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Determination of antioxidants in edible grain derivatives from the Canary Islands by capillary electrophoresis

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Abstract

A preliminary study of the antioxidant content of hand-produced grain derivatives from the Canary Islands, named gofio, is developed by means of a capillary electrophoresis (CE) method. Protocatechuic acid, salicylic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid were extracted from corn, wheat, barley and pea gofio samples by sonication with methanol and were successfully separated by CE using a polycation, hexadimetrine bromide (HDB), as electrosmotic flow modifier. The separation was achieved using a running buffer consisting of 125 mM boric acid, 49 mM disodium hydrogen phosphate, 0.002% (w/v) HDB and 2.5 mM α-cyclodextrin at pH 7.5. The developed procedure allowed the simultaneous determination of the selected antioxidants in less than 3.5 min. The electrophoretic profile obtained for each kind of flour (corn, wheat, barley and pea) allowed differentiation of the type of gofio. Among the analyzed samples, corn gofio showed the highest antioxidant content. Furthermore, highly roasted corn or wheat gofio samples were also analyzed and their antioxidant contents were lower than those of less roasted samples.

Keywords: Antioxidants; Roasted flour; Gofio; Canary Islands; Capillary electrophoresis

1. Introduction

Phenolic compounds are bioactive substances with antioxidant properties commonly found in food plants (Ho et al., 1992). Many of these compounds exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions (Cook & Samman, 1996). Some biological functions, such as antimutagenicity, anticarcinogenicity and antiaging are directly related to the consumption of a diet of high antioxidant content. So, it is well known that the Mediterranean diet which is rich in natural plant antioxidants, leads to a limited incidence of cerebral and cardiovascular diseases. Due to these interesting biological properties, their determination is important in nutrition, medicine or health (Buiarelli, Cartoni, Coccioli, & Levetsovitoum, 1995; Delage, Bohuon, Baron, & Drilleau, 1991; Klejdus & Kubán, 2000; Perry, Burgess, & Glennie, 2001; Shahidi & Naczk, 1995; Zieli'nski, Kozlowska, & Lewczuk, 2001). Recently, the determination of these compounds has been restricted to wine, tea or different plant extracts.

Cereal grains contain a large variety of substances, especially biologically active types, including antioxidants, dietary fibre, phytoestrogens, and lignans. The cereals of primary economic importance include wheat, corn, rice, barley, sorghum, oat, millet and rye. There

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are several studies (Miller, Rigelhof, Maguart, Prakash, & Kanter, 2000) that document the antioxidant potential of grain foods, particularly whole grain foods. The data indicate that cereals are rich in phenolic acids (Senter, Horvat, & Forbus, 1983; Zieli'nski et al., 2001). Moreover, ferulic, vanillic and *p*-coumaric acids are the major functional antioxidants in the whole grain. The free forms of salicylic, *p*-hydroxybenzoic, vanillic, protocatechuic, *p*-coumaric, syringic, ferulic and sinapic acids have also been identified in barley grains (Shahidi & Naczk, 1995). Gofio has been a typical food produced and used in the Canary Islands since pre-Hispanic times. It consists of roasted flours, alone or mixed in variable proportions of corn, wheat, barley or even legumes such as pea. Nowadays, the roasting procedure normally consists of 1 min 15 s at 300 °C of the whole grains. There are also highly roasted flours in which a two-step procedure takes place, consisting of 1 min 15 s at 300 °C each (between both procedures the grains are cooled). Gofio has nowadays gained significant importance in Canarian gastronomy. It has good nutritive properties, including antioxidant effects and, as a result, is even exported to the North of Africa. Unfortunately, few data have been published on Canary Island gofio antioxidant content.

The chromatographic technique most commonly applied to the determination of phenolic acids in foods, plant sediments and plant extracts (Arlorio, Coïson, & Martelli, 2000; Buiarelli et al., 1995; Chen & Adams, 1999; Delage et al., 1991; Klejdus & Kubán, 2000; Lobo, Mozeto, & Cass, 2000; Perry et al., 2001) is high performance liquid chromatography (HPLC) under reversed-phase or ion-exclusion conditions. However, capillary electrophoresis (CE) appears to be a complementary technique to HPLC or ion chromatography (IC) for the determination of these compounds, due to its special characteristics, such as separation efficiency, short analysis time and simplicity. Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are the two main modes used in CE for their determination (Chen, Krishnamurti, & Naidu, 2001; Pietta, Mauri, & Bauer, 1998; Pomponio, Gotti, Hudaib, & Cavrini, 2002; Schmitt-Koplin, Garrison, Perdue, Freitag, & Kettrup, 1998; Sheu, Chieh, & Weng, 2001; Zhao & Lunte, 1999). However, in order to obtain high-speed electrophoretic separations of anionic compounds, the electroosmotic flow (EOF) can be reversed with cationic surfactants added to the buffer electrolyte, such as, for instance, 1,5-dimethyl-1,5 diazaundecamethylene polymethobromide (hexadimetrine bromide, HDB), cetyltrimethylammonium bromide or chloride (CTAB and CTAC, respectively), which causes a fast migration as the anionic analytes co-migrate with the EOF (coelectroosmotic separations) (Masselter & Zemann, 1995; Volgger, Zemann, Bonn, & Antal, 1997).

The aim of the present work is to begin a preliminary study of the antioxidant content in different types of roasted flours from the Canary Islands, named gofio, by means of a capillary electrophoresis method, using HDB as EOF modifier. For this purpose, protocatechuic, salicylic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic and sinapic acids are determined in corn, wheat, barley and pea gofio samples.

2. Materials and methods

2.1. Apparatus

All CE analyses were carried out in a P/ACE 5510 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a diode array detector. The fused-silica capillaries were purchased from Composite Metal Services (Worcester, England) with 75 μm i.d., 47 cm of total length and 40 cm of effective length. Injections were made at the anodic end using a N_2 pressure of 0.5 psi for 2 s (1 psi = 6894.76 Pa). System GOLD software from Beckman was used to control the instrument. All measurements were carried out at 25 °C. Diode array detection was used over the range of 190–600 nm to obtain spectral data. Detection took place at 280 nm. Peak identification was achieved by comparing both migration time and spectral data obtained from real samples and standards. Besides, in order to assure the identification of the selected compounds, real samples were spiked with increasing amounts of each standard and injected into the PACE system.

2.2. Chemicals

All chemicals were of analytical reagent grade and used as received. 1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide (hexadimethine bromide, HDB) and α-cyclodextrin were from Aldrich (Milwaukee, WI, USA). Boric acid, disodium hydrogen phosphate, hydrochloric acid and sodium hydroxide from Merck (Darmstadt, Germany) were used to prepare CE running buffers. Methanol for preparation of standard solutions was obtained from Merck. All buffer solutions were prepared by dissolving them in ultra pure water from a Milli-Q gradient system A10 (Millipore, Bedford, MA, USA) with a conductivity of 18 MΩ.

Protocatechuic acid (**1**), salicylic acid (**2**), *p*-hydroxybenzoic acid (**3**), vanillic acid (**4**), syringic acid (**5**), *p*-coumaric acid (**6**), ferulic acid (**7**) and sinapic acid (**8**) were obtained from Aldrich. Stock solutions of these compounds (200 mg/l) were prepared by dissolving the appropriate amount in HPLC grade methanol. These solutions were wrapped in aluminium foil and stored at 4 °C. Working solutions were daily prepared by diluting the stock solutions with ultra pure water.

2.3. Capillary electrophoresis conditions

Separation electrolytes were prepared each week by adding appropriate aliquots of 0.2 M sodium tetraborate, 0.2 M phosphate, 10 mM α-cyclodextrin and HDB (0.2%, w/v) to Milli-Q water. The pH of the buffer solutions was adjusted by adding aliquots of 1.0 M sodium hydroxide or 1.0 M of hydrochloric acid and measured by a pH-meter (Profiline pH 197-S WTW). The final composition of the buffer was 125 mM borate, 48.6 mM phosphate, 0.002% (w/v) HDB, 2.5 mM α-CD and pH 7.5. The buffers were stored at 4 °C and warmed to room temperature before use.

Initially, a new capillary was preconditioned by rinsing with 1 M NaOH for 30 min, followed by a 15 min rinse with Milli-Q water. At the beginning of each day the capillary was washed with 1.0 M sodium hydroxide for 10 min and water for 5 min. Between injections, the next washing protocol was applied: 1.0 M sodium hydroxide for 1.0 min, separation electrolyte from one vial, 1.0 min, separation electrolyte from another vial, 2 min, and analysis which was less than 5 min under the optimum conditions. At the end of each day, the capillary was rinsed with deionized water for 5 min and N_2 for 1 min. In the reproducibility study, the capillary was conditioned every day by running four consecutive separations with the running buffer as injected sample. Triplicate injections of the solutions were performed and corrected average peak areas (area/migration time) were used for the quantification.

2.4. Sample analysis

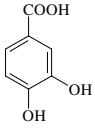
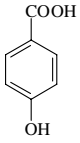
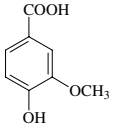
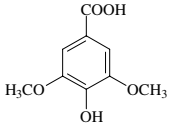
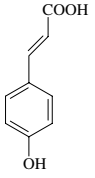
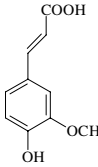
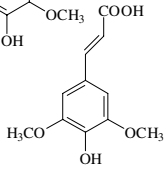
Different gofio samples (corn, wheat, barley and pea) were obtained from several supermarkets. After the samples were collected, they were stored in the refrigerator at 4 °C in order to maintain their original characteristics. Four grammes lots of cereal samples were weighed out and extracted with 18 ml of methanol in an ultrasonic bath for 10 min. After sonication, samples were centrifuged for 5 min (3000 rpm) and liquid phases were filtered through 0.45 μm filter (Millipore Corporation, Bedford, MA, USA). Liquid phases were taken to dryness in a rotary evaporator and redissolved in 1 ml of methanol. An aliquot ($t = 2$ s ≈ 2.5 nl) was injected into the P/ACE system.

3. Results and discussion

3.1. Capillary electrophoresis method

The selected antioxidants (protocatechuic, salicylic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic and sinapic acids) were chosen according to their presence in cereal samples (Ho et al., 1992). In order to develop a fast capillary electrophoresis method for their separation, a buffer containing a polycation, HDB, was used to reverse the EOF. The use of HDB causes a fast migration as the anionic analytes co-migrate with the EOF allowing shortening of analysis time (Masselter & Zemann, 1995). Buffer pH is an important factor that has to be taken into account for the separation of these compounds. According to the pK_a of the selected antioxidants (see Table 1 for pK_a values and chemical structures), pH values above 7 should be used in the separation of these compounds. After testing buffers with different pHs and compositions, the best separation was obtained at pH 7.5, using borate and phosphate as background electrolyte, together with HDB. However,

Table 1
Structures and ionization constants of the selected polyphenolic acids

Phenolic acid	Structure	pK_{a1}	pK_{a2}
Protocatechuic acid[a]		4.26	8.64
Salicylic acid[a]		2.99	12.95
p-Hydroxybenzoic acid[a]		4.50	9.11
Vanillic acid[a]		4.51	9.39
Syringic acid[a]		4.34	9.42
p-Coumaric acid[a]		4.64	9.45
Ferulic acid[a]		4.52	9.39
Sinapic acid[b]		4.47	9.21

[a] Serjeant and Dempsey (1979).
[b] Smyk and Drabent (1989).

as several peaks were overlapped, α-cyclodextrin, which has so far been demonstrated to be a buffer modifier, not only used in chiral separations, was added to the separation buffer. When relatively low amounts of α-cyclodextrin were used, a good separation in terms of selectivity and analysis time was achieved after an appropriate optimization of the selected buffer components. Optimum conditions of the separation were the following: 125 mM borate, 49 mM phosphate, 0.002% (w/v) HDB, 2.5 mM α-CD at pH 7.5. Fig. 1 shows the electropherogram of the separation of a standard solution of these compounds under the optimized conditions. As can be seen, the use of this buffer provided baseline resolution for all compounds within a relatively short analysis time (i.e., less than 3.5 min) and high efficiencies ($\geqslant$ 450,000 number of theoretical plates per metre).

The developed method was applied to the determination of phenolic compounds in different roasted flours samples (gofio) from the Canary Islands. However, when dealing with real samples, analysis time became longer in subsequent injections and a new washing protocol was necessary to provide reproducible EOF between injections. Consequently, a new washing protocol, consisting of 2 min washing with methanol, 1 min with 1 M NaOH, 1 min rising with the buffer, 2 min buffer fill (from a different vial) and 5 min analysis, provided very good reproducibility. In order to test the reproducibility of the method, spiked gofio samples were previously extracted with methanol in an ultrasonic bath, as described in Section 2, and injected instead of the standard mixture. These intra-day and inter-day reproducibility studies were carried out at three different concentrations (10, 60 and 100 mg/l). Intra-day precision was assessed by four replicates per concentration in one day ($n = 4$), while inter-day precision was measured for four replicates per concentration on three consecutive days ($n = 12$). Inter-day precision relative standard deviation (RSD) values for peak areas were below 6.5% for all compounds and below 5.4% for the intra-day precision. Calibration graphs were obtained by plotting concentration (mg/l) in the range 0.1–100 mg/l, against corrected peak areas (peak area/migration time), showing excellent correlation coefficients ($\geqslant$ 0.997) for all compounds. Table 2 shows the calibration data of the selected antioxidants. Limits of detection (LODs), calculated as three times the signal to noise ratio, ranged between 0.05 mg/l for protocatechuic acid and 0.07 mg/l for *p*-hydroxybenzoic and sinapic acids. Fig. 2 shows the electropherogram of a corn gofio sample spiked with the studied antioxidants. As can be seen, a good separation in terms of migration time and resolution was obtained. All selected compounds were well resolved and could be determined in the samples using the peak-purity capability of the diode array detector, which allows the detection and quantification of the selected compounds, and identified by co-injection with standards. Fig. 2 also shows the same real sample without spiking with standards. It can be seen in this sample that all compounds were detected except syringic acid and protocatechuic acid.

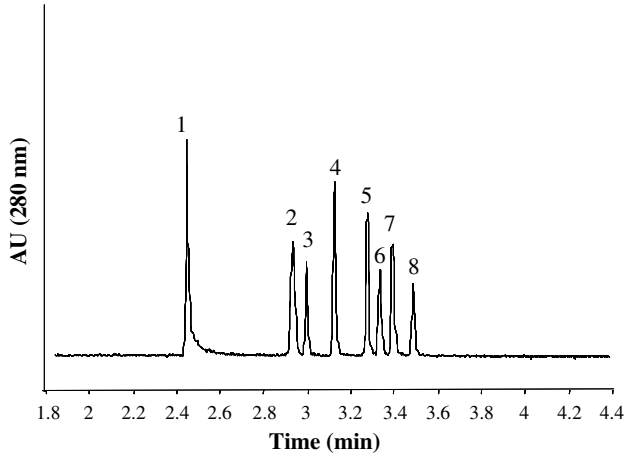


Fig. 1. Electropherogram of the separation of the eight selected antioxidants. Buffer: 125 mM borate, 48.6 mM phosphate, 0.002% (w/v) HDB, 2.5 mM α-CD and pH 7.5. Experimental conditions: total length of the capillary 47 cm, 40 cm of effective length, 75 μm i.d., run voltage −15 kV, detection at 280 nm, temperature 20 °C, hydrodynamic injection at the anode for 2 s at 0.5 psi. For identification of compounds, see Section 2.

Table 2
Calibration data for the selected antioxidants

Compound	Calibration curve	R	R^2	LOD[a] (mg/l)	LOQ[b] (mg/l)
Protocatechuic acid	$y = 5.583x - 0.016$	0.9988	0.9977	0.05	0.16
Salicylic acid	$y = 6.939x + 0.004$	0.9994	0.9989	0.04	0.13
p-Hydroxybenzoic acid	$y = 4.001x + 0.002$	0.9992	0.9985	0.07	0.22
Vanillic acid	$y = 6.865x + 0.003$	0.9992	0.9984	0.04	0.13
Syringic acid	$y = 6.818x - 0.003$	0.9994	0.9988	0.04	0.13
p-Coumaric acid	$y = 4.637x + 0.001$	0.9988	0.9977	0.06	0.19
Ferulic acid	$y = 4.195x - 0.001$	0.9989	0.9979	0.06	0.21
Sinapic acid	$y = 3.653x + 0.001$	0.9993	0.9986	0.07	0.24

Calibration graphs ranged between 0.1 and 100 mg/l.
[a] Calculated as three times the signal-to-noise ratio.
[b] Calculated as 10 times the signal-to-noise ratio.

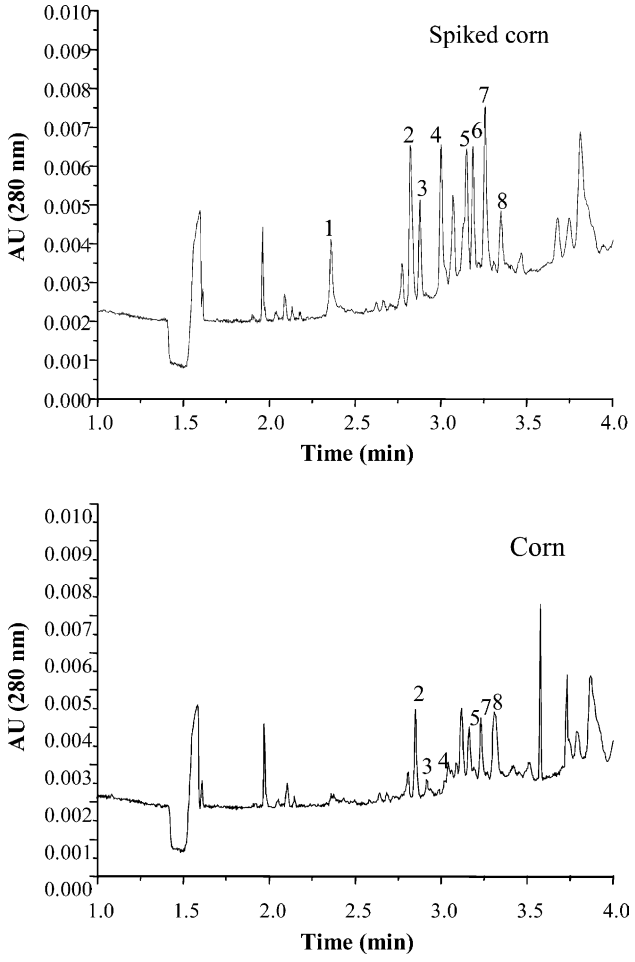


Fig. 2. Electropherogram of a spiked and non-spiked corn gofio sample. Buffer and experimental conditions as in Fig. 1. For identification of compounds, see Section 2.

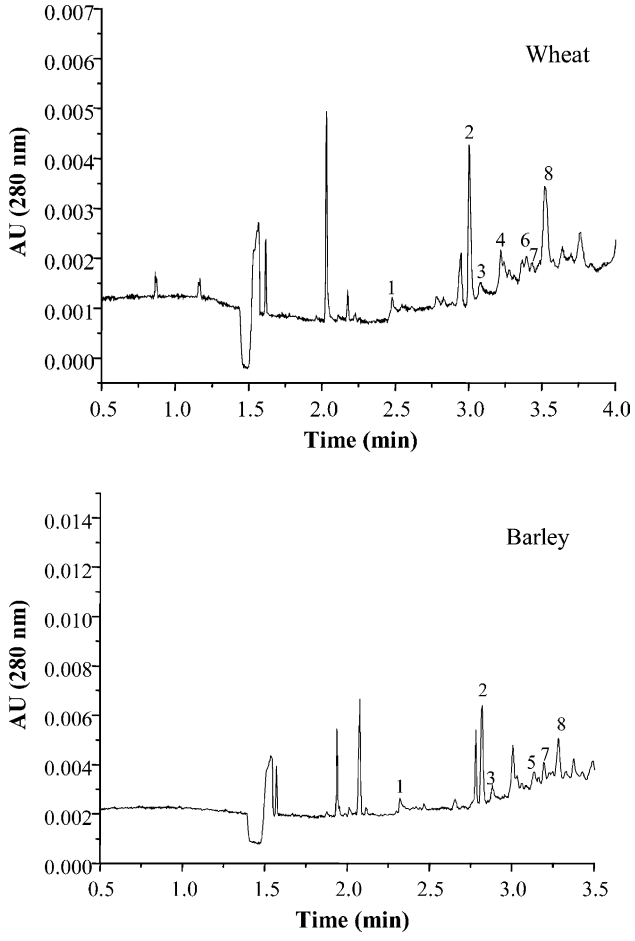


Fig. 3. Electropherogram of wheat and barley gofio samples. Buffer and experimental conditions as in Fig. 1. For identification of compounds, see Section 2.

3.2. Evaluation of the antioxidant content in gofio samples

In order to evaluate the antioxidant content of gofio, several gofio samples made with different cereals (namely wheat, corn and barley) were extracted with methanol in an ultrasonic bath (see Section 2) and introduced into the capillary. Fig. 3 shows the electropherogram of wheat and barley gofio samples. As can be seen, the electrophoretic profiles of corn (Fig. 2), wheat and barley gofio are different. Table 3 shows the phenolic contents of the different analyzed samples. Corn gofio samples had the highest amounts of phenolic acids, while those made with wheat had the lowest. The main phenolic compounds detected in corn samples were sinapic, syringic, ferulic, salicylic and *p*-hydroxybenzoic acids while, in wheat and barley samples, the major compounds were sinapic, salicylic and protocatechuic acids. It can also be seen that the total phenolic content in barley is also higher than in wheat samples, showing a difference between the two kinds of roasted flours. These data accord with results previously reported using flour as a reference sample (Senter et al., 1983; Shahidi & Naczk, 1995; Zieli'nski et al., 2001) and could be useful for differentiating the kind of cereal used to make gofio.

Frequently, gofio made with legumes can also be found in Canary Island gastronomy. In these preliminary studies, also, several samples made from roasted pea were extracted with methanol and introduced into the capillary in order to evaluate its antioxidant content and to compare it with the other types of samples. Fig. 4 shows the electropherogram of a pea gofio sample. A different electrophoretic profile can clearly be seen. In this case, only three phenolic acids, namely protocatechuic, salicylic and *p*-hydroxybenzoic acids, were found (see Table 3). Besides, in the analyzed pea gofio samples, the individual antioxidant content was slightly lower than in those previously analyzed, made from corn, wheat or barley, except for salicylic acid.

Highly roasted gofio is also manufactured in the Canary Islands and can easily be found in many supermarkets. As indicated above, these highly roasted samples are submitted to a heating procedure that consists of two steps of 1 min 15 s at 300 °C each (between both

Table 3
Antioxidant contents of the different cereal samples (μg/g)

	Corn[a]	Wheat[a]	Barley[a]	Pea[a]	Roasted corn[b]	Roasted wheat[b]
Protocatechuic acid	n.d.	1.09	1.43	1.17	1.89	n.d.
Salicylic acid	2.50	1.65	2.35	5.39	2.48	2.42
p-Hydroxybenzoic acid	1.26	0.50	0.97	1.46	0.58	0.94
Vanillic acid	0.74	0.21	n.d.	n.d.	0.58	0.38
Syringic acid	4.17	n.d.	0.40	n.d.	1.80	n.d.
p-Coumaric acid	n.d.	0.22	n.d.	n.d.	0.50	0.23
Ferullic acid	3.03	0.30	0.97	n.d.	0.37	0.27
Sinapic acid	4.63	4.12	2.87	n.d.	2.09	2.87
Total	16.3	8.09	8.99	8.02	10.3	7.11

Mean values of three determinations ($n = 3$). n.d. – Not detected.
[a] Roasting procedure: 1 min 15 s at 300 °C.
[b] Roasting procedure: 2 steps of 1 min 15 s at 300 °C each.

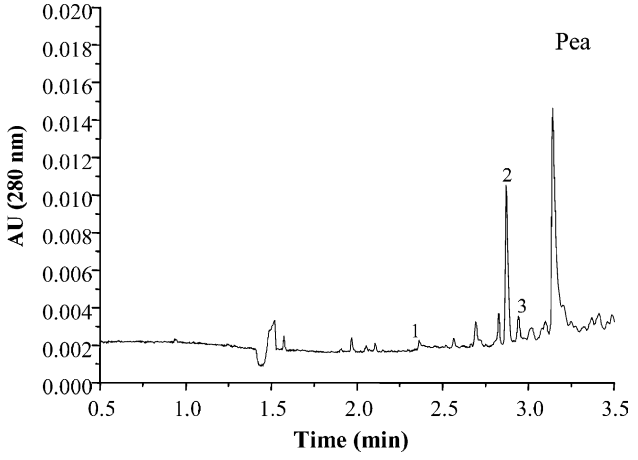


Fig. 4. Electropherogram of a sample of gofio made with pea. Buffer and experimental conditions as in Fig. 1. For identification of compounds, see Section 2.

steps the samples are cooled). This double heating procedure may have an influence on the antioxidant content. For this reason, also, highly roasted corn and wheat gofio was analyzed, following the same sample pretreatment as described above. Table 3 also shows the antioxidant content of these samples. As can be seen, in highly roasted corn samples, the total antioxidant content is slightly higher than in wheat samples. Besides, the antioxidant content of highly roasted gofio samples is lower than that of less roasted ones, due to the appearance of new forms of the free acids and disappearance or diminishing of others (Zieli'nski et al., 2001). This fact can be explained by the major thermal process in the manufacturing of the sample.

4. Conclusions

The determination of the phenolic compounds protocatechuic acid, salicylic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid, in edible cereal derivatives (gofio) from the Canary Island has been achieved by an capillary electrophoresis method. The method is reproducible and provides a good separation in terms of migration time and resolution. In this preliminary study, the antioxidant contents of corn, wheat, barley and pea gofio samples were determined, showing differences in their antioxidant contents and, therefore, their electrophoretic profiles. Among the analyzed samples, corn showed the highest antioxidant content. In gofio samples made with pea, only three of the selected antioxidants appeared, showing a clearly different electrophoretic profile. Also, highly roasted corn and wheat gofio samples were analyzed, and had a lower antioxidant content than less roasted samples.

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