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Bioactive phenolic compounds of cowpeas (*Vigna sinensis* L). Modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC 14917

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Abstract: In this work we have determined the phenolic composition of raw cowpeas (*Vigna sinensis* L) of the variety Carilla by HPLC/PAD/MS and have studied the effect of fermentation, both spontaneous and with *Lactobacillus plantarum* ATCC 14917, on the phenolic compounds. This variety contains mainly ferulic and *p*-coumaric acids esterified with aldaric acids, together with the *cis* and *trans* isomers of the corresponding free acids. Hydroxybenzoic acids such as gallic, vanillic, *p*-hydroxybenzoic and protocatechuic were also found, along with flavonols such as a myricetin glucoside, mono- and diglycosides of quercetin and a quercetin diglycoside acylated with ferulic acid. Fermentation, both spontaneous and inoculated, modifies the content of phenolic compounds, but differently in each case. The antioxidant activity as free radical-scavenging activity has also been evaluated. Fermentation followed by heating has been shown to be a very effective process to increase the functionality of this variety of *V sinensis*. For this reason, this cowpea variety could be used as an ingredient to obtain high value-added flours.

Keywords: cowpea; phenolic compounds; antioxidant activity; fermentation

INTRODUCTION

Cowpeas (*Vigna sinensis*) are recognised as a source of proteins as well as other nutrients and are especially consumed in developed countries.[1] In addition, they contain bioactive compounds such as vitamins, carotenoids and phenolics.[2,3] Their consumption, like that of other legumes, is limited by the presence of antinutritional factors which affect the digestibility and bioavailability of nutrients, and thus cowpeas need to be processed to reduce or even remove these factors.[4,5]

Various processes applied to legumes modify the chemical composition of the seeds, not only their antinutrient compounds but also other components. Fermentation has been proposed to improve the nutritive value of legumes, as it decreases the concentration of antinutritional factors.[6–8]

Legume seeds contain phenolic compounds in various forms: hydroxybenzoic and hydroxycinnamic acids, both free and bound to other molecules, as organic acids, in esterified forms,[2,9,10] and flavonoids, mainly flavan-3-ols, flavonols and flavones, which are present most frequently in glycosidios forms.[10–12]

Phenolic compounds are considered to be natural antioxidants and represent an important group of bioactive compounds in foods which may prevent the development of many diseases, including atherosclerosis, cancer, etc.[13,14] They also act as protective factors against oxidative damage[15–17] and possess antimutagenic activity,[18] with health benefits to prevent disease in humans.

Fermentation of legumes modifies the levels of various phenolic compounds. For example, in spontaneously fermented lentils, *p*-hydroxybenzoic and protocatechuic acids and (+)-catechin increase whereas hydroxycinnamic acids and procyanidin dimers decrease.[19] In beans the concentration of phenolic compounds also increases during this process.[20] In fermented red beans the changes in phenolic composition seem to be associated with a change in antioxidant activity; it was thus observed that fermented red beans possess antioxidant activities[21,22] and, when the fermentation was carried out by a controlled micro-organism, the beans showed radical-scavenging and Fe^{2+}-chelating ability and were safe regarding genotoxicity.[23]

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The antioxidant activity of phenolics is related to their chemical structure. In general, flavonoid compounds present a stronger antioxidant activity than non-flavonoids, and combined forms such as glycosides present a lower activity than the free forms.[24] Among the non-flavonoid compounds, the benzoic acids are somewhat less active than the homologous cinnamic acids;[25,26] in the case of derivatives of these acids the relative antioxidant activity indicates that cinnamic acid derivatives are more efficient that their benzoic acid counterparts.[27]

As a consequence of this activity, the presence of phenolic compounds in foods has in recent years come to be viewed in a positive light by both scientists and consumers and has resulted in a push to produce foods with specific beneficial effects, such as functional foods.

The aim of the present study was to determine the effect of spontaneous and inoculated (with *Lactobacillus plantarum*) fermentation on the phenolic composition of cowpea flours, and the evaluation of the antioxidant activity, in order to determine the optimal conditions for obtaining flours with higher functionality.

EXPERIMENTAL

Samples

Cowpeas (*Vigna sinensis* L var Carilla) (RB) were purchased from a wholesale market for use in the fermentation trials.

Preparation of cultures

The lactic acid bacterium *Lactobacillus plantarum* CECT 748 (ATCC 14917) was obtained from the Spanish Type Culture Collection (CECT) (Valencia, Spain). Stock cultures were grown and maintained on MRS agar (Difco, Detroit, MI, USA). Cultures were transferred from slants to MRS broth (Difco) and incubated for 24 h at 37 °C. The cells were washed twice with sterile saline solution (0.8% NaCl) and used as inoculum.

Fermentation

Cowpea seeds were washed three times with sterile distilled water under aseptic conditions and dried at 55 °C for 24 h. After drying, samples were ground in a ball mill (Glen Creston Ltd, Stanmore, UK), sieved and the 0.050–0.250 mm fraction was collected (HB).

Cowpea flour fermentation at the fermentor scale was carried out by suspending 900 g of flour (HB) in 3 l of sterile distilled water prepared aseptically. These suspensions were fermented, spontaneously, only with the micro-organisms present on the seeds (spontaneous fermentation, SFB), or inoculated with a 10% (v/v) inoculum representing 10^8 cells ml^{-1} of *Lactobacillus plantarum* CECT 748 (*L plantarum* fermentation, LFB) at 37 °C for 48 h, in a 5 l stirred fermentor (Infors ISF-100, Infors AG, Bottmingen, Switzerland) at 450 rpm. After fermentation the samples were freeze-dried as a whole.

The fermented cowpea flours (SFB and LFB) were heated in solid state and dry conditions (6% water content) as normal legume flours in an autoclave for 20 min at 121 °C in sealed containers. The quantity of autoclaved flour was 450 g each time. After heat treatment the samples were freeze-dried (HSFB and HLFB).

Extraction of phenolic compounds

The cowpea flours (10 g) corresponding to the different samples (RB, HB, SFB, LFB, HSFB and HLFB) were macerated with 3 × 80 ml of a solution of methanol-HCl (1‰)/water (80:20 v/v) following the method of Dueñas *et al*.[10] An aliquot of this methanol solution (200 ml) was extracted three times with diethyl ether and three times with ethyl acetate, and organic solutions were combined and dried with anhydrous Na_2SO_4 and evaporated to dryness under vacuum. The residue, dissolved in methanol/water (1:1 v/v), was analysed by high-performance liquid chromatography (HPLC). All samples were filtered through a 0.45 μm cellulose acetate filter (Millipore, Molsheim, France) before HPLC analysis. The samples were prepared and extracted in triplicate.

HPLC/PAD analysis

The chromatographic system was equipped with an autoinjector, a quaternary pump, a 2001 photodiode array detector (Waters, Milford, MA, USA) and a Nova-Pak (Waters, Milford, MA, USA) C_{18} column (300 mm × 3.9 mm, 4 μm). The conditions of analysis were those of Dueñas *et al*.[10] Two mobile phases were employed for elution: A, water/acetic acid (98:2 v/v); B, water/acetonitrile/acetic acid (78:20:2 v/v/v). The gradient profile was: 0–55 min, 100–20% A; 55–70 min, 20–10% A; 70–80 min, 10–5% A; 80–90 min, 100% B. The flow rate was 1 ml min^{-1} up to 55 min and 1.2 ml min^{-1} thereafter. The column was re-equilibrated between injections with 10 ml of acetonitrile and 25 ml of the initial mobile phase. Detection was performed by scanning from 210 to 400 nm with an acquisition speed of 1 s. A volume of 25 μl was injected. The samples were analysed in triplicate.

HPLC/MS analysis

Mass spectra were obtained using a Hewlett Packard 1100MS chromatograph (Palo Alto, CA, USA) equipped with an API source, using an ESI interface. The solvent gradient and column used were the same as for HPLC/PAD but with a flow rate of 0.7 ml min^{-1}. ESI conditions were as follows: negative mode; nitrogen as the nebulising pressure, 40 psi, drying gas, 10 l min^{-1} at 340 °C; voltage at capillary entrance, 4000 V; variable fragmentation voltage, 100 V ($m/z < 200$), 200 V (m/z 200–1000), 250 V (m/z 1000–2500). Mass spectra were recorded from m/z 100 to 2500.

Identification and quantification of compounds

Chromatographic peaks were identified by comparison of the retention times, UV spectra and data of UV spectral parameters[28,29] with those of standards. The standards gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, *trans*-*p*-coumaric and *trans*-ferulic acids, tyrosol and hydroxymethylfurfuraldehyde were from Aldrich Chimie (Munich, Germany) and the standards quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside and quercetin were from Extrasynthese (Genay, France). Other compounds for which no standards were available, such as hydroxycinnamic acid derivatives, were identified and confirmed by HPLC/PAD and HPLC/MS (ESI).

Quantification was carried out using the external standard method at 280 and 340 nm according to the maximum absorption of each compound. Calibration curves were constructed by injecting different volumes from the stock solutions (0.25 μg ml^{-1} for phenolic acids and 0.10 μg ml^{-1} for flavonoids) over the range of concentrations observed for each of the compounds, using a linear regression for the relationship of area sum versus concentration, under the same conditions as for the samples analysed. The hydroxycinnamic acid derivatives were quantified using the calibration curve of the corresponding free acid. Quercetin diglycoside and quercetin diglycoside acylated with ferulic acid were quantified using the curve of the corresponding quercetin 3-*O*-glucoside.

Antioxidant activity

The antioxidant activity (IC_{50}) was determined in methanol solution by the method of Brand-Williams *et al*[30] with 2,2′-diphenyl-1-picrylhydrazil (DPPH). The percentage of remaining DPPH was plotted against the sample concentration to obtain the amount of antioxidant (mg of legume flour) necessary to decrease the absorbance by 50%. A lower IC_{50} value corresponds to a higher antioxidant activity.

Statistical analysis

Analyses were performed in triplicate and data are presented as mean ± standard deviation (SD). Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Statgraphics Plus 5.0 (Graphics Software System, Rockville, MD, USA).

RESULTS AND DISCUSSION

Phenolic composition of raw cowpeas

Fig 1 shows the chromatogram of the raw cowpea (RB), in which has been identified a total of 26 phenolic compounds, including hydroxybenzoic acids gallic (peak 1), protocatechuic (peak 3), *p*-hydroxybenzoic (peak 8) and vanillic (peak 12), and hydroxycinnamic acids in free form, as *trans*- and *cis*-*p*-coumaric (peaks 17 and 19) and *trans*- and *cis*-ferulic (peaks 21 and 23). These compounds were identified by comparison of retention times and UV spectra with those of standards and confirmed by HPLC/MS (ESI) analysis (Table 1).

In addition to the free hydroxycinnamic acids, some esterified hydroxycinnamic acids have been identified. Peaks 2, 5, 7, 11 and 13 showed a UV spectrum similar to that of *trans*-*p*-coumaric acid (Table 1). In the analysis by HPLC/MS (Fig 2A), these peaks presented a negative molecular ion $[M-H]^-$ at *m*/*z* 355.1 corresponding to an aldaric acid (galactaric or glucaric acid) linked to *p*-coumaric acid, and two fragment ions, $[M-H]^-$ at *m*/*z* 163.1 corresponding to a *p*-coumaric acid residue, and $[M-H]^-$ at *m*/*z*

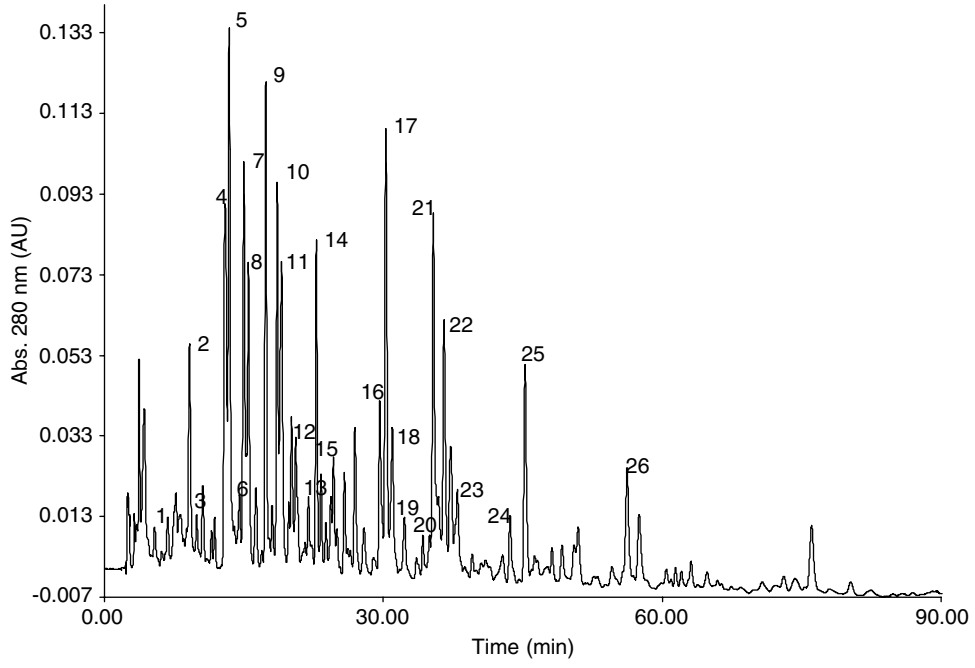

Figure 1. Chromatogram at 280 nm of raw cowpea (peak numbers correspond to those of Table 1).

Table 1. Spectral data of compounds identified in raw cowpea (from HPLC analysis)

Peak no	Compound	λ_{max} (nm)	$[M-H]^-$ (*m/z*)
1	Gallic acid	271.7	169.1
2	*trans*-*p*-Coumaroylaldaric acid	312.0	355.0
3	Protocatechuic acid	259.9–294.2	153.1
4	*trans*-Feruloylaldaric acid	325.1	385.0
5	*trans*-*p*-Coumaroylaldaric acid	312.0	355.1
6	*trans*-Feruloylaldaric acid	326.3	385.0
7	*trans*-*p*-Coumaroylaldaric acid	313.2	355.0
8	*p*-Hydroxybenzoic acid	255.2	136.9
9	*trans*-Feruloylaldaric acid	326.3	385.0
10	*trans*-Feruloylaldaric acid	326.3	385.0
11	*trans*-*p*-Coumaroylaldaric acid	313.2	355.0
12	Vanillic acid	261.1–293.0	167.1
13	*trans*-*p*-Coumaroylaldaric acid	313.2	355.0
14	*trans*-Feruloylaldaric acid	325.1	385.0
15	*trans*-Feruloylaldaric acid	326.3	385.4
16	*trans*-Feruloyl-methylaldaric acid	327.5	399.1
17	*trans*-*p*-Coumaric acid	309.6	162.9
18	*trans*-Feruloyl-methylaldaric acid	327.5	399.1
19	*cis*-*p*-Coumaric acid	295.4	162.9
20	Quercetin diglycoside	256.3–352.5	625.3
21	*trans*-Ferulic acid	322.7	193.1
22	Myricetin 3-*O*-glucoside	261.1–354.9	479.2
23	*cis*-Ferulic acid	310.8	193.1
24	Quercetin 3-*O*-galactoside	256.3–353.7	463.2
25	Quercetin 3-*O*-glucoside	256.3–353.7	463.1
26	Quercetin feruloyl-diglycoside	252.8–270(sh)–297(sh)–333.4	801.3

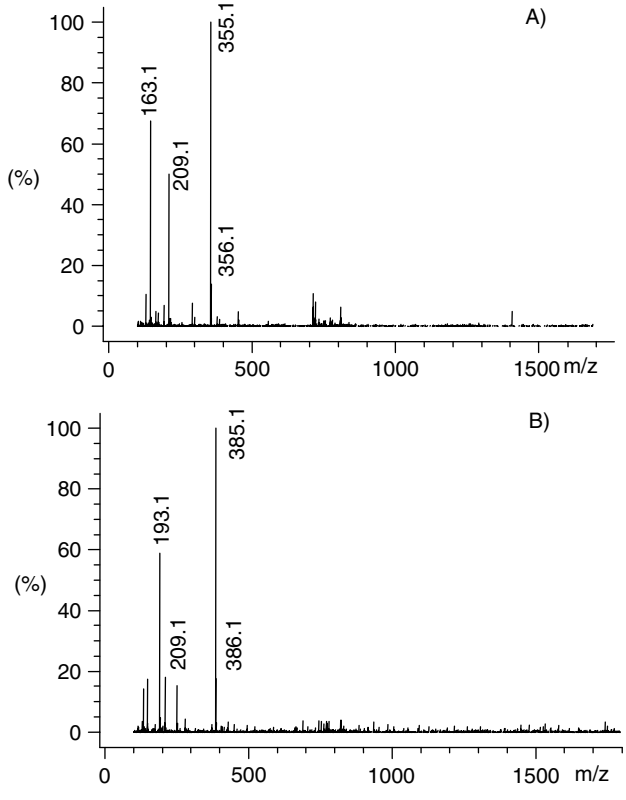

Figure 2. ESI mass spectra of *p*-coumaric (A) and ferulic (B) ester with aldaric acid.

209.1 corresponding to an aldaric acid residue. These compounds have been identified as isomeric forms of *trans*-*p*-coumaroylaldaric acid (Fig 3).

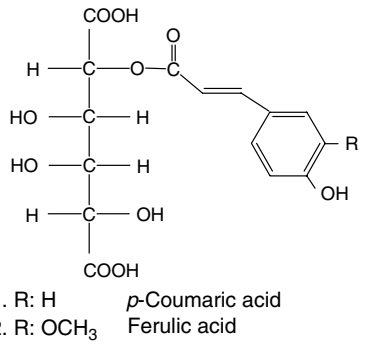

Figure 3. 2′-(*E*)-*O*-*p*-Coumaroylgalactaric acid (**1**) and 2′-(*E*)-*O*-feruloylgalactaric acid (**2**).

Peaks 4, 6, 9, 10, 14 and 15 showed a UV spectrum similar to that of ferulic acid (Table 1). In the HPLC/MS (ESI) analysis (Fig 2B), these peaks presented a negative molecular ion $[M-H]^-$ at *m/z* 385.1 corresponding to an aldaric acid (galactaric or glucaric acid) linked to ferulic acid, and two fragment ions, $[M-H]^-$ at *m/z* 193.1 corresponding to a ferulic acid residue, and $[M-H]^-$ at *m/z* 209.1 corresponding to an aldaric acid residue. These compounds have been identified as isomeric forms of *trans*-feruloylaldaric acid (Fig 3).

In the HPLC/MS (ESI) analysis, peaks 16 and 18 showed a molecular ion $[M-H]^-$ at *m/z* 399.1 corresponding to a feruloylaldaric acid derivative, with an $-OCH_3$ group instead of an $-OH$ group, and also a fragment ion $[M-H]^-$ at *m/z* 193.0 corresponding to cleavage of the aldaric acid moiety. These peaks have

been identified as isomeric forms of *trans*-feruloyl-methylaldaric acid (Table 1).

It is probable that the identified compounds, *trans-p*-coumaric or *trans*-ferulic acids conjugated with aldaric acid, correspond to different open chain isomeric forms resulting from positional isomerism, which occurs with esters of phenolic acids with polyhydroxy compounds.[31]

Cai *et al*[2] identified protocatechuic and *p*-hydroxybenzoic acids in 17 varieties of cowpeas, together with *trans-p*-coumaric and *trans*-ferulic acids, and found that these last hydroxycinnamic acids were the most abundant phenolics in these varieties. We have not found references about the presence of the compounds conjugated with aldaric acid in cowpeas. These conjugated cinnamates have been reported in orange peels[32,33] and leaves of rye (*Secale cereale*).[34] The presence of hydroxycinnamic acid conjugates with organic acids was also observed in other legumes, for example, *trans-p*-coumaric acid esterified with malic and glycolic acids in the cotyledon of lentil.[10] Cai *et al*[2] deduced the presence of some esterified feruloyl compounds in cowpeas by the increase in ferulic acid concentration after alkaline hydrolysis.

Several glycosides of quercetin and myricetin were identified in the analysis by HPLC/MS (Table 1). Peak 20 showed a negative molecular ion $[M-H]^-$ at *m/z* 625.3 corresponding to quercetin linked to a disaccharide (hexose+hexose), and a fragment ion $[M-H]^-$ at *m/z* 301.2 corresponding to a quercetin residue. Peak 22 showed a molecular ion $[M-H]^-$ at *m/z* 479.2 from myricetin 3-*O*-glucoside, and a fragment ion $[M-H]^-$ at *m/z* 317.0 corresponding to a myricetin residue. Peaks 24 and 25 showed a molecular ion $[M-H]^-$ at *m/z* 463.2 corresponding to quercetin linked to one hexose, and a fragment ion $[M-H]^-$ at *m/z* 301.1 from a quercetin aglycone. These two compounds have been confirmed as quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside by comparison of retention times and UV spectra with those of corresponding standards.

Peak 26 showed a UV spectrum whose shape and characteristics were those of a quercetin glycoside. The analysis by HPLC/MS showed a molecular ion $[M-H]^-$ at *m/z* 801.3 corresponding to a quercetin diglycoside linked to ferulic acid, and a fragment ion $[M-H]^-$ at *m/z* 193.1 corresponding to a ferulic acid residue. These data agree with the identification of acylated flavonol glycosides reported in cabbage leaves.[35] No data on the presence of these compounds in legumes were found.

Changes in phenolic composition during fermentation

Before fermentation the cowpea seeds were washed and dried (HB), consequently, their phenolic composition was slightly modified (Table 2). The free hydroxycinnamic acids *trans*-ferulic and *cis*- and *trans-p*-coumaric increased from 4.54% in raw cowpeas (RB) to 9.99% in washed and dried cowpeas (HB). The conjugated forms decreased slightly, from 33.77 to 26.35% for feruloyl derivatives and from 14.40 to 10.99% in the case of *p*-coumaric derivatives. Other authors also observed a decrease after soaking in the concentration of phenolic compounds[36] and other components such as carbohydrates, phytates, etc.[37]

In the cowpea flours obtained after spontaneous (SFB) and *L plantarum* (LFB) fermentation, the same compounds as identified in the raw sample (RB) were found, namely hydroxybenzoic and hydroxycinnamic compounds and quercetin and myricetin glycosides (Table 2). However, fermentation also gave rise to some phenolic compounds not detected in the raw flour, such as tyrosol, a compound generally produced as a consequence of the fermentation process, and quercetin (Table 2). These two compounds were much more abundant after inoculated than spontaneous fermentation. The decrease in some quercetin glycosides, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside, could be the origin of the strong increase in quercetin (Table 2). According to Sotomayor *et al*[38] and Reddy *et al*,[39] the microorganisms participating in natural fermentation produce a consistent pH lowering, which could activate some enzymes that hydrolyse the quercetin glycosides, thus yielding quercetin.

In relation to HB content, both an increase in *p*-hydroxybenzoic, vanillic and protocatechuic acids and a general decrease in gallic acid were observed. Most hydroxycinnamic derivatives underwent a decrease after both types of fermentation (SFB and LFB). After spontaneous fermentation we observed an increase in the free acids with respect to RB, but after fermentation with *L plantarum* a decrease in *trans-p*-coumaric and *cis*-ferulic acids was seen (Table 2). It has been suggested that the *L plantarum* strain imposes a phenolic acid decarboxylase (PAD) activity on *p*-coumaric and ferulic acids,[40] bringing about a decrease in these acids. From our results it seems that this activity could depend on the isomeric forms of the acids.

Heat treatment after both fermentation processes (HSFB and HLFB) produced hydroxymethylfurfuraldehyde (Table 2), a compound generally associated with the action of high temperature as consequence of Maillard's reaction, which was absent from the fermented samples that had not undergone heat treatment. An increase in the majority of hydroxycinnamic derivatives also took place. Free *trans-p*-coumaric and *trans*-ferulic acids increased greatly with the autoclaving treatment, after both natural and inoculated fermentation, while a decrease in *cis*-ferulic acid was observed.

Antioxidant activity

No changes in the antioxidant activity in RB and HB were observed (Table 3) by the evaluation with DPPH. Fermentation, both spontaneous (SFB) and inoculated (LFB), produced a slight increase in this activity. The increase was more marked when the

Table 2. Composition ($\mu g\, g^{-1}$) of phenolic compounds in raw and fermented cowpeas

Compound	RB	HB	SFB	HSFB	LFB	HLFB
Galllic acid	0.16 ± 0.08^{b}	0.21 ± 0.09^{b}	ND[a]	ND[a]	ND[a]	ND[a]
Hydroxymethylfurfuraldehyde	ND[a]	ND[a]	ND[a]	8.10 ± 0.09^{b}	ND[a]	10.00 ± 0.24^{c}
trans-p-Coumaroylaldaric acid	1.93 ± 0.10^{c}	1.48 ± 0.16^{b}	ND[a]	ND[a]	ND[a]	ND[a]
Protocatechuic acid	1.21 ± 0.07^{ab}	1.11 ± 0.04^{a}	1.80 ± 0.02^{c}	1.63 ± 0.00^{abc}	1.66 ± 0.03^{bc}	1.62 ± 0.48^{bc}
Tyrosol	ND[a]	ND[a]	6.98 ± 0.08^{b}	14.68 ± 0.11^{c}	89.42 ± 3.78^{e}	79.16 ± 1.92^{d}
trans-Feruloylaldaric acid	4.01 ± 0.15^{d}	3.42 ± 0.37^{c}	1.10 ± 0.19^{b}	ND[a]	ND[a]	ND[a]
trans-p-Coumaroylaldaric acid	3.66 ± 0.20^{c}	2.65 ± 0.85^{b}	2.73 ± 0.59^{b}	2.50 ± 0.30^{b}	1.29 ± 0.27^{a}	1.87 ± 0.19^{a}
trans-Feruloylaldaric acid	1.03 ± 0.07^{c}	0.64 ± 0.03^{b}	ND[a]	ND[a]	ND[a]	ND[a]
trans-p-Coumaroylaldaric acid	4.47 ± 0.10^{d}	1.75 ± 0.09^{c}	1.27 ± 0.02^{b}	1.64 ± 0.13^{c}	1.03 ± 0.26^{b}	0.74 ± 0.09^{a}
p-Hydroxybenzoic acid	4.49 ± 0.13^{a}	3.60 ± 0.63^{a}	4.65 ± 0.16^{a}	6.40 ± 0.67^{b}	13.66 ± 0.73^{c}	12.80 ± 0.58^{c}
trans-Feruloylaldaric acid	7.21 ± 1.23^{d}	4.94 ± 0.39^{c}	2.84 ± 0.02^{b}	3.03 ± 0.01^{b}	2.61 ± 0.31^{b}	1.06 ± 0.03^{a}
trans-Feruloylaldaric acid	5.90 ± 0.99^{d}	2.86 ± 0.73^{b}	2.47 ± 0.11^{ab}	4.76 ± 0.37^{c}	2.70 ± 0.14^{b}	1.79 ± 0.19^{a}
trans-p-Coumaroylaldaric acid	1.59 ± 0.21^{a}	1.92 ± 0.39^{a}	1.87 ± 0.10^{a}	2.70 ± 0.37^{b}	1.78 ± 0.51^{a}	1.67 ± 0.04^{a}
Vanillic acid	2.51 ± 0.87^{a}	1.99 ± 0.19^{a}	8.84 ± 0.02^{c}	10.14 ± 0.02^{c}	4.44 ± 1.00^{b}	2.01 ± 0.69^{a}
trans-p-Coumaroylaldaric acid	0.54 ± 0.10^{ab}	0.39 ± 0.01^{a}	0.42 ± 0.00^{a}	0.72 ± 0.00^{b}	1.05 ± 0.10^{c}	2.37 ± 0.15^{d}
trans-Feruloylaldaric acid	4.33 ± 0.01^{d}	3.45 ± 0.97^{b}	2.49 ± 0.02^{a}	3.67 ± 0.06^{bc}	2.21 ± 0.33^{a}	3.63 ± 0.16^{bc}
trans-Feruloyl-methylaldaric acid	1.02 ± 0.82^{a}	0.84 ± 0.03^{a}	1.00 ± 0.02^{a}	0.74 ± 0.06^{a}	1.62 ± 0.73^{a}	4.09 ± 0.04^{b}
trans-Feruloyl-methylaldaric acid	2.94 ± 0.51^{c}	2.14 ± 0.50^{b}	1.49 ± 0.06^{a}	1.68 ± 0.07^{a}	1.63 ± 0.29^{a}	1.79 ± 0.23^{a}
trans-p-Coumaric acid	0.86 ± 0.04^{a}	2.64 ± 0.27^{ab}	3.11 ± 0.01^{c}	4.47 ± 0.02^{d}	0.70 ± 0.12^{a}	2.43 ± 0.54^{b}
trans-Feruloyl-methylaldaric acid	2.15 ± 0.15^{c}	1.34 ± 0.31^{b}	1.26 ± 0.00^{b}	1.42 ± 0.00^{b}	ND[a]	ND[a]
cis-p-Coumaric acid	0.14 ± 0.01^{ab}	0.49 ± 0.08^{d}	0.88 ± 0.00^{e}	ND[a]	0.32 ± 0.17^{cd}	0.30 ± 0.01^{bc}
Quercetin diglycoside	1.18 ± 0.07^{a}	0.84 ± 0.14^{a}	0.77 ± 0.19^{a}	1.31 ± 0.04^{a}	4.14 ± 0.96^{b}	4.37 ± 0.32^{b}
trans-Ferulic acid	1.60 ± 0.07^{a}	3.20 ± 0.19^{b}	6.14 ± 0.00^{d}	8.01 ± 0.00^{e}	4.10 ± 0.14^{c}	8.64 ± 0.29^{f}
Myricetin 3-*O*-glucoside	9.64 ± 0.78^{d}	10.62 ± 1.05^{d}	1.10 ± 0.02^{b}	ND[a]	2.12 ± 0.62^{c}	ND[a]
cis-Ferulic acid	1.24 ± 0.09^{e}	1.11 ± 0.04^{d}	0.98 ± 0.01^{c}	0.55 ± 0.00^{b}	0.36 ± 0.02^{a}	0.39 ± 0.02^{a}
Quercetin 3-*O*-galactoside	3.64 ± 0.10^{e}	3.46 ± 0.24^{e}	1.98 ± 0.12^{c}	2.61 ± 0.14^{d}	1.60 ± 0.08^{b}	ND[a]
Quercetin 3-*O*-glucoside	11.45 ± 1.82^{c}	11.17 ± 1.18^{c}	0.39 ± 0.06^{a}	ND[a]	1.93 ± 0.06^{b}	0.89 ± 0.09^{ab}
Quercetin feruloyl-diglycoside	5.76 ± 0.10^{c}	6.22 ± 0.17^{c}	7.69 ± 0.01^{d}	8.13 ± 0.29^{d}	3.85 ± 0.53^{b}	ND[a]
Quercetin	ND[a]	ND[a]	11.10 ± 0.14^{b}	11.27 ± 0.43^{b}	22.02 ± 0.40^{c}	23.49 ± 0.36^{d}

ND, not detected. Values are mean ± SD ($n = 3$); means followed by the same letter in a row are not significantly different (LSD, 5%). RB, raw; HB, dried at 55 °C; SFB, spontaneous fermentation; HSFB, autoclave after spontaneous fermentation; LFB, *L plantarum* fermentation; HLFB, autoclave after *L plantarum* fermentation.

Table 3. Antioxidant activity (mg of sample) of cowpeas before and after fermentation

	RB	HB	SFB	HSFB	LFB	HLFB
IC_{50}	9.44 ± 0.02	9.47 ± 0.01	9.02 ± 0.05	5.06 ± 0.00	8.89 ± 0.02	6.44 ± 0.04

Values are ± SD ($n = 3$). RB, raw; HB, dried at 55 °C; SFB, spontaneous fermentation; HSFB, autoclave after spontaneous fermentation; LFB, *L plantarum* fermentation; HLFB, autoclave after *L plantarum* fermentation.

fermented flours were sterilised in the autoclave, and it seems that the spontaneously fermented sample that had undergone a later autoclaving treatment (HSFB) had the greatest antioxidant activity. This therefore appears to be the most convenient treatment from the point of view of this activity.

The increase in antioxidant activity after autoclaving (HSFB and HLFB) (Table 3) could be associated with the high temperature of this process leading to the formation of new compounds, mainly hydroxymethylfurfuraldehyde, as a consequence of Maillard's reaction, compounds that produce high antioxidant activity, as was observed by Piga *et al*[41] in the plum-drying process.

CONCLUSIONS

The Carilla variety of cowpea is a cheap bean whose consumption is not much appreciated. From the results obtained in this study, fermentation of cowpea flours seems to be an adequate and effective process for increasing their nutritional and biological quality,

owing to the improvement in phenolic compound concentration and the increase in antioxidant activity, resulting in foods with higher functionality.

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