC, GSH, and SOD-like activity) suffered a decrease as a consequence of the fermentation process (**Tables 2** and **3**). Dueñas et al. (*70*) also observed that the antioxidant capacity of raw cowpeas increased after fermentation, whereas the DPPH of raw cowpeas (*8*, *42*) decreased to 8.89 and 9.02 in the PF and NF samples, respectively. Tsuda et al. (*38*) reported that the total antioxidant capacity of methanolic extracts of some species of cowpeas is very low in comparison with that of other legumes.

In other legumes Fernández-Orozco et al. (*21*) observed that the TEAC of PBS extracts, from different varieties of lentils, ranged between 13 and 23 μmol of Trolox/g of dm. Frías et al. (*36*) reported that lupin fermentation with *L. plantarum* increased the antioxidant capacity (TEAC) of PBS extract by 5%, whereas TEAC did not show any modification after natural fermentation of lupins. Esaki et al. (*51*) observed that miso, natto, and tempeh are more stable against lipid peroxidation than steamed soybeans, due to the fact that during fermentation some compounds with antioxidative properties can be produced. Hoppe et al. (*50*) attributed the TEAC rise of tempeh to the synergic action between tocopherols and amino acids released during the soybean fermentation with *R. oligosporus*, whereas Murakami et al. (*49*) and Esaki et al. (*51*) suggested that it is due to an increment of isoflavones (daidzein and genistein). Yokota et al. (*53*) obtained an antioxidant-containing preparation from natto and suggested that it could prevent atherosclerosis development in rabbits by lowering the total cholesterol, low-density lipoproteins, and triacylglycerols.

In conclusion, fermentation or heat treatment in an autoclave after fermentation produced processed cowpea flours with lower PRTC, glutathione content, and SOD-like activity than the raw seeds. However, those processes increased the capacity to inhibit the lipid peroxidation in unilamellar lipoposomes and TEAC. According to the chemical and microbiological results obtained in this paper, the fermentation of cowpeas (naturally or with *L. plantarum*) produced a safe product that could be recommended to obtain functional cowpea flours with high antioxidant capacity.

LITERATURE CITED

(1) Halliwell, B.; Gutteridge, J. M. *Free Radicals in Biology and Medicine*; Oxford University Press: New York, 1989.

(2) Gutteridge, J. M. Free radicals in disease processes: a compilation of cause and consequence. *Free Radical Res. Commun.* **1993**, *19*, 141−158.

(3) Hochstein, P.; Atallah, A. H. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat. Res.* **1988**, *202*, 363−375.

(4) Halliwell, B. How to characterize a biological antioxidant. *Free Radical Res. Commun.* **1990**, *9*, 1−32.

(5) Antolovich, M.; Prenzler, P. D.; Patsalides, E.; McDonald, S.; Robards, K. Methods of testing antioxidant activity. *Analyst* **2002**, *127*, 183−198.

(6) Rice-Evans, C.; Miller, N. J. Total antioxidant status in plasma body fluids. *Methods Enzymol.* **1994**, *234*, 279−293.

(7) Arnao, M. B.; Cano, A.; Acosta, M. Total antioxidant activity in plant material and its interest in food technology. *Recent Res. Dev. Agric. Food Chem.* **1998**, *2*, 893−905.

(8) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532−2538.

(9) Arnao, B. M.; Cano, A.; Hernández-Ruiz, J.; García-Cánovas, F.; Acosta, M. Inhibition by L-ascorbic acid and other antioxidants of the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) oxidation catalysed by peroxidase: A new approach for determining total antioxidant status of foods. *Anal. Biochem.* **1996**, *236*, 255−261.

(10) Miller, N. J.; Rice-Evans, C.; Davies, M. J.; Gopinathan, V.; Milner, A. A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, *84*, 407−412.

(11) Miller, N. J.; Rice-Evans, C. A. Spectrophotometric determination of antioxidant activity. *Redox Rep.* **1996**, *2*, 161−171.

(12) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity and improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.

(13) Van den Berg, R.; Haenen, R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.

(14) Bohm, V.; Puspitasari-Nienaber, N.; Ferruzzi, M. G.; Schwartz, S. J. Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, β-carotene, lycopene, and zeaxanthin. *J. Agric. Food Chem.* **2002**, *50*, 221−226.

(15) Leong, L. P.; Shui, G. An investigation of antioxidant capacity of fruits in Singapore market. *Food Chem.* **2002**, *76*, 69−75.

(16) Valencia, E.; Marin, A.; Hardy, G. Glutathione. Nutritional and pharmacologic viewpoints. Part IV. *Nutrition* **2001**, *17*, 783−784.

(17) Kowalski, D. P.; Feely, R. M.; Jones, D. P. Use of exogenous glutathione for metabolism of peroxidized methyl linoleate in rat small intestine. *J. Nutr.* **1990**, *120*, 1115−1121.

(18) Aw, T. Y.; Williams, M. W. Intestinal absortion and lymphatin transport of peroxidized lipids in rats: effect of exogenous GSH. *Am. J. Physiol.* **1992**, *263*, G665-G672.

(19) Flagg, E. W.; Coates, R. J.; Jones, D. P. Dietary glutathione intake and the risk of oral and pharyngeal cancer. *Am. J. Epidemiol.* **1994**, *139*, 453−465.

(20) Hissin, J. P.; Hilf, R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal. Biochem.* **1976**, *74*, 214−226.

(21) Fernández-Orozco, R.; Zielinski, H.; Piskula, M. K. Contribution of low-molecular-weight antioxidants to the antioxidant capacity of raw and processed lentil seeds. *Nahrung/Food* **2003**, *47*, 291−299.

(22) Bartosz, G.; Janaszewska, A.; Ertel, D.; Bartosz, M. Simple determination of peroxyl radical-trapping capacity. *Biochem. Mol. Biol. Int.* **1998**, *46*, 519−528.

(23) Bartosz, G.; Bartosz, M. Antioxidant activity: what do we measure?. *Acta Biochim. Pol.* **1999**, *46*, 23−29.

(24) Zielinski, H.; Kozlowska, H. Superoxide scavening activity of cereal grains before and after hydrothermal processing. *Pol. J. Food Nutr. Sci.* **2000**, *9/50* (3S), 85−90.

(25) Asada, K. Ascorbare peroxidase—a hydrogen peroxide-scavenging enzyme in plants. *Physiol. Plant.* **1992**, *85*, 235−241.

(26) Machlin, L. J. *Handbook of Vitamins. Nutritional, Biochemical, and Clinical Aspects*; Machlin, L. J., Ed.; Dekker: New York, 1995.

(27) Cadenas, E.; Packer, L. *Handbook of Antioxidants*; Dekker: New York, 2002.

(28) Lundberg, W. O.; Jarvi, P. In *Progress in the Chemistry of Fats and Other Lipids*; Holmann, R. T., Ed.; Pergamon Press: Oxford, U.K., 1971; Vol. 9, pp 377−406.

(29) Keller, B. C. Liposomes in nutrition. *Trends Food Sci. Technol.* **2001**, *12*, 25−31.

(30) Koga, T.; Terao, J. Antioxidant behaviours of vitamin E analogues in unilamellar vesicles. *Biosci., Biotechnol., Biochem.* **1997**, *60*, 1043−1045.

(31) Azuma, K.; Ippoushi, K.; Ito, H.; Higashio, H.; Terao, J. Evaluation of antioxidative activity of vegetable extracts in linoleic acid emulsion and phospholipid bilayers. *J. Sci. Food Agric*. **1999**, *79*, 2010−2016.

(32) Terao, J.; Piskula, M.; Yao, Q. Protective effects of epicatechin, epicatechin gallate, and quercitin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.* **1994**, *308*, 278−284.

(33) Koga, T.; Takahashi, I.; Yamauchi, R.; Piskula, M.; Terao, J. *Chem. Phys. Lipids* **1996**, *86*, 85−93.

(34) Troszynska, A.; Ciska, E. Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. *Czech J. Food Sci*. **2002**, *20*, 15−21.

(35) Troszynska, A.; Estrella, I.; López-Amorós, M. L.; Hernández, T. Antioxidant activity of pea (*Pisum sativum L.*) seed coat acetone extracts. *Lebensm. Wiss. -Technol.* **2002**, *35*, 158−164.

(36) Frías, J.; Miranda-Zárate, M. L.; Doblado, R.; Vidal-Valverde, C. Effect of germination and fermentation in the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. Multolupa. *Food Chem*. **2005**, in press.

(37) Chavan, J. K.; Kadam, S. S.; Salunkhe, D. K. Cowpea. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. II, pp 1−21.

(38) Tsuda, T.; Makino, Y.; Kato, H.; Osawa, T.; Kawakishi, S. Screening for antioxidative active of edible pulses. *Biosci., Biotechnol., Biochem*. **1993**, *57*, 1606−1608.

(39) Lin, C. C.; Wu, S. J.; Wang, J. S.; Yang, J. J.; Chang, C. H. Evaluation of the antioxidant activity of legumes. *Pharm. Biol*. **2001**, *39*, 300−304.

(40) Carr, A. C.; Frei, B. Vitamin C and cardiovascular diseases. In *Handbook of Antioxidants*; Cadenas, E., Packer, L., Eds.; Dekker: New York, 2002.

(41) Landvik, S. V.; Diplock, A. T.; Packer, L. Efficacy of vitamin E in human health and disease. In *Handbook of Antioxidants*; Cadenas, E., Packer, L., Eds.; Dekker: New York, 2002.

(42) Krynsky, N. I. Actions of carotenoids in biological systems. *Annu. Rev. Nutr*. **1993**, *13*, 561−587.

(43) Sies, H.; Stahl, W. Vitamins E and C, β-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr*. **1995**, *62*, 1315S−1321S.

(44) Plaza, L.; De Ancos, B.; Cano, P. 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. *Eur. Food Res. Technol*. **2003**, *216*, 138−144.

(45) Monma, M.; Terao, J.; Ito, M.; Saito, M.; Chikuni, K. Carotenoid components in soybean seeds varying with seed color and maturation stage. *Biosci., Biotechnol., Biochem*. **1994**, *58*, 926−930.

(46) El-Difrawi, E. A.; Hudson, B. J. F. Identification and estimation of carotenoids in the seeds of four *Lupinus* species. *J. Sci. Food Agric.* **1979**, *30*, 1168−1170.

(47) Deshpande, S. S.; Salunkhe, D. K.; Oyewole, O. B.; Azam-Ali, S.; Battcock, M.; Bressani, R. *Fermented Grain Legumes, Seeds and Nuts. A Global Perspective*; FAO: Rome, Italy, 2000.

(48) Doblado, R.; Frías, J.; Muñoz, R.; Vidal-Valverde, C. Fermentation of *Vigna sinensis* var. carilla flours by natural microflora and *Lactobacillus* species. *J. Food Prot.* **2003**, *65*, 12, 2313−2320.

(49) Murakami, H.; Asakawa, T.; Terao, J.; Matsushita, S. Antioxidative stability of tempeh and liberation of isoflavones by fermentation. *Agric. Biol. Chem*. **1984**, *48*, 2971−2975.

(50) Hoppe, M. B.; Jha, H. C.; Egge, H. Structure of an antioxidant from fermented soybeans (tempeh). *J. Am. Oil Chem. Soc*. **1997**, *74*, 477−479.

(51) Esaki, H.; Onozaki, H.; Osawa, T. Antioxidative activity of fermented soybean products. In *Food Phytochemicals for Cancer Prevention I*; Huang, M. T., Osawa, T., Ho, C. T., Rosen, R. T., Eds.; ACS Symposium Series 546; American Chemical Society: Washington, DC, 1994.

(52) Chung, Y. C.; Chang, C. T.; Chao, W. W.; Lin, C. F.; Chou, S. T. Antioxidative activity and safety of the 50% ethanolic extracts from red bean fermented by *Bacillus subtillis* IMR-NK1. *J. Agric. Food Chem*. **2002**, *50*, 2454−2458.

(53) Yokota, T.; Hattori, T.; Ohishi, H.; Hasegawa, K.; Watanabe, K. The effect of antioxidant-containing fraction from fermented soybean food on atherosclerosis development in cholesterol-fed rabbits. *Lebensm. Wiss. -Technol.* **1996**, *29*, 751−755.

(54) Thompson, C. O.; Trenerry, V. C. A rapid method for the determination of total L-ascorbic acid in fruits and vegetables by micellar electrokinetic capillary chromatography. *Food Chem.* **1995**, *53*, 43−50.

(55) Sierra, I.; Prodanov, M.; Calvo, M.; Vidal-Valverde, C. Vitamin stability and growth of psychotrophic bacteria in refrigerated raw milk acidified with carbon dioxide. *J. Food Prot.* **1996**, *59*, 1305−1310.

(56) Eitenmiller, R. R.; Landen, W. O. *Vitamin Analysis for the Health and Food Sciences*; CRC Press: New York, 1999.

(57) Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. Structure−reactivity relationship in the oxidation of carotenoid pigments of pepper (*Capsicum annuum* L.) *J. Agric. Food Chem*. **2001**, *49*, 4864−4869.

(58) Smith, I. K.; Vierheller, T. L.; Thorne, C. A. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Anal. Biochem*. **1988**, *175*, 408−413.

(59) Wyatt, C. J.; Pérez-Carballido, S.; Méndez, R. O. α- and γ-tocopherol content of selected foods in the mexican diet: Effect of cooking losses. *J. Agric. Food Chem.* **1998**, *46*, 4657−4661.

(60) Fordham, J. R.; Wells, C. E.; Chen, L. H. Sprouting of seeds and nutrient composition of seeds and sprouts. *J. Food Sci*. **1975**, *40*, 552−556.

(61) Kantha, S. S.; Erdman Jr., J. W. Legume carotenoids. *Crit. Rev. Food Sci. Nutr*. **1987**, *26*, 137−155.

(62) Atienza, J.; Sanz, M.; Herguedas, A.; Alejos, J. A.; Jiménez, J. J. β-Carotene, α-tocopherol and γ-tocopherol contents in dry legumes. Influence of cooking. *Food Sci. Technol. Int*. **1998**, *4*, 437−441.

(63) Matsou, M. *In vivo* antioxidant activity of okara koji, a fermented okara, by *Aspergillus oryzae*. *Biosci., Biotechnol., Biochem*. **1997**, *61*, 1968−1972.

(64) Denter, J.; Rehm, H. J.; Bisping, B. Changes in the contents of fat-soluble vitamins and provitamins during tempe fermentation. *Int. J. Food Microbiol*. **1998**, *45*, 129−134.

(65) Burkey, K. O.; Wei, C.; Eason, G.; Ghosh, P.; Fenner, G. P. Antioxidant metabolite levels in ozone-sensitive and tolerant genotypes of snap bean. *Physiol. Planta.* **2000**, *110*, 195−200.

(66) Zielinski, H.; Honke, J.; Troszynska, A.; Kozlowska, H. Reduced-oxidized glutahione status as a potential index of oxidative stress in mature cereal grain. *Cereal Chem.* **1999**, *76*, 944−948.

(67) Zielinski, H.; Mudway, I.; Kozlowska, H.; Kelly, F. J. Impact of germination on glutathione content in cruciferous seeds. *Pol. J. Food Nutr. Sci.* **2002**, *11/52* (SI 1), 68−72.

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

(69) Arnao, M. B.; Cano, A., Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **2001**, *73*, 239−244.

(70) Dueñas, M.; Fernández, D.; Hernández, T.; Estrella, I.; Muñoz, R. Bioactive phenolic compounds of cowpeas (*Vigna sinensis L.*) modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC-14917. *J. Sci. Food Agric*. **2005**, *85*, 297−304.

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