

Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life

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Abstract

The application of pulsed electric fields (PEF) is one of the new non-thermal technologies being studied to evaluate their potential as alternative or complementary processes to thermal pasteurization. “Horchata de chufa” (tiger nut milk or earth almond milk) is of high nutritional quality and therefore has great potential in the food market, limited by its very short shelf-life. The present work studies whether PEF can be used to obtain a quality horchata and increase its shelf-life while maintaining its organoleptic characteristics. In order to do so we determined pH, total fat, peroxide index, thiobarbituric acid-reactive substances index, formol index, and peroxidase activity in natural (untreated) horchata and horchata subjected to various PEF treatments and studied their stability during refrigerated storage (2–4 °C). After PEF treatment, only peroxidase activity decreased significantly ($p < 0.05$). This parameter and pH varied during the shelf-life of the horchata, and a negative correlation was obtained between pH and peroxidase activity. © 2004 Elsevier Ltd. All rights reserved.

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1. Introduction

“Horchata de chufa” is a refreshing, non-alcoholic beverage of milky appearance, obtained from the tubers of the “chufa” (*Cyperus esculentus* L.). It is a typical product of Spain, and of great economic importance. The annual value of chufa production is close to 3 million euros (MAPA, 1997). According to the industrial production survey, 31,749 thousand litres of horchata were manufactured in 1998, representing a retail market value of some 20.1 million euros (INE, 1999). Natural horchata has a pH in the range 6.3–6.8 and is rich in starch. Consequently, it cannot be heated above 72 °C as this would cause the starch to gel and would alter the organoleptic characteristics of the product. Horchata de chufa is of high nutritional quality and therefore

has great potential in the food market, limited by its very short shelf-life (Selma, Fernández, Valero, & Salmorón, 2003). The fat is rich in oleic acid (75% of total fat) and linoleic acid (9–10% of total fat), and arginine is the major amino acid, followed by glutamic acid and aspartic acid. With the exception of histidine, the essential amino acids in natural horchata de chufa are higher than the amount in the model protein proposed for adults by the FAO/OMS (Morell & Barber, 1983; Navarro et al., 1984). Treatments to improve the stability of horchata are essential for its quality, but they have been applied after significant composition changes, consisting mainly in removal of starch. This has resulted in a loss of aroma and flavour with respect to natural horchata. Consequently, there is a need to explore new technologies that are less drastic and that can preserve product quality and stabilize the product.

Undesirable quality changes may take place during some food pasteurization processes that are used to increase the shelf-life of products in order to obtain an

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acceptable commercial duration. Preservation of these characteristics is the main aim in foods described as natural, because even mild pasteurization may cause considerable losses in some characteristic properties of natural products (Lu & Whitaker, 1974). Consequently, because of consumer demand for safe but natural foods, manufacturers have begun to take an interest in finding alternatives to thermal pasteurization (Gould, 1996).

The application of pulsed electric fields (PEF) is one of the non-thermal techniques that have proved capable of increasing the shelf-life of various pumpable liquid foods.

The main aim of any preservation process is the inactivation of pathogenic or sporulated microorganisms or reduction of their growth under controlled conditions. Various studies have been published which analyze the ability of PEF to inactivate microorganisms. Sale and Hamilton (1967) indicated that, of the microorganisms studied, *Saccharomyces cerevisiae* was the most sensitive to pulse treatment. Consequently, research has been carried out with this microorganism, achieving an inactivation range between 4 and 9 log reductions, depending on the treatment conditions and substrate used (Rodrigo, Martínez, Harte, Barbosa-Cánovas, & Rodrigo, 2001). Pothakamury (1995) achieved five decimal reductions with *Lactobacillus delbrueckii*, *Bacillus subtilis* and *Staphylococcus aureus*. Inactivation studies with PEF confirm the effectiveness of this treatment and its capability as an alternative technology for food processing.

An important consideration, in any technology used for the inactivation of microorganisms, is the interpretation of survival curves. The basic models used for interpreting survival curves are based on first-order relations. Hülshager, Potel, and Niemann (1981) were the first to propose a model that described survival curves based on the relation between the logarithm of the microbe survival and treatment time at a specific electric field intensity. Peleg (1995) proposed a model based on the Fermi equation, which related the percentage of surviving microorganisms to electric field intensity. Reina, Jin, Zhang, and Yousef (1998) found a relationship between the logarithm of the percentage of surviving microorganisms and treatment time at a determined field intensity.

Inactivation studies have concentrated both on model substrates and on real foods (orange juice, apple juice, and milk, among others).

For the study of horchata de chufa, the following parameters were selected: pH, total fat, peroxide index, thiobarbituric acid-reactive substances index (TBARS), formol index, and peroxidase activity. The possible variation of each parameter with storage was studied. As the shelf-life of natural horchata is considered to be about 48 h (Barber, 1981), we also studied storage for five days in order to establish a relationship between

variation in the parameters and reduction in the quality of the horchata with time.

The aim of this work is to study whether this new, non-thermal technology (PEF) can be used to obtain a quality horchata and increase its shelf-life while maintaining its organoleptic characteristics.

2. Materials and methods

2.1. Samples

Various batches of samples were obtained directly from the manufacturer involved in the project, which supplied containers of recently made natural horchata de chufa. In total, seven samples of natural horchata were analyzed, and each was subjected to various times and electric field intensities. The analyses were performed in duplicate, and during the period of the study the samples were stored in refrigeration (2–4 °C). In parallel, for each of the PEF treatments applied, a sample was analysed, in duplicate; for this, no treatment was applied and it was designated as the blank throughout the study.

2.2. Pulsed electric field treatment system

The sample treatments were applied in a continuous PEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers, with a diameter of 0.23 cm and an electrode gap of 0.293 cm, connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR).

The flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL). The treatment times varied between 100 and 475 μ s and the electric field intensity between 20 and 35 kV/cm. The temperatures during the treatments did not exceed 35 °C.

2.3. Analytic method

2.3.1. pH

The determination of pH was based on the potentiometric measurement at 20 °C (BOE, 1988). It was determined in a Crison GLP 21 pH meter equipped with a temperature compensation sensor at 20 °C. The results were expressed to two decimal places.

2.3.2. Total fat

The method established by Cortés, Esteve, Frigola, and Torregrosa (2004) was used for the extraction of fat in horchata de chufa, consisting in extraction of fat from the sample with a mixture of chloroform (Merck, Darmstadt, Germany) and methanol (J.T. Baker, Deventer, Holland) (2:1, vol/vol), in accordance with the method described by Angulo (1997) with various modifications.

2.3.3. Peroxide index

This is defined as the milliequivalents of active oxygen contained in 1 kg of fatty matter and it was calculated by iodometry. Iodine formed in the oxidation of iodide ion by the peroxides was determined with sodium thiosulphate. The fat was extracted in 10 ml of chloroform, and treated with 15 ml of glacial acetic acid and 0.7 g of potassium iodide. After 5 min 75 ml of water were added and some drops of starch 1% (vol/vol), and the iodine formed in the reaction determined with 0.01 N sodium thiosulphate (AOAC, 1990).

2.3.4. Thiobarbituric acid-reactive substances index (TBARS)

Hydroperoxides, formed by oxidation of lipids, start to degrade into various reaction products. One of the products formed during the oxidation process is malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) to give a coloured compound. In order to quantify MDA, initially, the method described by Angulo (1997) was used, which consists in determining the substances that react with TBA by means of fluorescence, with an excitation wavelength of 515 nm and an emission wavelength of 553 nm. However, when this method was applied, the spectrofluorimeter did not distinguish the different quantities of horchata. As a result, it was not possible to establish the optimum quantity to be taken, no reproducibility was achieved in the results and, with time, the fluorescence decreased rapidly. After several trials, there was a considerable influence of temperature on the medium.

After many tests and trials, given the impossibility of solving all the problems posed by the spectrofluorimetric method, the method for determination of TBA described by Salih, Smith, Price, and Dawson (1987) was used. It is a spectrophotometric technique and is based on the fact that the adduct that forms MDA with TBA absorbs at the same wavelength as the adduct formed by TBA with 1,1,3,3-tetramethoxypropane (TEP). The intensity of the colour is a measure of the concentration of MDA and has been correlated organoleptically with rancidity in fatty products.

For the analysis, 5 ml of horchata were homogenized in 15 ml of 0.38 M HClO₄ (Merck, Darmstadt, Germany) for 3 min in an ice bath; 0.5 ml of a solution of 0.19 M butylhydroxytoluene (Sigma, Steinheim, Ger-

many) in ethanol (J.T. Baker, Deventer, Holland) were added. The mixture was homogenized in an ice bath for 1 min and centrifuged at 3000g (Jouan GT 422 refrigerated centrifuge) for 15 min at 5 °C, and the supernatant was filtered through filter paper (Ø = 110 mm).

The original method employs 0.7 ml of this filtrate, but we studied the optimum volume for the determination, taking volumes ranging from 0.1 to 0.7 ml, and arrived at the conclusion that a volume of 0.2 ml should be used because the most reproducible results were obtained with this volume. Therefore 0.2 ml of filtrate was treated with 0.7 ml of a solution of 0.02 M TBA (Sigma, Steinheim, Germany) and made up to 1.4 ml with deionized water. The mixture was boiled at 100 °C for 30 min. It was allowed to cool and the absorbance was measured at 532 nm, using deionized water as a blank.

All compounds with aldehyde groups can react with TBA, so it is necessary to know whether the presence of matrix influences the behaviour of the standards. To determine whether or not there were matrix interferences, a least squares fit was performed and a covariance analysis was applied to confirm whether the curves were parallel or not. Parallel curves indicate that the solutions behave in a similar way and therefore there are no matrix interferences in the determination. In all cases studied, the curves were not parallel (Fig. 1), indicating that the solutions showed different behaviours and hence there were matrix interferences in the determination. Therefore, for all the TBA-reactive substances index determinations, curves of standards spiked with matrix should be used. In order to determine the goodness of the method selected the various analytical parameters were studied and gave a limit of detection about 0.076 nmol and a recovery percentage of 93.0 ± 0.76%. The instrumental precision, expressed as relative standard deviations (RSD%, *n* = 6), was determined in two different days. The interday precision was slightly high, but natural horchata de chufa has a shelf-life of only a few days (intraday precision = 4.3%, interday

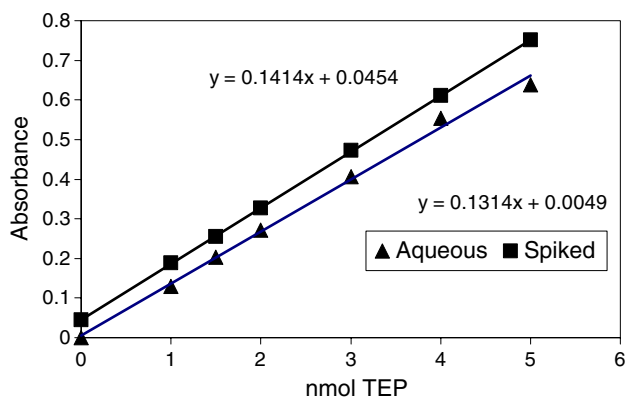


Fig. 1. Comparison of slopes in the matrix interference test.

precision = 13.9%). These parameters indicate that the method is suitable for the determination of TBA-reactive substances.

2.3.5. Formol index

Samples were analysed according to the “Ministerio de Agricultura, Pesca y Alimentación” (MAPA, 1993) methods, in order to determine the formol index.

2.3.6. Peroxidase activity

The method proposed by Moreno, Gasque, and Schwartz (1983) for the determination of peroxidase activity in horchata de chufa was used. About 4 ml of horchata were centrifuged at 4300 rpm for 15 min at 2 °C, and the supernatant was filtered through filter paper ($\varnothing = 110$ mm). After filtration of the homogenate, the filtrate was diluted 1:100 with deionized water. POD activity was assayed using 2 ml of the filtrate and adding 2 ml of 2% guaiacol in methanol (vol/vol) and 1 ml of 0.16% (vol/vol) H_2O_2 . The reaction was carried out for 12 min at 25 °C, and the change in absorbance at 480 nm with time was recorded, using a UV–Vis spectrophotometer (Perkin–Elmer, USA).

The initial velocity was calculated from the slope of the linear part of the curve obtained. The straight line section of the activity curve was used to express the enzyme activity. Enzymatic activity was defined as an increase in one absorbance unit per minute under the conditions of the assay.

2.4. Statistical analysis

The contents were compared using one-way analysis of variance (ANOVA). To determine differences between PEF treatment and the contents during storage of each of the PEF treatments, the LSD test ($p < 0.05$) was applied.

3. Results and discussion

The stability of horchata de chufa is related to pH so, when horchata is altered by the action of microorgan-

isms, there is generally a decrease in pH to a greater or lesser extent, according to the degree of alteration. Table 1 shows the results obtained, in which, at the start time, the pH of all the samples is above 6.3, which is the minimum pH established by the Technical Health Regulations (BOE, 1988). During storage, there was a clear reduction in these values with time, indicating a decrease in the stability of the horchata. This variation in pH can be fitted to a linear model (Table 2), and the slope of each curve indicates the rate of decrease of this parameter over time. The decrease in the pH of the natural horchata not subjected to any treatment (blank) was faster. Moreover, for a given electric field, when the treatment time increased, the rate of decrease in pH was less. This indicates that the longer the time over which pulses are applied (for a given field), the more effective is the treatment, allowing the stored horchata to have a longer shelf-time. There are almost no studies of the physicochemical characteristics of horchata de chufa subjected to PEF with which to compare our results, but some authors have carried out studies on other liquid foods (e.g. milk, juice) treated with PEF. Michalac, Alvarez, and Zhang (1999) studied the variation in various parameters, including pH, of milk subjected to PEF and concluded that no differences appeared between the values obtained before and after treatment. Yeom, Evrendilek, Jin, and Zhang (2001) studied a commercial plain low fat yogurt (87.6%) mixed with strawberry jelly (8.8%) and strawberry syrup (3.6%) and obtained pH values that did not vary with PEF treatment. In the present work, there were also no significant differences between the pH values of the blank and those of the samples subjected to PEF at the start time, but we cannot compare the evolution of pH during storage as there are no reported pH studies.

In the horchata samples, the fat content exceeded the minimum values established by the legislation for this product (2%) (Cano, Lobo, & De Ancos, 1998), and it did not undergo modifications as a result of PEF preservation treatment. Having observed, in preliminary tests, that the fat percentage did not vary with time, we did not study this parameter during storage in refrigeration. The results appear in Table 3. Dunn (1995) concentrated

Table 1
Mean pH values during the storage period, according to electric field strength (E) and treatment time (t)^a

E (kV/cm)	t (μ s)	Days					
		0	1	2	3	4	5
20	300	6.43 \pm 0.01	6.36 \pm 0.00	6.38 \pm 0.00	5.95 ^b \pm 0.00	5.56 \pm 0.01	5.10 \pm 0.00
20	450	6.53 \pm 0.02	6.40 \pm 0.01	6.35 \pm 0.01	6.10 ^b \pm 0.00	6.01 \pm 0.00	5.87 \pm 0.01
20	475	6.56 \pm 0.01	6.59 \pm 0.02	6.41 \pm 0.00	6.25 ^b \pm 0.00	6.19 \pm 0.01	6.10 \pm 0.02
25	300	6.59 \pm 0.01	6.62 \pm 0.01	6.35 \pm 0.00	6.38 \pm 0.00	6.30 \pm 0.02	6.25 ^b \pm 0.01
30	175	6.53 \pm 0.00	6.39 \pm 0.00	6.30 \pm 0.02	6.25 ^b \pm 0.01	5.91 \pm 0.01	5.63 \pm 0.01
35	100	6.48 \pm 0.00	6.33 \pm 0.01	6.27 ^b \pm 0.01	6.23 \pm 0.00	5.84 \pm 0.01	5.52 \pm 0.00
Blank		6.52 \pm 0.01	6.13 ^b \pm 0.01	5.94 \pm 0.00	5.61 \pm 0.01	5.25 \pm 0.02	4.91 \pm 0.00

^a Means \pm SD for three samples.

^b Indicates that they are higher than the minimum pH values established by the Technical Health Regulations.

Table 2
Linear fit of the pH values during storage, according to electric field strength (E) and treatment time (t)

E (kV/cm)	t (μ s)	Equation	r^a
20	300	$-0.271x + 6.641$	0.938 *
20	450	$-0.135x + 6.547$	0.988 **
20	475	$-0.104x + 6.611$	0.969 **
25	300	$-0.073x + 6.597$	0.913 *
30	175	$-0.171x + 6.596$	0.956 *
35	100	$-0.180x + 6.562$	0.938 *
Blank		$-0.315x + 6.514$	0.997 **

^a Mean correlation coefficient.

** Fit significant at 99% probability.

* Fit significant at 95% probability.

Table 3
Mean values of total fat (% wt/wt), according to electric field strength (E) and treatment time (t)^a

E (kV/cm)	t (μ s)	Total fat (% wt/wt)
20	300	3.04 \pm 0.03
20	450	3.05 \pm 0.06
20	475	3.09 \pm 0.02
25	300	3.05 \pm 0.04
30	175	3.04 \pm 0.08
35	100	3.05 \pm 0.06
Blank		3.03 \pm 0.04

^a Means \pm SD for three samples.

on studies of shelf-life and loss of organoleptic and physicochemical characteristics in milk and milk derivatives, showing as in the current work, that the fat content is not modified as a result of PEF preservation treatments or during the subsequent shelf-life of the product. Similarly, Qin et al. (1995) carried out a study of physicochemical properties and shelf-life of milk and did not observe changes in the percentage (wt/wt) of fat after treatment.

When the peroxide index during refrigerated storage was determined, none of the PEF-treated samples gave a positive result.

Table 4 shows that the TBARS did not undergo modifications as a result of the PEF treatments; in other words, there was no significant increase in this index due to processing with the new technology or subse-

quently during storage. It can be affirmed, therefore, that this treatment does not cause oxidation of fatty matter, and horchatas treated by this technique can be kept under refrigeration for 5 days without oxidation of fat taking place. Comparison of the TBA-reactive substances index of the samples of horchata subjected to PEF with the values obtained by Cortés, Esteve, Frgolá, and Torregrosa (2003), for various commercially available horchatas subjected to various preservation treatments, shows that the PEF-treated samples had the lowest TBARS index, and the index is much higher for all the other horchatas, which were subjected to severe heat treatments, owing to the oxidation of fat caused by the high treatment temperatures.

Similarly, Table 5 shows that the values of the formol index, and therefore the total content of free amino acids, did not undergo modifications as a result of PEF treatment or subsequently during the period of refrigerated storage.

Table 6 shows the peroxidase activities obtained for the horchata not treated by PEF and the horchatas subjected to PEF, and the evolution of this parameter during the period of refrigerated storage. In all cases, PEF treatment caused partial inactivation of the enzyme. The residual peroxidase activity (0.034–0.097 Δ Ab/min) changed during storage. In those cases in which it increased (which indicates regeneration of the enzyme), it never attained the peroxidase activity of the untreated horchata. Cano et al. (1998) studied peroxidase activity in long-term frozen stored papaya slices and found that, during preservation treatments, peroxidase was not totally destroyed but only reduced or reversibly inactivated, so that regeneration of its activity was often observed after long periods of storage, which may lead to deterioration of product quality. These results are similar to those found in our work.

The p -value, which tests the statistical significance of the correlation (-0.6279) is 0.0000 and it indicates statistically significant non-zero correlations at the 95% confidence level, so that there is a negative correlation between pH and peroxidase activity in the horchata de chufa samples subjected to PEF treatment (Fig. 2). As storage time increases, pH decreases, so that it can serve

Table 4
Mean values of TBARS index (mg MDA/l horchata) during storage period, according to electric field strength (E) and treatment time (t)^a

E (kV/cm)	t (μ s)	Days					
		0	1	2	3	4	5
20	300	0.385 \pm 0.024	0.358 \pm 0.018	0.366 \pm 0.020	0.387 \pm 0.011	0.374 \pm 0.014	0.377 \pm 0.010
20	450	0.398 \pm 0.014	0.374 \pm 0.021	0.401 \pm 0.007	0.384 \pm 0.023	0.395 \pm 0.008	0.391 \pm 0.017
20	475	0.401 \pm 0.008	0.393 \pm 0.014	0.405 \pm 0.010	0.416 \pm 0.005	0.416 \pm 0.010	0.411 \pm 0.014
25	300	0.480 \pm 0.008	0.463 \pm 0.009	0.425 \pm 0.012	0.498 \pm 0.007	0.455 \pm 0.020	0.474 \pm 0.021
30	175	0.445 \pm 0.007	0.463 \pm 0.015	0.453 \pm 0.005	0.447 \pm 0.017	0.461 \pm 0.011	0.457 \pm 0.023
35	100	0.464 \pm 0.010	0.443 \pm 0.018	0.466 \pm 0.011	0.457 \pm 0.013	0.440 \pm 0.012	0.452 \pm 0.025
Blank		0.479 \pm 0.015	0.450 \pm 0.007	0.477 \pm 0.013	0.466 \pm 0.024	0.462 \pm 0.009	0.461 \pm 0.017

^a Means \pm SD for three samples.

Table 5

Mean values of formol index (% vol/vol) during storage period, according to electric field strength (E) and treatment time (t)^a

E (kV/cm)	t (μ s)	Days					
		0	1	2	3	4	5
20	300	11.8 \pm 0.0	11.8 \pm 0.0	11.6 \pm 0.0	11.8 \pm 0.0	11.7 \pm 0.1	11.8 \pm 0.0
20	450	11.9 \pm 0.1	12.0 \pm 0.0	11.8 \pm 0.0	11.8 \pm 0.0	11.9 \pm 0.1	11.8 \pm 0.0
20	475	11.8 \pm 0.0	11.6 \pm 0.2	11.6 \pm 0.0	11.7 \pm 0.1	11.8 \pm 0.0	11.8 \pm 0.0
25	300	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0
30	175	11.9 \pm 0.2	11.8 \pm 0.0	11.9 \pm 0.2	12.0 \pm 0.0	12.0 \pm 0.0	11.8 \pm 0.0
35	100	11.8 \pm 0.0	11.8 \pm 0.0	11.7 \pm 0.1	11.8 \pm 0.0	11.8 \pm 0.0	11.6 \pm 0.0
Blank		11.4 \pm 0.0	11.4 \pm 0.2	11.6 \pm 0.2	11.4 \pm 0.0	11.5 \pm 0.1	11.4 \pm 0.0

^a Mean \pm SD for three samples.

Table 6

Mean values of peroxidase activity (Δ Abs/min) during storage period, according to electric field strength (E) and treatment time (t)^A

E (kV/cm)	t (μ s)	Days					
		0	1	2	3	4	5
20	300	0.063 \pm 0.002 ^a	0.110 \pm 0.001 ^b	0.090 \pm 0.002 ^c	0.112 \pm 0.002 ^b	0.112 \pm 0.003 ^b	0.084 \pm 0.001 ^d
20	450	0.067 \pm 0.001 ^a	0.073 \pm 0.002 ^b	0.086 \pm 0.004 ^c	0.078 \pm 0.002 ^d	0.087 \pm 0.005 ^c	0.080 \pm 0.001 ^d
20	475	0.075 \pm 0.002 ^a	0.072 \pm 0.002 ^a	0.085 \pm 0.004 ^{bc}	0.081 \pm 0.004 ^b	0.081 \pm 0.004 ^b	0.089 \pm 0.001 ^c
25	300	0.034 \pm 0.001 ^a	0.038 \pm 0.005 ^a	0.037 \pm 0.002 ^a	0.030 \pm 0.001 ^b	0.057 \pm 0.002 ^c	0.044 \pm 0.004 ^d
30	175	0.039 \pm 0.003 ^a	0.063 \pm 0.005 ^b	0.065 \pm 0.000 ^b	0.066 \pm 0.001 ^b	0.073 \pm 0.000 ^c	0.092 \pm 0.006 ^d
35	100	0.097 \pm 0.002 ^a	0.089 \pm 0.003 ^b	0.110 \pm 0.002 ^c	0.110 \pm 0.001 ^c	0.116 \pm 0.003 ^d	0.108 \pm 0.001 ^c
Blank		0.123 \pm 0.002 ^a	0.130 \pm 0.006 ^b	0.135 \pm 0.002 ^c	0.138 \pm 0.003 ^c	0.187 \pm 0.000 ^d	0.153 \pm 0.002 ^e

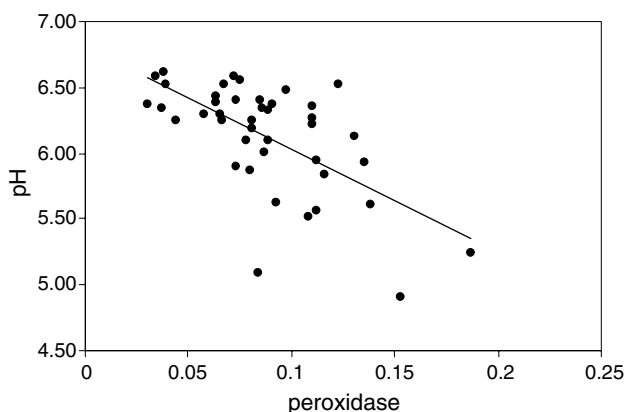
^A Mean \pm SD for three samples. Differences in letters within a row indicate significant ($p < 0.05$) differences.

Fig. 2. Negative correlation between pH and peroxidase activity.

as an indicator of deterioration of horchata in terms of quality, in relation to an increase in peroxidase activity. Lu and Whitaker (1974) state that the oxidative activity of peroxidase is affected by pH, depending on the kind of food.

Selma et al. (2003) studied inactivation of *Enterobacter aerogenes* in horchata de chufa subjected to PEF treatments and its possible reactivation or growth after an incubation period, concluding that, to prevent growth of this microorganism in horchata treated with PEF, it is necessary to monitor contamination of the product in the production line and guarantee its refrigeration during distribution and storage, as recontamination has been detected in the process of extraction of this

beverage and in samples incubated at temperatures above 8 °C. These two factors are critical (low contamination and refrigeration) for obtaining this new product, PEF-treated horchata de chufa, which would potentially increase the market for this product, as it fulfils consumer requirements as a natural, healthy, additive-free vegetable beverage.

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