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Obtention of Enzymatically Hydrolyzed Product from Cactus (*Opuntia boldinghii* Britton and Rose) Cladodes Whole Flour

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Abstract: The objective of this work was to obtain an enzymatically hydrolyzed product from cactus cladodes whole flour (*Opuntia boldinghii* Britton and Rose). The whole flour was subjected to the action of the commercially prepared enzymes Pectinex® Ultra SP-L and Cellubrix® L (1:1 ratio). The experiments were carried out under fixed conditions of temperature of 50°C ± 1°C and with pH of 5.5±0.1 under continuous agitation at 150 rpm. The kinetic parameters: enzyme and substrate optimal concentration, reaction time, K_M, V_{max} and K_3 were established. It was observed that the fibrous constituents in the hydrolized flour decreased ($P<0.05$) with the exception of the reducing sugars ($P<0.05$) which increased. The yield in sugars was 6.96% (w/w). It is presumed a non competitive inhibition caused by the mucilage and there was an evident synergism between the enzymes. The hydrolysis produced modifications of the functional properties of the flour.

Key words: Cactus, cladodes, enzymatic hydrolysis, flour, *Opuntia boldinghii*

INTRODUCTION

The cacti are endemic species from semi-arid and arid regions. They are found in Venezuela in the coastal zones and in the states of Lara, Falcon, Sucre and Carabobo (Ponce, 1989). The cacti do not offer much commercial interest due to the difficult post-harvest handling and for the lack of knowledge of their alimentary potential (Viloria *et al*., 2002). They are greatly adaptable, being able to grow in dry and desertic environments, in forests and in sandy beaches (Sandi, 2000).

The cacti have been studied as a potential source of colourants for food, such as betalains (Stintzing *et al*., 2002, Moreno-Alvarez *et al*., 2003), they have also been used as forage (McMillan *et al*., 2002), as emerging food and as provider of water in arid and semi-arid regions (Ben - Salem *et al*., 2002). The fruits and cladodes of the cacti have been studied for consumption in human diet, where their fruits have been used mainly for their juice, whose chemical and mineral composition was described by various authors (Saenz and Sepulveda, 2001; Moreno-Alvarez *et al*., 2003). The cladodes represent an important source of fiber, calcium and mucilage; three components which are necessary for a healthy diet. The dietary fiber content of the cladodes compares to that found in many vegetables and fruits, such as: Spinach, artichoke, chard, eggplant fruit, mango and melon, among others (Saenz *et al*., 2006). Moreno - Alvarez *et al*. (2006) determined raw fiber content in cladodes from 8.02 to 8.68% and Saenz (1996), found values of 12 to 17.5%. Saenz (1996) found total dietary fiber content in the flour of the species *Opuntia ficus-indica*, of 43%; likewise, Rosado and Diaz (1995) indicated, for dehydrated cactus, a dietary fiber content of 50.4%. For the species *Opuntia boldinghii*, Moreno - Alvarez *et al*. (2006) determined the dietary fiber content to be 41.5%.

Various formulations have been tried satisfactorily in the development of new types of foods high in fiber content using cactus whole flour; nevertheless, the addition of large amounts of flour deteriorates the functional properties, such as texture and gel firmness, of the products obtained. In this respect, Saenz *et al*. (2002) formulated a powder to prepare a pudding like dessert using cactus flour as a source of fiber; observing that proportions greater than 16% affected unfavorably the texture of the desert.

One way to make good use of cactus flour in food formulation, without affecting the mechanical properties of the final product due to high fiber content, is by the use of enzymes to degrade the constituents of the fibers, which are found mainly in the cellular walls (pectins, hemicelluloses and celluloses), into simpler forms of carbohydrates.

There have been various investigations on vegetable species in that respect. Olle *et al*. (2000) used commercial enzymatic preparations with activity of pectinases and cellulases, for the purpose of degrading the polysaccharides of the cellular walls of a mango puree (*Mangifera indica* L); achieving depolymerization and solubilization of the pectins in 76 to 88%, and of the celluloses in 40 to 50%. In cactus flour (*O. ficus-indica*), Medina *et al*. (2006), evaluated the effect of a preparation of exogenous fibrolytic enzymes (cellulases and xilanases) in the degradability *in situ* of the dry matter (DM), of the neutral detergent fiber (NDF) and of the acid

detergent fiber (ADF), in diets high or low in flour; it was determined that with the application of 1 to 3 g of enzymes in the low cactus flour content diet, had effect on increasing the total volatile fatty acids.

The enzymes used for the present investigation are a combination of Pectinex® Ultra SP-L with Cellubrix® L, both commercial enzymatic preparations from Novozymes (The Netherlands). The purpose of employing said enzymatic combination is to hydrolyze the fiber from the cladodes whole flour to increase the value of the simpler sugars; among them, monosaccharides like galactose, arabinose (associated to the pectic substances), cellulosic glucose and cellobiose, among others, that produce in food formulation improvements in the functional properties (reduction in viscosity, increment in °Brix, greater gel firmness, solubility and better texture).

The objectives of this work are: To obtain a hydrolyzed product of low fiber content from whole flour of cladodes of *Opuntia boldinghii* (Britton and Rose) by the use of enzymes; as well as to analyze the effects of the variation of enzyme concentration, of substrate concentration and of hydrolysis time; to establish the kinetic parameters: Michaelis-Menten constant (K_M), maximum velocity (V_{max}) and turnover number (K_3). In addition, to evaluate the functional properties: Water absorption capacity (WAC), oil absorption capacity (OAC), solubility in water (WS) and cations interchange capacity (CIC) of the hydrolyzed product obtained from cactus cladodes whole flour.

MATERIALS AND METHODS

Selection and treatment of the raw material: A lot of 40 Kg of cladodes of the cactus *Opuntia boldinghii* (Britton and Rose) were collected from its natural environment, located in the village of Guama, sector Los Chucos, state of Yaracuy, Venezuela. The criteria for selection were the following: From the ramifications of cladodes from plants of 1.0 to 2.0 m in height were collected three, starting from the most apical. The plants were harvested at the same place during afternoon hours (3 pm to 5 pm), when the desacidification of the plant is greater, due to descarboxilation of malic acid (Hernandez, 2002; Flores-Hernandez *et al*., 2004). The samples were washed with water and dried with absorbing paper and then stored in a polystyrene cooler and translated to the Laboratory of Biomolecules of the "Simon Rodriguez" University, Canoabo Campus, state of Carabobo, where were stored in a refrigerator at 6°C±1°C (Moreno - Alvarez *et al*., 2003).

Preparation of the samples: *Cutting*. The cladodes were cut manually with a knife into pieces of approximately 1 cm by 1 cm. *Drying I*. This operation was carried out in a FELISA oven at 78°C ± 5°C. *Grinding I*. A VICTORIA mechanical mill was used. *Sieving I*. Was made through a WS TYLER sieve, model RX-812. To obtain the cladodes whole flour (CWF), the ground material was sieved through an Nº 18 mesh with 1 mm apertures.

Chemical and microbiological characterization of the raw material: Representative samples of the CWF were processed to evaluate humidity, ethereal extract, protein (Kjeldahl N x 6.25), ashes, raw fiber, titrable and ionic acidity, soluble solids and calcium; all the evaluations were made following established procedures according to the AOAC norms (1990); neutral detergent fiber (NDF); acid detergent fiber (ADF), cellulose, hemicellulose and lignin, were evaluated according to the methods of Van Soest and Wine (1967); pectin by Lees' method (1990), reducing sugars by AOAC (1990 - Method 923.09) and phosphorus by Fiske and Subbarow's method (1925). Microbiological evaluations were made for molds and yeasts, total coliforms and *Escherichia coli* and *Salmonella*, using the methodologies of the COVENIN norms (1337, 3276 and 1291, respectively). The same analyses were performed to the obtained enzymatically hydrolyzed product. All the data was expressed in dry base.

Enzymes: A combination of two commercial enzymatic preparations from Novozymes (The Netherlands) was employed: Pectinex® Ultra SP-L and Cellubrix® L. The active components of Pectinex® are based mainly in pectolitic activity, as well as in a diverse number of hemicellulases activities. Pectinex® is a brown colored water-soluble liquid with a light fermentation odor and ρ=1.12 g/mL. The standard activity declared of Pectinex® is 9,500 polygalacturonase units per milliliter (PGU/mL), and its optimal conditions of pH and temperature are 5.5 to 6.4 and 40 to 60°C respectively. Cellubrix® L is based mainly in cellulase activity. It is a brown colored liquid with a light fermentation odor, it is water soluble and presents ρ=1.2 g/mL. Its standard activity declared is 700 endoglucanases units per gram (EGU/g) and its optimal conditions of pH and temperature are: 5.5 and 50 to 55°C respectively (Novozymes, 2006).

The Pectinex® Ultra SP-L and Cellubrix® L combination was made in a proportion of 1:1 (Diaz *et al*., 2004; Siddiq *et al*., 2005). The pH and temperature suggested in the literature for the enzymatic combination of Pectinex®=and Cellubrix® is 5.5 and 50°C respectively (Cabrera *et al*., 1997; Diaz *et al*., 2004; Ruijten, 2004; Siddiq *et al*., 2005).

Hydrolysis conditions: In fixed conditions of temperature 50°C±1°C, pH 5.5±0.1, continuous agitation of 150 rpm and mixture of Pectinex® Ultra SP-L and Cellubrix® L in a proportion 1:1, were determined the following optimal conditions for the hydrolysis of the constituents of the fiber in the CWF: Effect of the variation of the enzymes concentration ((E) = mL E/100g fresh mass), effect of the

variation of the substrate concentration ((S_o) = g dry mass/100mL), calculation of kinetic parameters K_M, V_{max} and K_3 using the method of Lineweaver-Burk ($1/V_o = f(1/S_0)$) and effect of the variation of the hydrolysis time.
In all the tests the content of reducing sugars (RS) was expressed or referred to as glucose.

Analysis of the effects of variations in enzymes concentration: Samples of 25 g of CWF were mixed with 500 mL of water in a 600 mL beaker, relation that represented a dry mass concentration of 5% (w/v). The beaker was immersed in water contained in a cylindrical equipment of 2 L capacity fitted with a jacket heated by a thermic resistor. The equipment had a temperature regulator that was set to 50°C±1°C. A HANNA potentiometer, model PH211, was used; the pH of the samples were adjusted to 5.5±0.1 by adding drops of 4 N HCl solution. When the conditions were reached, the samples were dosed with the enzymatic combination of Pectinex® Ultra SP-L and Cellubrix® L (1:1 ratio), in percentages of: 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mL E/100g of fresh mass (% v/w). The samples were agitated at 150 rpm. A control sample with no addition of enzymes was used.
The time of enzymatic action was 60 min. For deactivation of the enzymes, the samples were heated to 85°C for 5 min on a previously heated hot plate. The RS percentage was calculated following the Lane-Eynon method (AOAC, 1990), modified and adapted for this process. The tests were carried out in triplicate. This test permitted to define the optimal enzyme concentration for the hydrolysis (E_0).

Effect of the variation of substrate concentration and calculation of the kinetic parameters K_M, V_{max} and K_3: Samples of CWF were mixed with 500 mL of water in a 600 mL beaker in a proportion that represented initial concentrations of substrate ((S_o)=g of dry mass/100 mL) of 1.0%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 5.5%, 6.0%, 6.5%, 7.0% and 7.5% (w/v). The temperature was increased and controlled at 50°C±1°C and the pH was adjusted to 5.5±0.1. The enzyme concentrations selected in the previous test (E_0) were added to the samples and then were agitated at 150 rpm, with an enzymatic action time (t) for each sample of 5 min. Proceeding afterwards to deactivate the enzymes at 85°C for 5 min.
The RS percentage (w/w) was determined to the samples with the before mentioned method, using the (RS) obtained on each sample and with 5 min of hydrolysis time (t). The initial velocities (V_o) were established; that is: V_o = (RS)/t.
The values of V_o and S_o were plotted ($V_o = f(S)$), verifying if the data obtained from the reaction adjusted to Michaelis - Menten kinetics and if $1/V_o = f(1/S_o)$ according to Lineweaver-Burk method. A straight line was plotted in this last graph by means of linear regression analysis, where the value of R^2 indicated if it adjusted to the model.
In the equation of the straight line: y = mx + b, the inverse of the value "b" defined V_{max} (V_{max} = 1/b). The slope of the straight line (m) allowed the calculation of Michaelis-Menten constant ($K_M = m * V_{max}$) and finally, the turnover number (K_3) was calculated with the formula $K_3 = V_{max}/(E_o)$. These tests were carried out in duplicate. V_{max} in the graph $V_o = f(S_o)$ allowed to define the optimal substrate concentration (S).

Evaluation of the effect of the variation of the hydrolysis time: Samples of CWF were taken in a proportion that represented the (S) by mixing them with water (w/v); then, their temperature was adjusted to 50°C±1°C and the pH to 5.5±0.1. It was dosed with the (E_o). The hydrolysis was carried out with agitation at 150 rpm during 150 min and was monitored every 15 min by means of the determination of the % RS (w/w). The tests were carried out in duplicate. This experience permitted to define the maximum hydrolysis time necessary for obtaining the greatest amount of hydrolyzed product.

Obtainment of the enzymatically hydrolyzed product (EHP) from CWF in previously established conditions: The technological scheme for obtaining the EHP from CWF, as a trial run, is described as follows: *Conditioning*. The CWF was placed in a 600 mL beaker; to it, water was added in a proportion according to the (S) (w/v) and then, it was immersed in water contained in a 2 L capacity cylindrical apparatus equipped with a jacket heated by a thermic resistor. The equipment had a temperature regulator that was set to 50°C±1°C; a HANNA potentiometer model PH211 was used. The pH of the samples were adjusted to 5.5±0.1 by adding drops of 4N HCl solution agitated continuously at 150 rpm to homogenize the mixture. *Enzymatic hydrolysis*. To the CWF in the beaker contained in the apparatus was added the enzymatic combination of Pectinex® Ultra SP-L and Cellubrix® L, according to the (E_o) (v/w), with temperature, pH and was agitated continuously during the time established as maximum for obtaining the greatest amount of hydrolyzed product. *Enzymatic inactivation*. Culminated the hydrolysis time, the enzymes were inactivated by placing the beaker on a preheated hot plate at 85°C for 20 minutes under continuous agitation. *Drying II*. The hydrolyzed mixture was kept in the beaker on the hot plate, decreasing the temperature to 80°C under continuous agitation for 3 to 4 hours, obtaining a concentrated liquid (reducing the volume from 500 mL to about 150 to 200 mL). Then, the concentrated liquid was spread on a stainless steel tray and subjected to drying in an oven at 80°C until dry, obtaining this way the EHP. *Grinding II and Sieving II*. The EHP was ground in a mechanical mill and then was

sieved through an Nº 18 mesh of 1 mm openings, what produced the enzymatically hydrolyzed flour (EHF). *Packaging*. The EHF was placed in airtight polyethylene bags and stored in darkness at room temperature.

Evaluation of the functional properties of EHF and CWF: The following functional properties were determined to the EHF and CWF (control): WAC, OAC, WS, and CIC (Rosado and Diaz, 1995).

Enzymatic tests evaluated: To evaluate the enzymatic activity of Pectinex® Ultra SP-L, pectin (Biosynth, Riedel-de Haën) was used as substrate. This test was repeated using the CWF as substrate. The activity of Cellubrix® L was measured using as substrate microcrystalline cellulose of 50 microns (Sigmacell, Sigma Chemical) and CWF. The enzymatic activity was measured to the combination of Pectinex® Ultra SP-L and Cellubrix® L in, 1:1 ratio, using CWF as substrate with two purposes: One, to evaluate the synergistic effect, and the other, to determine the enzymatic activity of the mixture under the optimal conditions of hydrolysis determined in this investigation.

Statistical analysis: The statistical analysis was made with the help of the Statistix 1.0 software. The significance of the difference between the means of the values of the chemical composition and of the functional properties of the CWF and EHF, were established with the Student's t test for two independent samples. Values of $P<0.05$ were considered as significative.

RESULTS AND DISCUSSION

Chemical and microbiological characterization of the EHF and the CWF: The CWF and EHF showed humidity values of 7.69% and 6.48% respectively. The chemical constituents are presented in Table 1. The EHF had small increments in the parameters of proteins, ashes calcium and phosphorus ($P<0.05$). This can be attributed to the percentage decrease of the values of the raw fiber ($P<0.05$) and of the nitrogen free extract ($P<0.05$), due to enzymatic action. The protein content obtained was: 9.5% (CWF) and 9.71% (EHF). Lopez *et al.* (1997), indicate protein percent values for 23 species of *Opuntia* of 2.78 to 8.92%. The percent values of ashes in the samples were 20.34% (CWF) and 23.25% (EHF). In general, the species of *Opuntia* had high ash content values. Values of up to 40.11% have been determined (Lopez *et al.*, 1997). Rodriguez (1997), published ash values of 13.1% for the species *O. ficus-indica*, 18.6% for the species *O. robusta* and 25.5% for the species *O. lindheimeri*. In the case of the species *O. boldinghii*, Moreno - Alvarez *et al.* (2006), determined an ash value of 26.0%. The soluble solids content increased in EHF ($P<0.05$) for the effect of the hydrolysis of insoluble fibrous compounds into soluble fractions. The ethereal extract decreased due to the effect of the enzymatic treatment ($P<0.05$). In this sense, possibly, oxidation reactions were generated that affected the fatty acids, due to their contact with the oxygen provided by the air while agitation was carried out during the enzymatic hydrolysis and to the effect of temperature. To this respect, Aikenss and Dix (1991) maintain that the lipids present high reactivity to the reactive oxygen species (ROS) because they have carbon-carbon double bonds, that are extremely prone to oxidation, suffering lipoperoxidation. Min and Boff (2002) describe that under aerobic conditions, the chemical degradation of the foods is produced only through the ROS and on the other hand, the heat generates molecular agitation that causes rupture of the oxygen-oxygen bonds of the stable peroxides, always existing in small quantities in any system where lipids are present. At room temperature, the process is slow, but it increases noticeably when the temperature increases.

Table 1: Chemical composition of cladodes flours before and after of enzymatic treatment

	Parameters	
Determination*	Cladodes whole flour (CWF)	Enzymatically hydrolyzed flour (EHF)
Ethereal extract (%)	0.95[a]	0.49[b]
Protein (N x 6,25) (%)	9.5[a]	9.71[b]
Ashes (%)	20.34[a]	23.25[b]
Raw fiber (%)	9.33[a]	8.82[b]
Free-nitrogen extract (%)	59.87[a]	57.74[b]
Neutral detergent fiber (%)**	22.07[a]	20.51[b]
Acid detergent fiber (%)***	14.81[a]	13.76[b]
Cellulose (%)	9.49[a]	8.46[b]
Hemicellulose (%)	7.26[a]	6.75[b]
Lignin (%)	5.32[a]	5.30[a]
Pectin (%)	0.39[a]	0.19[b]
Titrable acidity (%)****	1.32[a]	2.28[b]
pH*****	5.68[a]	5.56[a]
Reducing sugars (%)	0.57[a]	7.53[b]
Soluble solids (ºBrix to 29 ºC)	8.34[a]	11.07[b]
Calcium (%)	5.52[a]	5.84[b]
Phosphorus (%)	0.19[a]	0.26[b]

*Percentages values are expressed in dry basis. **Value without ashes (The method included the utilization of α-amylase). ***Value without ashes. ****g anhydrous citric acid / 100 g dry mass. *****CWF, pH units at 23ºC. EHF, pH units at 29ºC. Different letters in superindexes in the same row indicate significant differences ($P<0.05$).

The fibrous constituents in the EHF decreased, except for the lignin content ($P>0.05$) that stood the same and the RS ($P<0.05$) which increased. In relation to the cellulose, the decrease in content from 9.49 to 8.46% ($P<0.05$), suggests a synergistic effect between the evaluated enzymes. The endoglucanases acted over the amorphous regions of the cellulose fibrils, converting the long chains into oligosaccharides, increasing the availability of the not reducing extremes of the cellulose molecule (Bravo *et al.*, 2002; Lemos *et al.*, 2003). The exogluconases, acting from the not reducing extreme of

Table 2: Microbiological characterization of cladodes flours

Determination*	Results: Cladodes whole flour (CWF)	Results: Enzymatically hydrolyzed flour (EHF)
Molds (Estimated standard count/g)	< 10	< 10
Yeast (Estimated standard count/g)	< 10	< 10
Total coliforms (CFU/g)	< 1	< 1
Escherichia coli (CFU/g)	< 1	< 1
Salmonella (in 25 g)	Not detected	Not detected

* The determinations were carried out in duplicate.

the cellulose and cellodextrin molecules, caused the liberation of units of cellobiose (reducing sugar) on which acted the cellobiases, degrading them to form monomers of glucose (Ward, 1989; Klyosov, 1990; Vilches, 2002; Ovando - Chacon *et al*., 2005); contributing this way to increase the value of the RS. The cellulose fibrils are in tight association with the hemicelluloses and the pectins and when under the action of the enzymatic mixture, underwent, by different mechanisms, transformation into simpler sugars that contributed to elevate the RS content. In respect to the hemicelluloses ($P<0.05$), their enzymatic degradation produced oligomers of up to 2 to 6 units (Carrillo, 2000) and xylose monomers, among others. In the case of pectins ($P<0.05$), the resulting production of oligogalacturonides, pectic fragments, from polygalacturonic acid due to action of the polygalacturonase (Cabrera *et al*., 1997) and monomers of galactose and arabinose, among others.

In the CWF were determined reductions in the contents of cellulose (10.85%), hemicelluloses (7.02%) and pectins (51.28%) ($P<0.05$). This effect of substrate degradation can be considered modest and is attributed possibly to the inhibitory effect of the substrate for cause of the mucilage. The reduction of the contents of cellulose, hemicelluloses and pectin, previously indicated, is equivalent to degradation into simpler sugars of 1.74%; what conflicts with the increment of RS of 6.9%, for that, it was considered that other components not evaluated, became degraded, reflected on the nitrogen free extract.

In reference to the microbiological characterization of the CWF and EHF, (Table 2) it can be noticed that viable forms of microorganisms were not found. Saenz *et al*. (2006) show for nopal flour a total recount of 3.3 UFC/g, and for moulds and yeasts of 4.6 UFC/g. The low microbial activity in the flours is due to the low water availability.

Enzymatic kinetics: The effect of variation of substrate concentration on the speed of product formation is shown in Fig. 1. The speed of formation of product suggests increments that tend to diminish until a substrate concentration of 5%, then experiments

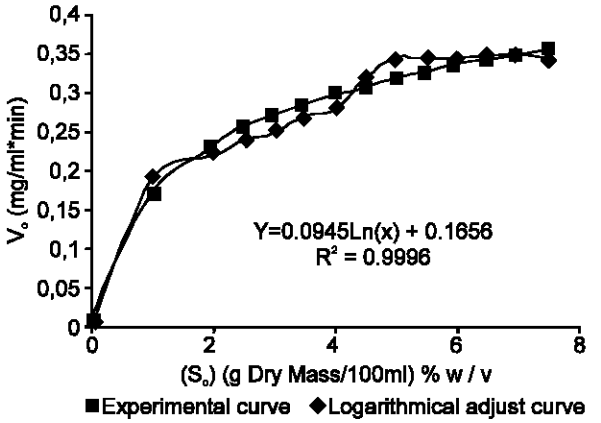

Fig. 1: Effect of variation of substrate concentration on the cladodes whole flour hydrolysis.

abruptly a tendency to remain constant (experimental V_{max} = 0.346 mg/mL*min). The behavior of the curve in the second phase (kinetic behavior of mixed order) suffers attenuation; this effect has been described before and there exist various proposals that could explain this conduct. The cellobiohydrolases are inhibited by one of the products of the enzymatic hydrolysis, the cellobiose, what decreases hydrolysis efficiency (Vilches, 2002; Lemos *et al*., 2003; Ovando-Chacon *et al*., 2005) and consequently, due to the produced glucose, the absorptive loss of the enzyme caused by the lignin (Gregg and Saddler, 1996) reduces the extension of the hydrolysis (Sewalt *et al*., 1997) and the loss of mobility of the cellulolitic enzymes, tightly or narrowly absorbed in the surface of cellulose (Sinitsyn *et al*., 1993). Carrillo (2000) points out that one of the aspects from which depends the catalytic activity is the complex enzyme-substrate formation, that is controlled by factors which affect the accessibility of the substrate to the characteristics of the enzyme, such as: morphology, physical configuration and chemical structure; expressing as well that the high specifity of the enzymatic reaction is sensitive to the changes in the structural properties of the substrates (degree of polymerization and pore dimension, among others), influencing the development of the process of enzymatic degradation. In that respect, it is important to point out the methodological difficulties found during the enzymatic hydrolysis of the CWF attributed to the substrate that could have been related to the behavior of the curve in the second phase (mixed order). The cladodes of the cacti contain mucilage without gelification capacity, which is a polysaccharide whose chemical composition is similar to that of the pectins, particularly, in the rhamnogalacturonan-I fraction, and for this reason, it is generally associated to them (Cardenas *et al*., 1997) that polysaccharide has the characteristic of forming solutions with high degree of

Table 3: Kinetic parameters to obtain an enzymatically hydrolyzed product from cactus cladodes whole flour

Kinetic parameters	Results
Enzymes concentration (% v/w)*	0.8
Substrate concentration (% w/v)**	5
Hydrolysis time (min)	60
Pectinex® Ultra SP-L + Cellubrix® L Mix (proportion)***	1:1
Hydrolysis temperature (°C)	50°C±1
pH	5.5±0,1
Continuous agitation (rpm)	150
Enzymatic deactivation temperature (°C)	85±1
Enzymatic deactivation time (min)	20
K_M (% w/v)	1.1821
K_M (mol/L)	0.0657
Vmax experimental (mg/mL*min)	0.346
Vmax graphic (mg/mL*min)	0.3872
K_3 (mg/mg*min)	0.0678

* (E_0) = mL E/100g fresh mass. **(S) = g dry mass/100mL *** The density (ρ) of enzymatic mix was esteemed in 1.16 g/mL.

viscosity when mixed with water (Cardenas *et al.*, 1998) increasing said viscosity when the concentration of substrate increases; this effect can be minimized, in certain way, augmenting the drying temperature for the production of the CWF between 75 and 80°C (Saenz, 1997). The sample of cladodes in this investigation was dried at 78°C (initially were made tests at 60°C) and during the enzymatic treatments (increasing the substrate concentration) preliminarily it was observed that the mucilage content could be affected by the increment in temperature, what translates into a noticeable improvement of the processes due to decrease in viscosity; however, it is possible that the mucilage content, still present, produced an inhibitory effect of non competitive type (Cornish-Bowden, 1974; Iyer and Lee, 1999), when combined with the enzymes and the complexes enzyme-substrate, lowering the speed of the reaction.

In the representation of Lineweaver - Burk (Fig. 2) it could be determined a value for R^2 of 0.8533; what makes evident that the obtained values follow a kinetic of Michaelis-Menten (Nunez, 2001; Whitaker *et al.*, 2003). In the straight-line equation y = 3.0527x + 2.5824, the inverse of the value 2.5824, defined V_{max}, that was 0.3872 mg/mL*min. The slope of the line (3.0527) permitted the calculation of Michaelis-Menten constant ($K_M = m * V_{max}$), giving a value of 1.1821% (w/v), what is equivalent to 0.0657 mol/L. The turnover number, defined by the equation $K_3 = V_{max}/ (E_0)$, where (E_0) is the optimum enzymes concentration (0.8 mL E/100 g fresh mass), was represented by a value of 0.0678 mg/mg*min. In relation to the V_{max}, the value obtained graphically of 0.3872 mg/mL*min is greater than the value determined experimentally of 0.346 mg/mL*min; what suggests again that there was a decrease in the velocity due to an inhibitor effect of the substrate. (Gregg and Saddler, 1996; Sewalt *et al.*, 1997; Iyer and Lee, 1999). In Table 3, are shown the established kinetic parameters, as concluded in the enzymatic hydrolysis of the CWF.

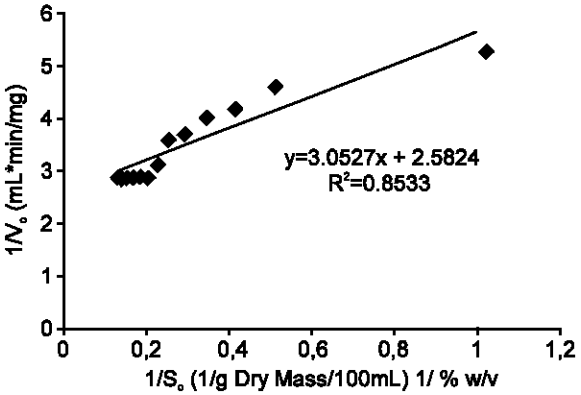

Fig. 2: Lineweaver-Burk model.

Table 4: Process yield steps

Steps	Yield (%)	Reducing sugar (g glucose/100g Dry Mass)
Fresh cladodes	100	ND
Cut	99.47	ND
Dried I	9.32	ND
Grinding I-Sifting I*	8.96	0.57
Dried II	8.61	ND
Grinding II-Sifting II**	8.48	7.53

* Cladodes whole flour. ** Enzymatically hydrolyzed flour. ND: not determined values.

Process yield: During all the process carried out for obtaining the EHF from fresh cladodes, a production yield of the raw material of 8.48% was obtained (Table 4). Basically, the losses are represented as a decrease of water during *Drying I*, this is normal in the sense that the fresh samples of cladodes presented humidity of 90.63%. The CWF (after the *Grinding I and Sieving I*) presented an RS content of 0.57 g glucose/100 g dry mass and after the enzymatic hydrolysis and the operational steps of *Drying II*, *Grinding II* and *Sieving II* (obtainment of EHF), the RS content increased to 7.53 g glucose/100 g dry mass, what represents an increase, or yield in production of sugars of 6.96% (w/w). The productivity obtained under optimal conditions of hydrolysis of 6.96 g glucose/100 g dry mass, is equivalent to 3.48 g glucose/L of solution*h. Sinitsyn *et al.* (1993) expressed that the productivity resulting in the enzymatic hydrolysis of cellulose, usually does not exceed 1 to 5 g/L*h and published, from an experience using a reactor agitated at 150 rpm, a productivity of 6.5 g/L*h and from another experience, using an intensive mass transference reactor, productivities of 32 to 49 g/L*h. In this investigation, the conditions of agitation were of 150 rpm, and the productivity obtained was less than what published Sinitsyn *et al.* (1993) under the same conditions of agitation, and it is probable that the

Table 5: Functional properties of cladodes flours

Parameters	Cladodes whole flour (CWF)	Enzymatically hydrolyzed Flour (EHF)
Water absorption capacity (WAC) (g retained water/g dry mass)	3.54[a]	2.16[b]
Oil absorption capacity (OAC) (g oil/g dry mass)	1.56[a]	1.42[b]
Water solubility (WS) (%)	19.21[a]	19.64[b]
Cations interchange capacity (CIC) (meq OH/g dry mass)	0.46[a]	0.36[b]

Different letters in superindexes in the same row indicate significant differences (P<0.05).

inhibitory effect of the substrate, before mentioned, influenced in obtaining a low productivity (Iyer and Lee, 1999).

Functional properties: The EHF decreased in the WAC (2.16 g retained water/g dry mass) in relation to the CWF (3.54 g retained water/g dry mass), showing significant differences (P<0.05) (Table 5); this could be explained by the lesser fiber content found in the EHF, as a result of the conversion of the fiber into simpler carbohydrates by enzymatic action. The WAC is related, among other factors, to the presence of fiber. The reduction in the content in the EHF of pectins that form gel type colloids, which collect water due to their osmotic action (Salgado-Cruz *et al*., 2005); could be one of the most influential contributing factors that decrease the WAC in the EHF. The CWF and EHF gave values of WAC lower than the ones referred to by Rosado and Diaz (1995) and Saenz (1997) for dehydrated nopal (*Opuntia* sp.) of 11.1 and 5.6 g/g dry mass, respectively; what may be attributed, in addition, to the decrease in mucilage content (characterized for forming viscous mixtures) for effect of the drying treatment at 78^{0}C and even due to the plant species. From the technological point of view, the EHF, for presenting a lower WAC, could require a lower quantity of water for the preparation of compound flour mixtures in the kneading operation. Rosado and Diaz (1995) say that from a physiological point of view, the higher the WAC of a flour, the higher the benefits relative to the increase in viscosity of the intestinal fluids. In comparison, the lowest the WAC, the easier it is to augment the fecal weight and to help the treatment of problems of reduction on the frequency of intestinal evacuation.

Significant differences were found for the obtained values of OAC in the CWF and EHF (P<0.05). The EHF showed a lower OAC (1.42 g oil/g dry mass) in relation to the CWF (1.56 g oil/g dry mass). The decrease in the OAC of the EHF can be attributed to the decrease of the insoluble fiber content. It has been determined that the insoluble fibers have greater values of absorption of organic molecules than the soluble fibers, in part, due to their content in lignin. (Villarroel *et al*., 2003). Rosado and Diaz (1995) indicate an OAC value for dehydrated nopal (*Opuntia* sp.) of 3.7 g/g dry mass, which is higher than the values obtained (1.42 g in the EHF and 1.56 g in CWF). Other sources of fiber have the following values: 2.7 g/g dry mass in carrot bagasse (Chavez *et al*., 2005) and 4 g/g dry mass in wheat bran (Rosado and Diaz, 1995). From the technological point of view, the CWF would have a greater capacity for fixing organic molecules such as lipids and proteins, in relation to the EHF at the time of its possible utilization in the preparation of mixtures of compound flours.

In Table 5 can be appreciated the values of WS, for the samples of CWF and EHF. In EHF, the solubility value increased to 19.64% against 19.21% in CWF. Between both samples existed significant differences (P<0.05). The WS shows the amount of solids dissolved by water when a sample of flour is subjected to an excess of that liquid (Gonzalez *et al*., 1991). The increment in the solubility of the EHF could be attributed to the conversion into simpler sugars, for the effect of enzymatic action on the fibrous material, to which the flour was subjected. It is well known that when the hydrates of carbon decrease in size, their solubility increases. Torres and Guerra (2003), in compound flours with pigeon pea (*Cajanus Cajan*) with and without husk, determined solubility values of 10.93%; showing that the greater solubility value is a consequence of the lower fiber content of the flour without husk. Both values of WS (19.21% in the CWF and 19.64% in the EHF) are lower than the ones published by Rosado and Diaz (1995) for dehydrated nopal (*Opuntia* sp.) which was situated in 34.2% and which was greater than the values determined by Torres and Guerra (2003) of 5.2% for pre cooked corn flour.

In relation to the values of CIC of the samples of CWF and EHF, significant differences were determined between the samples (P<0.05). The EHF presented a lower CIC (0.36 meqOH/g dry mass) in relation to the CWF (0.46 meqOH/g dry mass). The CIC is a property related to the fiber and its capacity to form complexes with some minerals and electrolytes (Salgado-Cruz *et al*., 2005). The increment in fiber content in the diet determines an increment in the loss of sodium, potassium, calcium and magnesium in the fecal matter. (Arrieta *et al*., 2006); Rodriguez - Palenzuela *et al*. (1996) show as well, that the fiber interferes with divalent cations absorption, iron and zinc, what could aggravate deficiency situations. The decrease of the CIC in the EHF is a consequence of fiber degraded by the enzymatic action, what indicates, from a physiological point of view, that it is the flour with the smallest risk of interfering with the absorption of minerals and electrolytes in the intestinal tract, when compared to the

Table 6: Enzymatic activity of Pectinex® and Cellubrix®

Enzymatic preparation	Enzyme volume (mL)	Substrate	Formed glucose*(mg)	Enzymatic activity (mg glucose/mL*min*g)
Pectinex	1.0	Pectin	437.13	5.3
Cellubrix	1.0	Cellulose	265.95	3.55
Pectinex	1.1	CWF**	945.22	0.57
Cellubrix	1.1	CWF**	800	0.48
Pectinex+Cellubrix	0.55+0.55	CWF**	1260	0.84
Pectinex+Cellubrix	1.1+1.1	CWF**	1760	0.53

* Reducing sugars expressed as glucose. **CWF = Cladodes whole flour.

CWF. The value of CIC obtained for the EHF (0.36 meqOH/g dry mass), is smaller than the one determined by Rosado and Diaz (1995) for dehydrated cactus (*Opuntia* sp.) of 4.25 meq OH/g dry mass and similar to the value indicated by Chavez *et al*. (2005) in carrot bagasse of 0.31 meqOH/g dry mass.

Enzymatic activity: Pectinex®, acting on the substrate (pectin) produced 437.13 mg of glucose, what represents 2/3 more than the glucose formed by the action of Cellubrix® on cellulose (used as substrate) that was 265.95 mg (Table 6). The smaller productivity of Cellubrix® can be attributed to the high degree of pure crystallinity and molecular orientation of the substrate (Carrillo, 2002). Under the same conditions, the enzymatic action of Pectinex®, acting individually on the CWF, produced a greater amount of glucose (945.22 mg) in comparison with Cellubrix® (800 mg). The combination of Pectinex® + Cellubrix® in a proportion 1:1, and in the same individual test conditions, produced 1/3 more (1,260 mg) than the amount of glucose formed with Pectinex® (945.22 mg). This is because both enzymatic preparations hydrolyze molecules of different nature producing simple sugars. Finally, the enzymatic activity of the combined action of Pectinex® and Cellubrix® over the CWF, under the optimal conditions determined in this investigation, also was evaluated, using the (E_0), the (S) and the best hydrolysis time, obtaining a value of 0.53 mg glucose/mL*min*g. The reasons expressed justify the evidence that the enzymatic combination of Pectinex® and Cellubrix® produce a synergic effect that benefits a greater production of sugars and supports what pointed out Santamaria *et al*. (2000) who holds that in substrates with high pectin content, the synergic effect between Pectinex® and a commercial cellulose, favors outstandingly the hydrolysis of the cellulosic compounds.

Conclusions: The hydrolysis of the CWF with fibrolytic enzymes increased the sugar output by 6.96% (w/w) as a consequence of the degradation of the fibrous constituents present in the CWF, due to the evident synergistic action between Pectinex® Ultra SP-L and Cellubrix® L, which favored the enzymatic hydrolysis. If in fact is presumed that there was inhibition of the non competitive type, as effect of the mucilage content, during the CWF hydrolysis, it could have been affected by the temperature of 78°C of the initial drying during the obtainment of the CWF, improving the hydrolysis. The EHF is a new product which presented modifications in its functional properties, decreasing the capacity of water and oil absorption and of cation interchange, presenting greater water solubility. This makes feasible its use in the formulation of commercial compound mixtures of flour, being a resource of low cost.

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REFERENCES

Aikenss, J. and T. Dix, 1991. Perhydroxyl radical (HOO●) initiated lipid peroxidation. J. Biol. Chem., 266: 15091-15098.

AOAC, 1990. Official Methods of Analysis. 13th Edn. Washington D.C.

Arrieta, M., M. Di Federico and F. Yaroschevsky, 2006. Protocolo: Constipacion. Rev. Asoc. Coloproct. del. Sur., 1: 117-126.

Ben-Salem, H., A. Nefzaoui and L. Ben-Salem, 2002. Supplementing spineless cactus (Opuntia ficus-indica F. inermis) based diets with urea-treated straw or oldman saltbush (*Atriplex nummularia*). Effects on intake digestion and sheep growth. J. Agric. Sci., 38: 85-92.

Bravo, V., M. Paez, M. El-Hadj, A. Reyes and A. Garcia, 2002. Hydrolysis of carboxymethyl cellulose with mixtures of cellulase and β-1,4-glucosidase. J. Chem. Technol. and Biotechnol., 77: 15-20.

Cabrera, J., D. Paz and A. Gutierrez, 1997. Study of commercial pectic enzyme preparations. I-Polygalacturonase activity of Pectinex Ultra SPL. http://www.inca.edu.cu/otras_web/revista/CT18(1), %201997-INTERNET.htm

Cardenas, A., W. Arguelles and F. Goycoolea, 1998. On the possible role of *Opuntia ficus-indica* mucilage in lime mortar performance in the protection of historical buildings. J. Profess. Assoc. Cactus Develop., 3: 64-71.

Cardenas, A., I. Higuera - Ciapara and F. Goycoolea, 1997. Rheology and aggregation of cactus (*Opuntia ficus-indica*) mucilage in solution. J. Profess. Assoc. Cactus Develop., 2: 152-157.

Carrillo, L., 2000. Microbiologia agricola. http://www.unsa.edu.ar/mata.edu.ar/matbib/micagricap3.pdf

Chavez, Y., L. Flores and Y. Gallardo, 2005. Elaboracion de alimentos a base de fibra de zanahoria por el proceso de extrusion. RESPYN. 13 (CNA-08): 43-48. http://www.respyn.uanl.mx/especiales/2005/ee-13-2005/index.html

Cornish - Bowden, A., 1974. A simple graphical method for determining the inhibition constants of mixed, uncompetitive and noncompetitive inhibitors. Biochem. J., 137: 143-144.

Diaz, V., E. Hedren, J. Ruales and U. Svanberg, 2004. Effect of cell wall degrading enzymes on *in vitro* carotene accessibility in lactic acid fermented carrot beverage. J. Food Sci., 69: 79-84.

Fiske, H. and Y. Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-384.

Flores - Hernandez, A., I. Orona - Castillo, B. Murillo - Amador, R. Valdez - Cepeda and J. Garcia - Hernandez, 2004. Produccion y calidad de nopalito en la region de la Comarca Lagunera de Mexico y su relacion con el precio en el mercado nacional. J. Profess. Assoc. Cactus Develop., 6: 23-34.

Gonzalez, R.J., R.L. Torres, D.M. De Greef, N.A. Gordon and M.E. Velocci, 1991. Influencia de las condiciones de extrusion en las caracteristicas de la harina de maiz para elaborar sopas instantaneas. Rev. Agroquim. Tecnol. Aliment., 31: 87-96.

Gregg, D. and J. Saddler, 1996. Factors affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the effciency of an integrated wood ethanol process. Biotech. Bioeng., 51: 375-383.

Hernandez, R., 2002. Plantas con el metabolismo acido de crasulaceas (MAC o CAM). http://www.forest.ula.ve/~rubenhg/fotosintesis/

Iyer, P. and Y. Lee, 1999. Product inhibition in simultaneous saccharification and fermentation of cellulose into lactic acid. Biotech. Lett., 21: 371-373.

Klyosov, A., 1990. Trends in biochemistry and enzymology of cellulose degradation. Biochem., 29: 10577-10585.

Lees, R., 1990. Analisis de los alimentos. Metodos analiticos y de control de calidad. Acribia, Espana.

Lemos, M., J. Teixeira, M. Domingues, M. Mota and F. Gama, 2003. The enhacement of the cellulolytic activity of cellobiohydrolase I and endoglucanase by the addition of cellulose binding domains derived from *Trichoderma reesei*. Enzyme Microb. Technol., 32: 35-40.

Lopez, J., J. Fuentes, V. Rodriguez and A. Rodriguez, 1997. Establecimiento, utilizacion y manejo del nopal rastero (*Opuntia rastrera* Weber) en el sur de Coahuila, Mexico. En: Memorias de la 7ma Reunion Nacional y 5ta Reunion Internacional sobre el conocimiento y aprovechamiento del nopal. Monterrey, Nuevo Leon, Mexico.

McMillan, Z., C. Scott, C. Taylor and J. Huston, 2002. Nutritional value and intake of prickly pear by goats. J. Range Manage., 55: 139-143.

Min, D. and J. Boff, 2002. Chemistry and reaction of singlet oxygen in foods. Comp. Rev. Food Sci. Food Saf., 1: 58-72.

Medina, M., G. Tirado, I. Mejia, I. Camarillo and C. Cruz-Vazquez, 2006. Digestibilidad *in situ* de dietas con harina de nopal deshidratado conteniendo un preparado de enzimas fibroliticas exogenas. Pesq. Agropec. Bras., 41: 1173-1177.

Moreno - Alvarez, M.J., D.M. Garcia, D.R. Belen, C.A. Medina, N. Munoz, I. Herrera and C. Espinoza, 2006. Evaluacion bromatologica de frutos y cladodios de la tuna (*Opuntia boldinghii* Britton y Rose). Bol. Nakari, 17: 9-12.

Moreno - Alvarez, M.J., C.A. Medina, L. Anton, D.M. Garcia and D.R. Belen, 2003. Uso de pulpa de tuna (*Opuntia boldinghii*) en la elaboracion de bebidas citricas pigmentadas. Interciencia, 28: 539-543.

Nunez, I., 2001. Enzimologia. Piramide, Madrid.

Olle, D., A. Baron, Y. Lozano and J.M. Brillouet, 2000. Enzymatic degradation of cell wall polysaccharides from mango (*Mangifera indica* L.) puree. J. Agric. Food Chem., 48: 2713-2716.

Ovando - Chacon, S., K. Waliszewski and V. Pardio, 2005. The effect of hydration time and ethanol concentration on the rate of hydrolysis of extracted vanilla beans by commercial cellulose preparations. Int. J. Food Sci. Technol., 40: 1011-1018.

Ponce, M., 1989. Distribucion de cactaceas en Venezuela y su ambito mundial. Trabajo de Ascenso. Facultad de Agronomia, Universidad Central de Venezuela, Maracay, pp: 84.

Rodriguez, J., 1997. A comparison of the nutritional value of *Opuntia* and *Agave* plants for ruminants. J. Profess. Assoc. Cactus Develop., 2: 20-24.

Rodriguez - Palenzuela, P., J. Garcia and C. de Blas, 1996. Fibra soluble y su implicacion en nutricion animal: Enzimas y probioticos. http://www. etsia. upm. es/fedna /capitulos/98CAPXIV.pdf

Rosado, J. and M. Diaz, 1995. Propiedades fisicoquimicas relacionadas con funcion gastrointestinal de seis fuentes de fibra dietetica. Rev. Invest. Clin., 47: 283-289.

Ruijten, H., 2004. Extraction method, pharmaceutical composition and a cosmetic composition. Xenobiosis. 6759065. http://www. freepatentsonline. com/6759065.

Saenz, C., 1996. Nopal and cactus pear processing alternatives. http : // ag. arizona. edu / OALS / oals / proj /linkages /cactus /processingalternatives.html.

Saenz, C., 1997. Cladodes: A source of dietary fiber. J. Profess. Assoc. Cactus Develop., 2: 117-123.

Saenz, C., H. Berger, J. Corrales, L. Galletti, V. Garcia, I. Higuera, C. Mondragon, A. Rodriguez - Felix, E. Sepulveda and M. Varnero, 2006. Utilizacion agroindustrial del nopal. Boletin de Servicios Agricolas de la FAO, Nº 162, pp: 168.

Saenz, C. and E. Sepulveda, 2001. Cactus-pear juices. J. Profess. Assoc. Cactus Develop., 4: 3-11.

Saenz, C., E. Sepulveda, N. Pak and X. Vallejos, 2002. Uso de fibra dietetica de nopal en la formulacion de un polvo para flan. Arch. Latinoam. Nutr., 52: 387-392.

Salgado - Cruz, M., D. Cedillo - Lopez and M. Beltran - Orozco, 2005. Estudio de las propiedades funcionales de la semilla de chia (*Salvia hispanica*) y de la fibra dietaria obtenida de la misma. RESPYN., 13 (CNA-53): 358-366. http://www.respyn.ualn.mx/especiales/2005 /ee-13-2005/index.html.

Sandi, M., 2000. Jornadas de nopal. http://www.laneta. apc.org/emis/jornada/2000/enero/nopales.htm.

Santamaria, R., M. Reyes - Duarte, E. Barzana, D. Fernando, F. Gama, M. Mota and A. Lopez - Munguia, 2000. Selective enzyme - mediated extraction of capsaicinoids and carotenoids from chili guajillo puya (*Capsicum annuum* L.) using ethanol as solvent. J. Agric. Food Chem., 48: 3063-3067.

Sewalt, V., W. Glasser and K. Beauchemin, 1997. Lignin impact on fiber degradation. 3. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. J. Agric. Food Chem., 45:1823-1828.

Siddiq, M., A. Khan, J. Harte, K. Dolan, J. Collins and P. Perkins - Veazie, 2005. Impact of extraction methods on the physicochemical and phytochemical quality of blueberry juice. Second COST 926 International Conference. Abstracts.

Sinitsyn, A., A. Gusakov, I. Davydkin, V. Davydkin and O. Protas, 1993. A hyperefficient process for enzymatic cellulose hydrolysis in the intensive transfer reactor. Biotech. Lett., 15: 283-288.

Stintzing, F., A. Schieber and R. Carle, 2002. Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear (*Opuntia ficus-indica* L. Mill.) by high-performance liquid chromatography - electrospray ionization mass spectrometry. J. Agric. Food. Chem., 50: 2302-2307.

Torres, A. and M. Guerra, 2003. Sustitucion parcial de harina de maiz precocida con harina de quinchoncho (*Cajanus cajan*) para la elaboracion de arepas. Interciencia., 28: 660-664.

Van Soest, P. and R. Wine, 1967. Uses of detergent in the analysis of fibrous forages. IV. Determination of plant cell-wall constituents. J. Assoc. off. Anal. Chem., 50: 50-55.

Vilches, L., 2002. Determinacion de la actividad de exoglucanasas de cepas fungicas nativas de las provincias de Huaylas y Huaraz. http://sisbib.unmsm. edu.pe/bibvirtual /tesis/Basic /Vilches_P_L/introduccion.htm.

Villarroel, M., C. Acevedo, E. Yanez and E. Biolley, 2003. Propiedades funcionales de la fibra del musgo *Sphagnum magellanicum* y su utilizacion en la formulacion de productos de panaderia. Arch. Latinoam. Nutr., 53: 400-407.

Viloria - Matos, A., D. Corbelli - Moreno and M.J. Moreno - Alvarez, 2002. Estabilidad de betalainas en pulpa de tuna (*Opuntia boldinghii* Br. *et* R.) sometidas a un proceso de liofilizacion. Rev. Fac. Agron., 19: 324-331.

Ward, O., 1989. Biotecnologia de la fermentacion. Principios, procesos y productos. Acribia, Espana, pp: 238-239.

Whitaker, J.R., A.G.J. Voragen and D.W.S. Wong, 2003. Handbook of food enzymology. Marcel Dekker, New York.