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Chemical Evaluation and Sensory Quality of Sauerkrauts Obtained by Natural and Induced Fermentations at Different NaCl Levels from *Brassica oleracea* Var. *capitata* Cv. Bronco Grown in Eastern Spain. Effect of Storage

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The aim of the present work was to optimize fermentation conditions of white cabbage (*Brassica oleracea* L. var. *capitata* cv. Bronco) grown in winter in eastern Spain. The influence of two salt concentrations (0.5 and 1.5% NaCl) in combination with spontaneous or induced cabbage fermentation on the content of ascorbigen (ABG) and vitamin C as well as on the sensory quality of sauerkraut was investigated. The effect of storage at 4 °C for 1−3 months was also studied. ABG content increased from 14 μmol/100 g of dm in raw cabbage to 63−137 μmol/100 g of dm during fermentation, whereas vitamin C decreased from 354 to 236−277 mg/100 g of dm, and the variations depended on the fermentation conditions. Sauerkrauts obtained by *Leuconostoc mesenteroides* at 0.5% NaCl showed the highest ABG content and a large amount of vitamin C. Refrigeration for 1−3 months led to a reduction of ABG and vitamin C levels, but *L. mesenteroides* sauerkrauts presented considerable amounts of both compounds at the end of the storage period (74−82 μmol/100 g of dm and 33−44 mg/100 g of dm, respectively), higher than those found with *Lactobacillus plantarum* and the mixed starter culture before storage. Experimental sauerkrauts presented better organoleptic properties than the commercial products, and no differences in overall acceptability were found among natural fermentations and those performed with starter cultures. These results suggest than low-salted sauerkraut produced with *L. mesenteroides* provided highly beneficial antioxidant and anticarcinogenic compounds and low sodium content, which is in accordance with the general trend in industrialized countries of reducing the salt level of foods to prevent cardiovascular diseases.

KEYWORDS: Sauerkraut; ascorbigen; vitamin C; fermentation; sensory quality

INTRODUCTION

Increasing consumer demands for healthful foods have fostered the development of an active functional foods market. Numerous vegetable foods are already associated with health promotion and disease prevention. One group of vegetables that has been widely regarded for their antioxidant and anticarcinogenic properties are *Brassica* vegetables, including all cabbage-like vegetables (*1*, *2*). These health-promoting properties are attributed to their high content in antioxidant vitamins and polyphenols and also in glucosinolates (GLS), which are biologically inactive but, after disruption of plant cell, rapidly hydrolyzed by a β-thioglucosidase endogenous enzyme, called myrosinase, to release a complex variety of breakdown products (*3*). These compounds seem to be responsible for the cancer-protecting effects of *Brassica* vegetables (*4*−*6*) through different mechanisms, all involving their ability to modulate the phase I and phase II xenobiotic metabolizing enzyme activities (*7*).

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One of the most important commercial products obtained from *Brassica* vegetables is sauerkraut, which results from the lactic acid fermentation of shredded and salted white cabbage. It has usually been prepared by spontaneous fermentation caused by the lactic acid bacteria (LAB) present on cabbage leaves (*Leuconostoc mesenteroides* and *Lactobacillus plantarum*, predominantly) in a correct succession (*8*). This process represents a cheap cabbage preservation method, and also consumers appreciate traditionally fermented products for their outstanding gastronomic qualities. However, the quality of the end product varies depending on the indigenous microbial populations present on the raw material. The addition of starter cultures has been proposed for sauerkraut production to minimize the impact of this source of variation, improving the product uniformity and quality (*9*, *10*). Salt addition is a critical factor during cabbage fermentation, because the microbial growth and sensory properties of the final product are affected by the amount of salt used. The salt content in sauerkraut usually ranges between 0.6 and 2% NaCl, but it can even exceed 2%. However, consumers nowadays prefer to lower their sodium intake (*11*) for health purposes, which has led to research work aimed at reducing NaCl in fermentation trials.

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Ascorbigen (ABG) is the major GLS derived product found in sauerkraut (*12*), and it is considered to be one of the most potent anticarcinogens of the GLS family (*13*, *14*). ABG is not present in intact plant tissues, and it is formed during cabbage processing by the enzymatic hydrolysis of glucobrassicin, followed by the spontaneous reaction of the intermediate indol-3-carbinol with L-ascorbic acid (*15*, *16*). The formation of ABG is strongly dependent on the pH and the rate of ABG formation increases as the pH in the medium decreases, and the already formed ABG remains relatively stable at acid pH (*16*). ABG content in sauerkraut depends on the content of native glucobrassicin of the raw cabbage, as well as on the fermentation conditions such as the salt concentration and the starter culture used, which also affect the sensory quality of the final product.

In a previous paper (*17*), we have studied the influence of different seasons on the contents of ascorbigen and vitamin C in fermented white cabbage (*Brassica oleracea* var. *capitata* cv. Taler) obtained with two NaCl levels by natural fermentation or by *L. plantarum* or *L. mesenteroides* as starter cultures, and it was shown that the winter cabbage produced the highest level of ascorbigen in sauerkrauts.

The aim of the present work was to study in a different cultivar of winter white cabbage (*B. oleracea* var. *capitata* cv. Bronco) not only the fermentation conditions carried out previously (*17*) but also the influence of *L. plantarum*−*L. mesenteroides* mixed culture (1:1) and storage at 4 °C for up to 3 months on the contents of ascorbigen and vitamin C. To determine consumer acceptability, sensory analyses of the experimental and commercial sauerkrauts were also carried out.

MATERIALS AND METHODS

Plant Material. White cabbages (*B. oleracea* L. var. *capitata* cv. Bronco) grown in the winter of 2007−2008 in eastern Spain (Levante) were selected among five different Spanish cultivars, on the basis of their highest glucobrassicin content. Fresh cabbage heads were provided by Bejo Iberica S.L. (Madrid, Spain) and were stored for < 5 days at 4 °C before fermentation.

Starter Culture Preparation. *L. plantarum* (CECT 748) and *L. mesenteroides* (CECT 219) strains were supplied by the Spanish Type Culture Collection (CECT, Valencia, Spain). They were multiplied twice in MRS broth (Difco Laboratories, Detroit, MI) and incubated overnight at 30 °C. The cells were harvested by centrifugation (5000 rpm, 10 min) and then washed twice in a sterile saline solution (0.9% NaCl). Finally, the starter cultures were inoculated at approximately 10^6 colony-forming units (cfu)/g of cabbage. Three different starter cultures were separately used in the fermentation process: *L. plantarum*, *L. mesenteroides*, and a mixed starter culture containing equal proportions of both strains.

Sauerkraut Production. Cabbage heads were trimmed of outer leaves and their central cores were removed. The edible part of cabbages was then shredded into about 2 mm thick strips using a domestic shredder (Moka Express, Barcelona, Spain). Different salt concentrations (0.5 or 1.5% NaCl) were added onto shredded cabbage and mixed vigorously. Subsequently, cabbage and brine were transferred to autoclaved polyethylene vessels (8 L) and tightly pressed together to exclude air so that the subsequent lactic acid fermentation takes place. Fermentations were performed spontaneously by the indigenous microbiota present on raw cabbage or by using three different starter cultures (induced fermentation): *L. plantarum*, *L. mesenteroides*, or a mixed culture of both microorganisms (1:1). Each type of fermentation was run in three parallel batches (4 kg per batch) at room temperature (22−25 °C) for 7 days. On the third day, cabbage was pricked to remove releasing gases.

Raw and fermented cabbages were freeze-dried, milled, and stored at −20 °C until their analysis.

Storage Conditions. Three samples of sauerkraut corresponding to each fermentation batch were placed in sterile capped glass vessels (500 mL) at the end of the fermentation process, simulating the packaging and storage in households. Then, they were stored at 4 °C for 1, 2, and 3 months.

Chemical Analysis. *Determination of pH during Fermentation.* Brine samples from each fermentation vessel (2 mL) were collected at 0, 3, and 7 days of fermentation and their pH was measured with a pH-meter Basic 20 (Crison, Barcelona, Spain). The mean values and the standard deviation of the three batches of each type of fermentation were calculated.

Analysis of Ascorbigen. The content of ABG in raw and fermented cabbage was determined as in Martinez-Villaluenga et al. (*17*) with slight modifications. Briefly, 0.5 g of freeze-dried material was homogenized with a solution containing distilled water/acetone (1:1) using an Ultra Turrax homogenizer T-25 Digital (Ika Werke GMBH & Co. KG, Staufen, Germany), and the mix was centrifuged for 7 min at 5000 rpm and 5 °C. The supernatant was collected, and the pellet was extracted twice with 10 mL of acetone (Carlo Erba, Rodano, Italy). Then the supernatants were combined and, after filtration, concentrated to a total volume of ∼7 mL. The concentrate was extracted twice with 15 mL of ethyl acetate (LAB-SCAN, Gliwice, Poland). The combined organic layers were dried over anhydrous sodium sulfate (Panreac, Barcelona, Spain), filtered, and evaporated under vacuum to dryness. The residue was dissolved in acetonitrile (LAB-SCAN) and made up to 5 mL with 0.1 M ammonium acetate (Merck, Darmstadt, Germany), pH 5.7, as mobile phase A composition.

Quantification of ABG was performed by HPLC using an Alliance Separation Module 2695 (Waters, Milford, MA), a photodiode array detector 996 at 280 nm (Waters), and a personal computer running Empower 2 for Microsoft Windows chromatographic software (Waters). The sample (20 μL) was injected into an ODS-2 column, 150 × 4.6 mm i.d., 5 μm size column (Waters), at 30 °C. The chromatogram was developed at a flow rate of 1.2 mL/min by elution in a gradient of mobile phase A (0.1 M ammonium acetate, pH 5.7, containing 10% acetonitrile) and mobile phase B (0.1 M ammonium acetate, pH 5.7, containing 80% acetonitrile) as follows: linear gradient of 100% A−100% B for 25 min, isocratic 100% B for 5 min, linear gradient of 100% B−100% A for 5 min, equilibrate for 5 min.

Standard ABG was used for identification and quantification by HPLC. The synthesis was performed according to the method of Kiss and Neukon (*18*) with some modifications. Briefly, 440 mg of ascorbic acid (2.5 mM) (Merck) was dissolved in 20 mL of phosphate buffer (pH 4) (Merck), and 370 mg of 3-hydroxymethylindole (2.5 mM) (Sigma, Steinheim, Germany) was added. The mixture was kept at room temperature during 1 h, under nitrogen and protected from light. The solids formed were filtered, and the solution was washed with ethyl ether (Carlo Erba) and ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated under vacuum. Evaporation of the ethyl acetate extract yielded ABG (∼200 mg), and the purity of standard ABG was determined by HPLC. The compound was frozen (−20 °C) under nitrogen and protected from light. A calibration curve was plotted and adjusted by using the method of least-squares. The regression coefficient of the ascorbigen curve was > 0.990.

Determination of Vitamin C. The quantification of ascorbic acid content in raw cabbage and sauerkrauts was carried out by capillary electrophoresis (CE) using a fused silica capillary TSP075375 (47 cm × 75 μm) purchased from Composite Metal Services Ltd. (The Chase, Hallow, Worcester, U.K.). A P/ACE system 2050 (Beckman Instruments, Fullerton, CA) and UV detection at 254 nm (*19*) were used for the analysis. Briefly, 0.5 g of freeze-dried cabbage was extracted with 20 mL of 3% metaphosphoric acid (Sigma-Aldrich, Steinheim, Germany), and after homogenization for 2 min using a Ultra Turrax homogenizer T25 Digital (Ika Werke GmbH & Co. KG, Staufen, Germany), the volume was adjusted to 25 mL with 3% metaphosphoric acid. The resultant slurry was filtered through a Whatman no. 1 filter paper, and 1.5 mL of the filtrate was added to 100 μL of isoascorbic acid (Fluka, Steinheim, Germany) as internal standard (0.6 mg/mL) in aqueous 0.2% D,D-dithiothreitol (Sigma-Aldrich), made up to 2 mL with aqueous 0.2% D,L-dithiothreitol, mixed thoroughly, and filtered through a 0.45 μm membrane. D,L-Dithiothreitol is added to prevent oxidation of the ascorbic acid to the corresponding dehydroascorbic acid. Extractions were performed in triplicate. Ascorbic acid was quantified from a calibration curve built with pure ascorbic acid standard (Fluka) and with the response factor relative to internal standard.

Sensory Analysis. Ten panelists from the Institute of Industrial Fermentations (CSIC) were selected on the basis of availability, sauerkraut

Table 1. Defined Attributes for Sensory Analysis of Sauerkraut Using Category Scaling

attribute[a]	definition
flavor/aroma raw cabbage	green, vegetative aroma and flavor of raw cabbage
flavor/aroma kraut sulfur	strong sulfur note characteristic of properly fermented sauerkraut
acid flavor	sour taste associated with organic acids in solution
saltiness	basic taste associated with sodium chloride in solution
firmness	amount of effort required to masticate the sample
color	graduated scale from green to creamy color
others	any other perceptive attribute that is not anticipated
overall acceptability	overall qualification

[a] Scale from 0 = not detectable to 10 = very strong. For firmness 0 = very soft to 10 = very strong. For color 0 = green to 10 = creamy color. For overall acceptability 0 = not acceptable to 10 = excellent acceptability.

acceptance, prior panel experience, and ability to distinguish the basic scale tastes.

A scale from 0 = not detectable to 10 = very strong was used for aroma and flavor attributes of sauerkraut, as shown in **Table 1** and described by Johanningsmeier et al. (*20*). Firmness was scored using a scale from 0 = very soft to 10 = very firm. Color was scored with the values ranging from 0 = green, 2 = green-yellowish, 4 = light yellow, 6 = dark yellow, 8 = yellow−creamy to 10 = creamy. Overall acceptability was scored from 0 = not acceptable to 10 = excellent acceptability.

Every evaluation was carried out with the three experimental batches of each of the experimental sauerkrauts stored under refrigeration for 1 month and three commercial sauerkrauts (A, B, and C). A total of five samples were presented to each panelist in a random order at each testing section. Commercial drinking water and unsalted crackers were provided to the panel for palate cleansing between samples.

Statistical Analysis. Data were subjected to multifactor ANOVA using the least-squared difference test (LSD) with the Statgraphic 5.0 Program (Statistical Graphics Corp., Rockville, MD) for Windows.

RESULTS AND DISCUSSION

Evolution of pH during Cabbage Fermentation. The decrease of pH during fermentation can be considered a key variable for monitoring the success of vegetable fermentations, as Johanningsmeier et al. (*19*) reported. In the present study, the pH modifications of sauerkraut during spontaneous or induced cabbage fermentation using different starter cultures were measured, and the results are depicted in **Figure 1**. Before fermentation started, the pH of shredded cabbage ranged between 5.7 and 6. In the course of the fermentation, the pH value of sauerkraut decreased to 3.2−3.9, and a similar trend in the rate of pH reduction was found in sauerkrauts obtained at both salt concentrations. The pH values attained at the end of fermentation are in line with those reported by other authors in sauerkrauts obtained by natural and induced fermentations (*11*, *19*, *20*), and they were higher than those of studied commercial sauerkrauts (pH 3.1−3.2), with the exception of sauerkrauts obtained with mixed culture at 0.5% NaCl, which showed a pH of 3.22.

During the first 3 days, the pH decrease was faster in induced fermentations than in natural ones, regardless of the starter culture used. These results indicated that fermentation was accelerated by the addition of starter cultures because LAB are present in higher numbers than in natural fermentations, and also they lack the lag phase unlike LAB populations naturally present in raw cabbage as Desai and Sheth (*22*) and Harris et al. (*10*) reported. A rapid decrease in pH at the beginning of fermentation is of great importance for sauerkraut quality, because it minimizes the influence of spoilage bacteria (*11*) and improves the quality of the final product.

From day 3 to day 7, the pH value of natural fermented cabbage continued decreasing, whereas this parameter did not

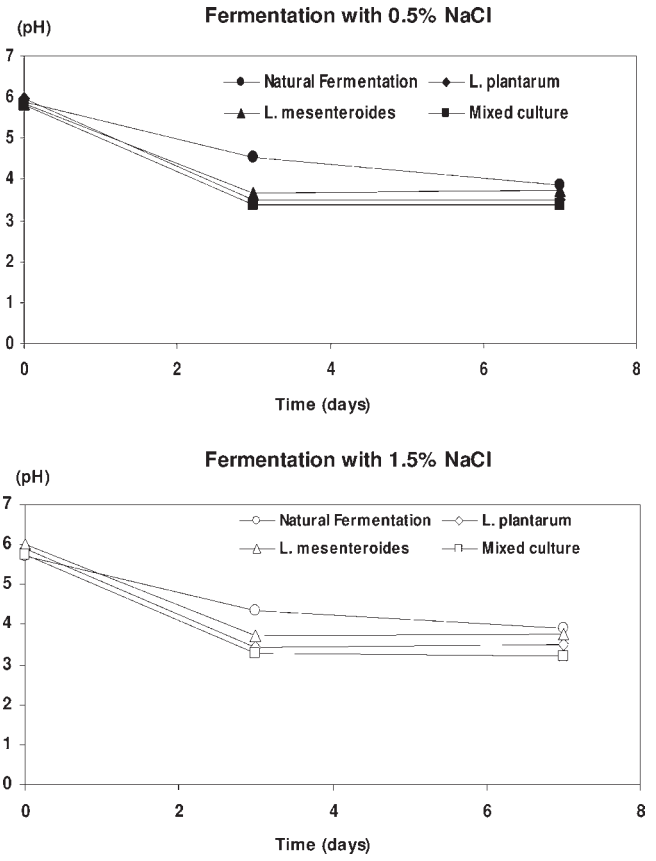

Figure 1. Evolution of pH during spontaneous or induced cabbage fermentation using different starter cultures at 0.5 and 1.5% NaCl.

significantly change in all of the starter-induced fermentations. Cabbage inoculated with the mixed starter culture showed lower pH levels than sauerkraut obtained in the other conditions. These results agree with those reported by Kohajdová and Karovicová (*21*), who found a higher decrease of pH in cabbage juice fermented by a *L. plantarum*−*Saccharomyces cerevisiae* mixed culture than in those fermented spontaneously or by *L. plantarum*.

Effect of Fermentation Conditions and Storage on the Content of ABG in Sauerkraut. The content of ABG in raw cabbage (*Brassica oleracea* var. *capitata* cv. Bronco) and sauerkrauts obtained under different fermentation conditions is show *L. plantarum* in **Tables 2**−**5**. Raw shredded cabbage was found to contain low amounts of ABG (14 μmol/100 g of dry weight). Despite the fact that this phytochemical is reported to be not present in intact plant tissues (*4*), ABG was found in shredded cabbage because a certain amount of glucobrassicin might have been hydrolyzed by the endogenous myrosinase as a consequence of cell damage, resulting in the production of low amounts of ABG in raw cabbage. ABG levels found in the present work are slightly higher than those reported by Ciska and Pathak (*12*) and Martínez-Villaluenga et al. (*17*) for cultivars Kamienna Glowa and Taler, respectively, of white cabbage.

During fermentation, ABG content increased significantly ($P \leq 0.05$) and the extent of such increment depended on the fermentation conditions (**Tables 2**−**5**). Sauerkrauts obtained at low salt concentration (0.5%) showed significantly ($P \leq 0.05$) higher ABG concentrations than those obtained at higher NaCl level (1.5%), regardless of the starter culture used for sauerkraut production. These findings agree with those reported previously by our group for sauerkrauts obtained from white cabbage cv. Taler grown in different seasons (winter and summer) (*17*).

Table 2. Ascorbigen and Ascorbic Acid Contents of Natural Fermented *Brassica oleracea* Var. *capitata* Cv. Bronco Cabbage: Effect of Storage[a]

fermented cabbage	ascorbigen (μmol/100 g of dm)	ascorbic acid (mg/100 g of dm)	water (%)
raw cabbage	13.94 ± 2.44	325.78 ± 14.32	91.7
natural fermentation, 0.5% NaCl			
0 time of storage	100.84 ± 2.76	$263.29 \pm 13.03_{5}$	92.0
1 month of storage	96.14 ± 2.97	$181.91 \pm 5.64_{5}$	92.2
2 months of storage	54.34 ± 2.81	$88.29 \pm 3.35^{b}{}_{2}$	92.2
3 months of storage	42.64 ± 1.03	$38.38 \pm 3.33^{a}{}_{1}$	92.1
natural fermentation, 1.5% NaCl			
0 time of storage	75.05 ± 3.13	$242.72 \pm 17.84_{12}$	91.3
1 month of storage	72.20 ± 2.95	$174.77 \pm 11.03_{23}$	91.4
2 months of storage	42.14 ± 1.94	$86.26 \pm 4.84^{b}{}_{1}$	91.5
3 months of storage	35.12 ± 1.43	$37.71 \pm 2.52^{a}{}_{1}$	91.3

[a] Mean value $\pm$ SD. The same superscript in the same column means no significant difference ($P \leq 0.05$). The same subscript for the same storage time of each column among **Tables 2**−**5** means no significant difference ($P \leq 0.05$).

Table 3. Ascorbigen and Ascorbic Acid Contents of *Brassica oleracea* Var. *capitata* Cv. Bronco Cabbage Fermented with *L. plantarum*: Effect of Storage[a]

fermented cabbage	ascorbigen (μmol/100 g of dm)	ascorbic acid (mg/100 g of dm)	water (%)
raw cabbage	13.94 ± 2.44	325.78 ± 14.32	91.7
L. plantarum, 0.5% NaCl			
0 time of storage	79.22 ± 2.78	$256.98 \pm 5.42_{45}$	91.7
1 month of storage	60.58 ± 0.83	$181.21 \pm 4.93_{45}$	92.3
2 months of storage	37.88 ± 3.48	103.35 ± 2.55	92.3
3 months of storage	26.44 ± 4.50	45.65 ± 1.38	92.3
L. plantarum, 1.5% NaCl			
0 time of storage	44.19 ± 1.07	$247.11 \pm 5.97_{23}$	91.6
1 month of storage	37.22 ± 2.12	$169.89 \pm 1.42_{1}$	91.0
2 months of storage	29.83 ± 0.90	95.78 ± 2.70	91.0
3 months of storage	$23.96 \pm 0.69_{1}$	$38.92 \pm 2.14_{1}$	90.8

[a] Mean value $\pm$ SD. The same superscript in the same column means no significant difference ($P \leq 0.05$). The same subscript for the same storage time of each column among **Tables 2**−**5** means no significant difference ($P \leq 0.05$).

Table 4. Ascorbigen and Ascorbic Acid Contents of *Brassica oleracea* Var. *capitata* Cv. Bronco Cabbage Fermented with *L. mesenteroides*: Effect of Storage[a]

fermented cabbage	ascorbigen (μmol/100 g of dm)	ascorbic acid (mg/100 g of dm)	water (%)
raw cabbage	13.94 ± 2.44	325.78 ± 14.32	91.7
L. mesenteroides, 0.5% NaCl			
0 time of storage	137.12 ± 1.70	$254.18 \pm 13.11_{34}$	92.0
1 month of storage	114.92 ± 5.43	$174.92 \pm 2.04^{b}{}_{23}$	91.8
2 months of storage	89.53 ± 2.40	$86.45 \pm 2.09^{a}{}_{12}$	91.9
3 months of storage	82.08 ± 1.44	43.58 ± 1.96	91.8
L. mesenteroides, 1.5% NaCl			
0 time of storage	124.84 ± 1.57	$235.55 \pm 5.58_{1}$	91.8
1 month of storage	106.56 ± 10.71	$178.10 \pm 2.81^{b}{}_{34}$	91.6
2 months of storage	78.98 ± 2.48	$87.46 \pm 1.93^{a}{}_{12}$	91.7
3 months of storage	73.71 ± 1.70	29.85 ± 2.22	91.6

[a] Mean value $\pm$ SD. The same superscript in the same column means no significant difference ($P \leq 0.05$). The same subscript for the same storage time of each column between **Tables 2-5** means no significant difference ($P \leq 0.05$).

Table 5. Ascorbigen and Ascorbic Acid Contents of *Brassica oleracea* Var. *capitata* Cv. Bronco Cabbage Fermented with *L. plantarum* and *L. mesenteroides*: Effect of Storage[a]

fermented cabbage	ascorbigen (μmol/100 g of dm)	ascorbic acid (mg/100 g of dm)	water (%)
raw cabbage	13.94 ± 2.44	325.78 ± 14.32	91.7
L. plantarum and *L. mesenteroides*, 0.5% NaCl			
0 time of storage	62.52 ± 1.58	$263.71 \pm 10.46_{5}$	92.2
1 month of storage	42.07 ± 5.45	$171.69 \pm 4.35^{c}{}_{12}$	92.8
2 months of storage	33.14 ± 4.59	$86.57 \pm 2.49^{b}{}_{12}$	92.6
3 months of storage	$23.82 \pm 1.48_{1}$	33.95 ± 2.95^{a}	92.8
L. plantarum and *L. mesenteroides*, 1.5% NaCl			
0 time of storage	59.80 ± 1.95	277.37 ± 9.01	90.4
1 month of storage	30.58 ± 0.87	$176.14 \pm 4.67^{c}{}_{3}$	90.7
2 months of storage	20.81 ± 0.86	$87.42 \pm 3.18^{b}{}_{12}$	90.8
3 months of storage	15.53 ± 1.26	$37.72 \pm 3.35^{a}{}_{1}$	90.6

[a] Mean value $\pm$ SD. The same superscript in the same column means no significant difference ($P \leq 0.05$). The same subscript for the same storage time of each column among **Tables 2**−**5** means no significant difference ($P \leq 0.05$).

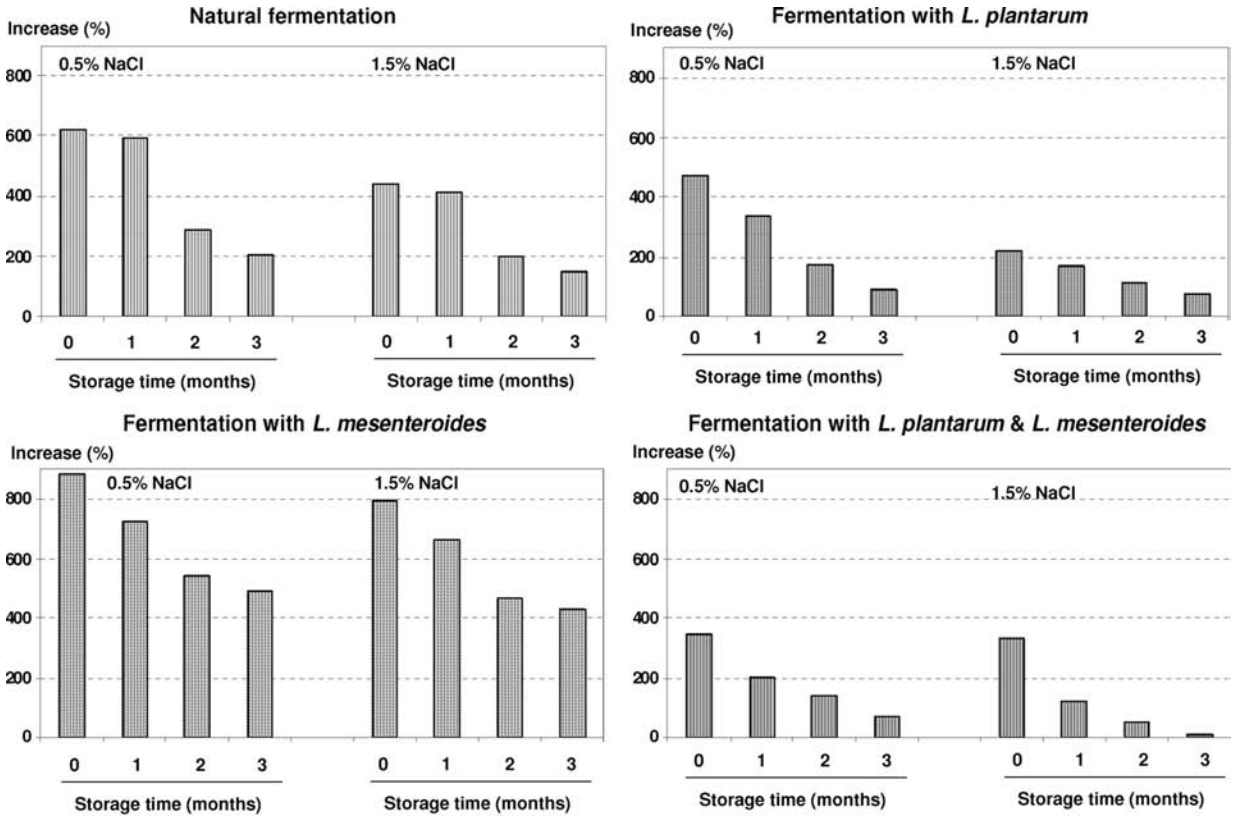

Figure 2. Increase of ascorbigen content in cabbage fermented spontaneously or using different starter cultures at 0.5 and 1.5% NaCl.

The reduction of salt concentration may reduce the volume of brine formed during fermentation, thereby increasing the sauerkraut yield. Furthermore, the brine constitutes an environmental and industrial problem because it is a nondegradable byproduct (*20*).

The LAB species used as starter culture during the fermentation process had also an important influence on the formation of ABG in sauerkraut. Thus, the highest ABG content was observed in sauerkraut produced by *L. mesenteroides* at 0.5 and 1.5% NaCl (137 and 125 μmol/100 g of dm, respectively) (**Table 4**), whereas sauerkrauts obtained using the mixed culture (**Table 5**) presented the lowest ABG concentrations (63 and 60 μmol/100 g of dm at 0.5 and 1.5% NaCl, respectively). Natural fermentations (**Table 2**) and those performed by *L. plantarum* (**Table 3**) showed intermediate ABG amounts, ranging between 75 and 101 μmol/100 g of dm and between 44 and 79 μmol/100 g of dm, respectively. Ciska and Pathak (*12*) reported ABG values of 14 μmol/100 g of fresh weight in natural fermented cabbage, whereas Martinez-Villaluenga et al. (*17*) found levels which ranged between 75 and 109 μmol/100 g of dm in white cabbage cv. Taler fermented spontaneously or by using *L. plantarum* or *L. mesenteroides* as starter cultures. The different levels of ABG found by different authors not only could be caused by different initial levels of glucobrassicin, but also other factors such as pH and temperature considerably affect the formation and stability of ABG (*16*). In addition, the findings of the present work suggest that the hydrolysis of glucobrassicin and, consequently, the ABG formation may be affected by the starter bacteria used in the fermentation process. In this sense, Tolonen et al. (*23*) observed that sauerkrauts obtained during fermentation with a starter mixture containing *L. mesenteroides* and *Pediococcus dextrinicus* (1:1) presented higher concentration of indol-3-carbinol, a precursor of ABG, than that produced by spontaneous fermentation.

The influence of refrigerated storage on the ABG content of sauerkrauts produced under different conditions is shown in **Tables 2−5** and **Figure 2**. A gradual but significant ($P \leq 0.05$) decrease of ABG levels was observed during storage in all experimental sauerkrauts, reaching the lowest values at the end of the storage period. At the end of storage period, the content of this phytochemical in natural fermented cabbage salted at 0.5 or 1.5% NaCl decreased about 58 and 53%, respectively, compared with unstored products. Sauerkrauts obtained by *L. plantarum* at the same salt concentrations and kept for 3 months at 4 °C exhibited a diminution of ABG content around 66 and 46%, respectively, whereas for those fermented with the mixed starter culture the losses were around 62% at 0.5% NaCl and 74% for 1.5% NaCl content compared with time zero of storage. For *L. mesenteroides* fermentations, the ABG amount was about 40% lower in 3-months-stored sauerkrauts than in unstored ones. However, it should be noted that even after 3 months of storage, *L. mesenteroides* sauerkrauts presented high ABG concentration, especially at 0.5% NaCl, a condition in which ABG levels were higher than those obtained in sauerkrauts with *La. plantarum* or mixed starter culture before storage. There is scarce information about the effect of storage on the ABG content in sauerkraut obtained with different starter cultures. In naturally fermented cabbage stored for 2−17 weeks at 5 °C, Ciska and Pathak (*12*) did not find appreciable changes in ABG content. These results show the different stabilities of ABG depending on the fermentation conditions used during sauerkraut manufacture.

Effect of Fermentation Conditions and Storage on the Content of Ascorbic Acid in Sauerkraut. Tables 2−5 present the influence of fermentation conditions and refrigerated storage on the content of vitamin C, measured as ascorbic acid. Raw white cabbage showed a large vitamin C amount (~326 mg/100 g of dm), which is in accordance with the levels previously reported for raw

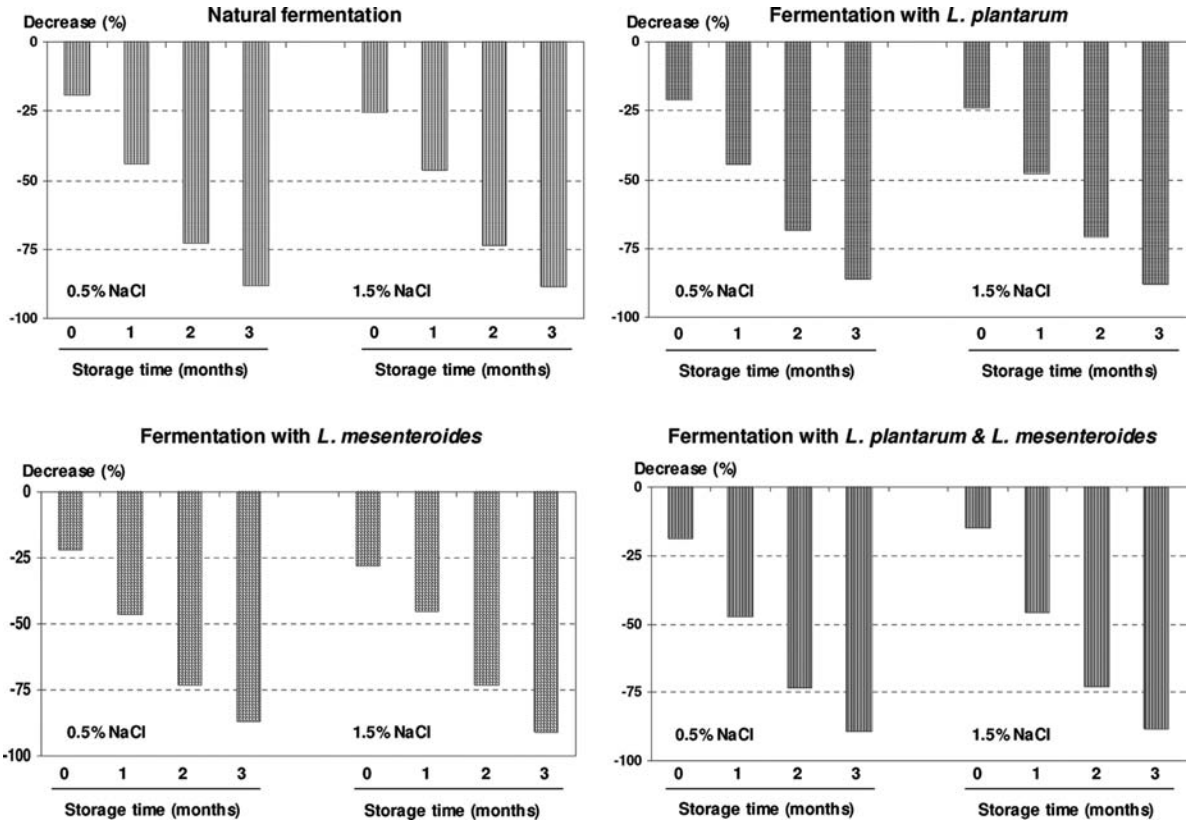

Figure 3. Decrease of ascorbic acid in cabbage fermented spontaneously or using different starter cultures at 0.5 and 1.5% NaCl.

cabbage in the literature (*17*, *24*, *25*) and higher than that found in other *Brassica* vegetables such as cauliflower, Brussels sprouts, and Chinese cabbage (*26*). Vitamin C is an important dietary antioxidant that is able to scavenge the free radicals than can cause oxidative damage to macromolecules and is implicated in chronic diseases (*27*). Epidemiological data as well as in vitro studies strongly suggest that the consumption of vegetables having antioxidant compounds, such as vitamin C, has strong protective effects against degenerative diseases such as cancer and cardiovascular diseases (*28*).

During cabbage fermentation, vitamin C content dropped 15−28% depending on the fermentation conditions (**Tables 2−5** and **Figure 3**), and levels between 236 and 277 mg/100 g of dm were found. In general, sauerkrauts obtained with 1.5% NaCl presented significantly ($P \leq 0.05$) lower vitamin C levels than those obtained at 0.5% NaCl, with the exception of fermented cabbage obtained by mixed starter culture at 1.5% NaCl that exhibited the highest ascorbic acid content (277 mg/100 g of dm). Despite these results, the data indicate that the type of LAB used during the fermentation process did not have an important influence on the ascorbic acid content of sauerkraut. Similar levels of vitamin C were reported earlier by our group in white cabbage cv. Taler grown in winter (*17*).

Losses of vitamin C content during fermentation can be attributed principally to its reaction with indol-3-carbinol to form ABG. Hrncirik et al. (*29*) reported that the decrease of ascorbic acid content as a result of its transformation into ABG will probably not reach more than 10%. The additional drops of this compound observed in the present work might be related to its chemical and enzymatic oxidation during sauerkraut production. The removal of outer leaves before fermentation could also contribute to the decrease of vitamin C in sauerkraut, because they contain higher vitamin C amounts than the inner ones (*30*).

Storage at 4 °C for 1, 2, and 3 months led to a gradual decrease in vitamin C content (**Tables 2−5**; **Figure 3**), reaching values between 30 and 46 mg/100 g of dm at the end of the storage period, depending on the starter culture used. Cabbage fermented by *L. mesenteroides* and salted with 1.5% NaCl presented the lowest vitamin C amount (30 mg/100 g of dm), whereas naturally fermented cabbage obtained at 0.5% NaCl showed the highest value (46 mg/100 g of dm) after 3 months of storage (**Tables 2−5**). In general, significantly ($P \leq 0.05$) higher vitamin C levels were observed for 0.5% NaCl fermentations, with the exception of those performed with the mixed starter culture (**Tables 2−5**; **Figure 3**). No information has been found about the effect of storage on the vitamin C levels of sauerkraut, and the losses observed in the present work could be due to oxidation reactions occurred during storage, as has been reported previously in stored leafy vegetables (*28*).

Evaluation of Sauerkraut Sensory Properties. Although the change in pH is a good parameter for monitoring the fermentation process, as was described above, it is not necessarily indicative of the sensory quality of the sauerkrauts produced. For this reason, we considered it necessary to evaluate the sensory properties of the end products obtained in the present work and thus determine the most acceptable sauerkraut for consumers.

Acceptance of flavor and aroma, firmness, color, and overall acceptability were the parameters chosen to evaluate the sensory quality of sauerkrauts. Three commercial products were also included in the sensory analysis and were considered reference samples. The scores obtained for different sensory attributes in experimental and commercial sauerkrauts are shown in **Table 6** and **Figure 4**.

Flavor Evaluation. Raw cabbage flavor, kraut sulfur flavor, acid flavor, and saltiness were the descriptors selected for the evaluation of taste and odor in sauerkrauts.

Table 6. Sensorial Analysis of Commercial Sauerkraut and Cabbage Fermented at Laboratory Scale[a]

	raw cabbage flavor	kraut sulfur flavor	acid flavor	saltiness	firmness	color	overall acceptability
commercial sauerkrauts							
A	2.5 ± 2.2^{ab}	4.7 ± 2.6^{c}	8.0 ± 1.3^{f}	5.0 ± 2.4^{d}	6.0 ± 1.9^{b}	7.2 ± 2.1^{d}	4.0 ± 2.0^{a}
B	3.0 ± 2.4^{ab}	4.6 ± 2.1^{c}	6.8 ± 1.4^{de}	5.0 ± 1.9^{d}	6.0 ± 1.7^{b}	7.9 ± 1.7^{e}	5.6 ± 1.5^{b}
C	2.9 ± 2.3^{ab}	5.0 ± 1.8^{c}	6.7 ± 1.4^{d}	5.1 ± 1.8^{d}	5.3 ± 1.8^{a}	8.7 ± 1.8^{f}	5.5 ± 1.2^{b}
pilot plant sauerkrauts							
natural fermentation, 0.5% NaCl	2.3 ± 2.3^{ab}	2.6 ± 1.8^{a}	5.6 ± 1.9^{bc}	3.4 ± 1.8^{ab}	6.5 ± 1.4^{bc}	3.6 ± 2.0^{ab}	6.8 ± 1.9^{c}
natural fermentation, 1.5% NaCl	4.9 ± 1.9^{c}	3.6 ± 2.2^{ab}	4.3 ± 1.9^{a}	4.0 ± 1.7^{abc}	6.8 ± 1.5^{cd}	4.1 ± 1.0^{abc}	6.8 ± 1.1^{c}
L. plantarum, 0.5% NaCl	3.2 ± 2.3^{bc}	3.6 ± 2.5^{ab}	4.9 ± 1.9^{ab}	3.2 ± 1.3^{a}	7.0 ± 1.2^{cd}	4.2 ± 1.1^{bc}	7.2 ± 1.2^{c}
L. plantarum, 1.5% NaCl	4.0 ± 2.2^{cd}	4.4 ± 1.3^{bc}	5.9 ± 1.1^{c}	4.2 ± 1.0^{bcd}	7.0 ± 1.4^{cd}	4.2 ± 1.1^{abc}	7.5 ± 1.5^{c}
L. mesenteroides, 0.5% NaCl	2.9 ± 1.9^{ab}	3.3 ± 1.4^{ab}	4.8 ± 1.4^{ab}	3.2 ± 1.4^{a}	7.5 ± 1.0^{de}	4.3 ± 1.3^{bc}	6.9 ± 1.5^{c}
L. mesenteroides, 1.5% NaCl	2.9 ± 2.2^{abc}	4.1 ± 1.6^{bc}	5.8 ± 1.1^{c}	4.4 ± 1.5^{cd}	7.2 ± 0.9^{cde}	4.5 ± 1.2^{c}	7.0 ± 1.3^{c}
mixed starter culture, 0.5% NaCl	2.1 ± 1.9^{a}	3.3 ± 2.3^{ab}	7.5 ± 0.9^{ef}	4.0 ± 2.4^{abc}	8.0 ± 0.5^{e}	3.5 ± 0.8^{ab}	6.9 ± 1.0^{c}
mixed starter culture, 1.5% NaCl	2.8 ± 2.5^{ab}	4.1 ± 1.9^{bc}	6.3 ± 1.2^{cd}	4.8 ± 2.1^{cd}	7.5 ± 0.9^{de}	3.4 ± 1.2^{a}	7.1 ± 0.8^{c}

[a] The same superscript in the same column means no significant differences ($P \leq 0.05\%$).

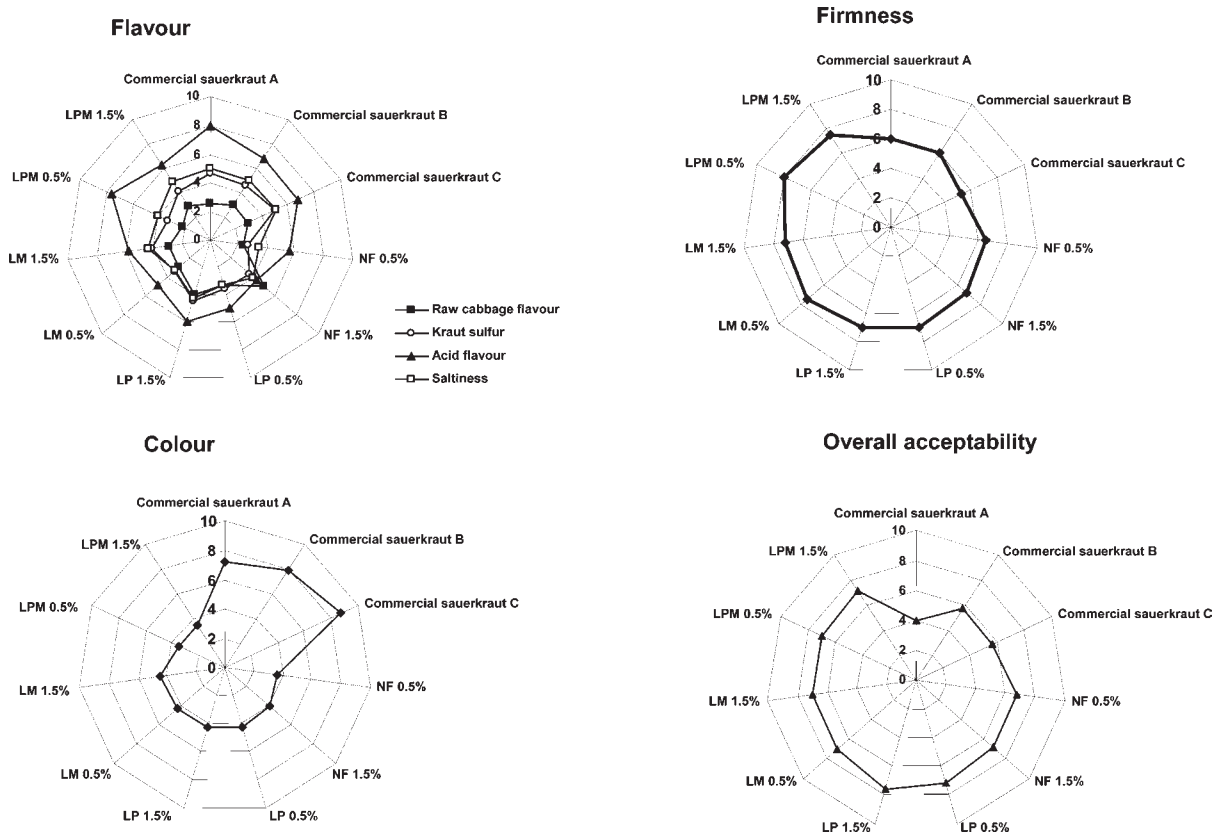

Figure 4. Diagrams of sensorial evaluation of commercial and experimental sauerkrauts. NF: natural fermentation; LP: fermentation with *L. plantarum*; LM: fermentation with *L. mesenteroides*; LPM: fermentation with *L. plantarum* and *L. mesenteroides*.

Panelists perceived the raw cabbage-like flavor in all sauerkraut assessed with low intensity, as **Table 6** and **Figure 4** show. Low scores for this attribute can be considered a positive characteristic for consumers, because raw cabbage-like taste is associated with green or immature sauerkraut (*8*). Natural fermentation performed at 1.5% NaCl received the highest score for this descriptor, and no significant differences ($P \leq 0.05$) were found between this sauerkraut and those obtained by *L. plantarum* at both salt concentrations. In addition, no significant differences were found ($P \leq 0.05$) for raw cabbage flavor between commercial sauerkrauts and *L. mesenteroides* and the mixed starter culture at both NaCl levels and with natural fermentation and *L. plantarum* at 0.5% NaCl. Isothiocyanates generated from glucosinolates by the action of myrosinase have been reported to be responsible for this pungent raw-cabbage flavor (*31*).

Kraut sulfur, which is the typical sulfurous flavor associated with properly fermented sauerkraut, was slightly lower at 0.5% NaCl than at 1.5% NaCl, in all fermentation trials, although no significant differences ($P \leq 0.05$) between salt concentrations were observed (**Table 6**; **Figure 4**). These results agree with those reported by Johanningsmeier et al. (*32*) for sauerkraut obtained at three salt concentrations (0.5, 1.0, and 2.0% NaCl) using *L. mesenteroides* as the starter culture. Sulfur compounds derived from *S*-methylcysteine sulfoxide and some glucosinolates in raw cabbage appeared to be important for kraut flavor developed during fermentation.

With respect to the acid flavor, commercial sauerkrauts and those obtained in the presence of the mixed culture were awarded the highest scores (**Table 6**; **Figure 4**). These results are directly linked to the lowest pH values obtained at the end of fermentation (**Figure 1**), as was stated above. Among them, commercial sauerkraut A and sauerkraut obtained with the mixed starter culture at 0.5% NaCl presented significantly ($P \leq 0.05$) higher values for this attribute than the other three sauerkrauts, possibly

due to their similar pH values. In contrast, natural fermented sauerkraut at 1.5% NaCl and sauerkrauts obtained at 0.5% using *L. plantarum* and *L. mesenteroides* showed the lowest values. These fermented products earned higher ratings for overall acceptability than the more acid commercial sauerkrauts, indicating that consumers prefer mild-flavored sauerkrauts, in terms of acid content, as Viander et al. (*11*) suggested. In general, the lowest raw cabbage flavor scores corresponded with the highest acid flavor scores, and these results thus indicate that the acid flavor may be responsible for masking the typical cabbage like-flavor.

The intensity of the salty taste was more acute with the increment of salt concentration when the process was performed with *L. plantarum* or *L. mesenteroides*, whereas no significant differences were found ($P \leq 0.05$) in natural sauerkraut and those obtained with the mixed starter culture at both NaCl levels. In addition, the saltiness scores for the laboratory-scale sauerkrauts were lower than for the commercial sauerkrauts, but the differences were significant ($P \leq 0.05$) only for natural fermentations and those performed by using the starter culture at 0.5% NaCl. No significant differences ($P \leq 0.05$) were perceived between the three commercial products by the consumer panel either. Low saltiness can be considered a positive characteristic of sauerkraut because consumers prefer mildly salted sauerkrauts (*8*). Moreover, the general trend in industrialized countries is to reduce the salt level of foods to prevent cardiovascular diseases. This consumer preference is usually taken into account by sauerkraut manufacturers, who increasingly reduce the salt concentration of their products, as Trail et al. (*33*) reported.

Firmness Evaluation. Commercial fermented products were awarded the lowest firmness scores, and these were followed by naturally fermented sauerkraut at 0.5% NaCl (**Table 6**; **Figure 4**). Sauerkraut obtained by natural fermentation at 1.5% NaCl and those acquired by the addition of starter cultures showed significantly ($P \leq 0.05$) greater scores for firmness than the commercial products. Johanningsmeier et al. (*20*) reported that decreasing the salt concentration from 2.0 to 0.5% in natural cabbage fermentations resulted in a significant softening of sauerkraut. Our data, however, did not show differences between 0.5 and 1.5% NaCl.

Color Evaluation. The evaluation of the sauerkraut color showed significant ($P \leq 0.05$) differences between commercial and experimental sauerkrauts (**Table 6**; **Figure 4**). Commercial sauerkrauts presented creamy colors and earned significantly higher ratings than our experimental sauerkrauts, which showed colors closer to light yellow. The panelists did not perceive important differences between cabbage fermented with 0.5 and 1.5% NaCl, nor did they perceive a difference between natural fermented products and those obtained by *L. plantarum* and mixed culture at both salt concentration and by *L. mesenteroides* at 0.5%.

Overall Acceptability. The evaluation of the global acceptability of sauerkrauts by the sensory panel showed that those obtained at laboratory scale presented a significantly ($P \leq 0.05$) higher acceptability than commercial sauerkrauts (**Table 6**; **Figure 4**). These results may be attributed, in general, to higher acid flavor and saltiness and lower firmness of commercial sauerkrauts. Commercial sauerkraut A, which presented significantly ($P \leq 0.05$) higher acidity than the other commercial sauerkrauts, received the worst evaluation, whereas no significant differences were observed between commercial sauerkrauts B and C.

All experimental trials presented high acceptability by the sensory panel, and no significant differences ($P \leq 0.05$) were observed between naturally fermented products and those obtained by starter culture inoculation. However, it is important to point out that it is hard to achieve good repeatability in natural fermentations due to variations of the natural microbiota in raw cabbage, which depend on environmental (agronomic and climatic conditions) factors. The microbial population variability in cabbage may lead to important changes in sauerkraut sensory properties. The use of starter cultures ensures the uniformity of the final product, irrespective of the natural microbial population in raw cabbage. Moreover, several authors have reported better sensory properties in sauerkrauts fermented by using a starter culture than in naturally fermented products. In this sense, Breidt et al. (*9*) and Harris et al. (*10*) used *L. mesenteroides* as a starter culture in cabbage fermentation and obtained sauerkraut characterized by better sensory quality compared to the control variant. On the other hand, Kohajdová and Karovicová (*21*) found that cabbage juices fermented with a selected strain of *L. plantarum* showed better organoleptic properties than those spontaneously fermented by the autochthonous cabbage microbiota.

On the basis of the results obtained, it can be stated that fermentation enhanced the formation of ascorbigen, which is known to present beneficial effects on human health. The concentration of this phytochemical in sauerkrauts depended on the salt concentration and on the starter culture used during fermentation. Sauerkraut obtained by *L. mesenteroides* at 0.5% NaCl showed the highest ABG content and a large amount of vitamin C. Refrigerated storage led to a reduction of ABG and vitamin C contents, but *L. mesenteroides* sauerkrauts presented high amounts of both bioactive compounds at the end of the storage period. Experimental sauerkrauts presented better organoleptic properties, such as flavor, firmness, and overall acceptability than the commercial products studied, and no differences in overall acceptability were found among natural fermentations and those performed with starter cultures. In conclusion, low salted sauerkraut produced with *L. mesenteroides* provided high beneficial antioxidant and anticarcinogenic compounds and low sodium content, which is in accordance with the general trend in industrialized countries of reducing the salt level of foods to prevent cardiovascular diseases.

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