Evaluation of refrigerated storage in nitrogen-enriched atmospheres on the microbial quality, content of bioactive compounds and antioxidant activity of sauerkrauts.

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ABSTRACT

The aim of this work was to investigate the influence of storage at 4 ºC in conventional or nitrogen (N_2)-enriched atmospheres for 3 months on the microbial status of sauerkraut obtained by natural fermentation or by *L. mesenteroides* inoculation. The content of vitamin C, glucosinolate derivatives and the antioxidant activity of stored sauerkrauts were also evaluated. Aerobic/anaerobic mesophilic bacteria and lactic acid bacteria populations decreased sharply during N_2 storage, whilst they increased during conventional storage. Ascorbigen and vitamin C levels decreased gradually during storage and no significant differences were found between both storage types. The concentration of nitriles and isothiocyanates decreased during storage and, in general, lower content of these compounds were found in N_2-stored sauerkrauts. The antioxidant capacity of fermented cabbages was retained after storage at both conditions, and *L. mesenteroides* sauerkrauts presented significantly higher antioxidant activity at the end of the storage period when N_2 atmosphere were used. Thus, the use of N_2-atmosphere during refrigerated storage is a promising and cost-effective approach to improve the microbial quality of sauerkraut, and consequently, to extend its shelf-life. Sauerkrauts stored in these conditions had large antioxidant activity and retained high phytochemical concentrations.

Keywords: cabbage fermentation, modified atmosphere, storage, sauerkraut quality, GLS breakdown compounds

INTRODUCTION

Brassicaceous crops are among the most consumed vegetables worldwide due to their availability in local food markets and the high consumer acceptance. Brassica vegetables are excellent sources of fiber, vitamins and minerals, and they have been the focus of intense research based on their potential health benefits (Björkman et al., 2011), which include protective properties against cancer and degenerative diseases as well as antioxidant and antimicrobial activities. These health-promoting properties can be attributed to their high content of glucosinolates (GLS), a group of sulphur-containing plant secondary metabolites, and to the presence of antioxidant compounds such as vitamin C, carotenoids and phenolic compounds (Jaiswal, Raiauria, Abu-Ghannam, & Gupta, 2011; Singh, Upadhyay, Bahadur, Singh, Singh, & Rai, 2006).

Sauerkraut is one of the most common cabbage-derived products that results from the lactic acid fermentation of white cabbage. Fermentation involves many physical and chemical changes and a rapid turnover of lactic acid bacteria (LAB) that influence the quality of the product. GLS have no direct biological activity but during fermentation they are hydrolysed by myrosinase enzyme resulting in a wide range of biologically active GLS breakdown products (Dinkova-Kostova & Kostov, 2012). The most abundant GLS derived compound in sauerkraut is ascorbigen (ABG) (Peñas, Frias, Sidro, & Vidal-Valverde, 2010), which shows an important anticarcinogenic activity (Stephensen, Bonnesen, Bjeldanes, & Vang, 1999) and is formed by reaction of indol-3-carbinol (I3C) and vitamin C. A broad range of other GLS breakdown products are also released during cabbage fermentation, such as sulforaphane (SFN, 1-isothiocyanate-4(methylsulfinyl)-butane), derived from glucoraphanin; allyl isothiocyanate (AITC, 3-isothiocyanate-1-propene) and allyl cyanide (AC, 3-butenenitrile) derived from sinigrin; iberin isothiocyanate (IB, 1-isothiocyanate-3(methylsulfinyl)-propane) and iberin nitrile (IBN, 4-(methylsulfinyl)-butane nitrile) derived from glucoiberin (Peñas, Pihlava, Vidal-Valverde, & Frias, 2012), among others. All these compounds have shown potential cancer-protective properties (Jahgangir, Kim, Choi, & Verpoorte, 2009).

Sauerkraut can be stored for long periods since LAB produce acids during fermentation that inhibit the growth of spoilage microorganisms. However, the high populations of LAB in fermented cabbage can lead to an excessive acidification of the product, reducing the consumer acceptability, since European consumers prefer mild acidified products (Holzapfel, Schillinger, & Buckenhüskes, 2003).

In the last decades, the use of different modified atmosphere conditions during low-temperature storage has gained popularity for extending the shelf-life of fresh-cut fruits and vegetables. Controlled atmosphere (CA) storage involves the alteration of the proportion of normal atmospheric gases through the storage of the product under atmospheres generally enriched in CO_2 and with reduced levels of O_2 (Guo et al., 2013). In the last years, the use of non-conventional atmospheres enriched in Argon (Ar), nitric oxide (NO) or nitrogen (N_2) has gained interest (Char, Silveira, Inestroza-Lizardo, Hinojosa, Machuca, & Escalona, 2012; Yang, Zhou, Wu, & Cheng, 2010). The modification of the normal atmosphere allows to extent the shelf-life of the product and to prevent the development of enzymatic browning reactions. Although CA storage is frequently used for fresh-cut vegetables, CA storage of fermented cabbage could represent a valuable approach to extend its shelf-life by improving the microbial quality, and consequently, to avoid the excessive decrease of sauerkraut pH, phenomenon that could increase the acceptance of the product by consumers. CA storage of sauerkraut could also prevent the loss by oxidation of bioactive sauerkraut phytochemicals such as vitamin C and ABG that occurs during conventional refrigerated storage, as it was previously reported (Peñas et al., 2010).

N_2-enriched atmospheres are commonly industrially applied in beverages to prevent oxidation (Koseki & Itoh, 2002), but there is little information on their use during vegetables storage. Therefore, this work was aimed to examine the effect of refrigerated storage in N_2-enriched atmospheres for 3 months on sauerkraut, with particular attention on their effects on sauerkraut microbial quality and on the content of bioactive compounds. Additionally, the influence of N_2-storage on the antioxidant activity of sauerkraut was evaluated.

MATERIALS AND METHODS

2.1. Plant material. White cabbages (*Brassica oleracea* L. var. *capitata* cv. Megaton) grown in the North region of Spain (Calahorra, La Rioja) were provided by Bejo Iberica S. L. (Madrid, Spain).

2.2. Starter culture preparation. L. mesenteroides (CECT 219) strain was supplied by the Spanish Type Culture Collection (CECT, Valencia, Spain) and was inoculated (1%) in MRS broth (Difco Laboratories, Detroit, MI, USA) and incubated at 30 ºC for 16 h. After centrifugation (6429 g, 10 min), cells were harvested and then washed twice in a sterile saline solution. The starter culture was inoculated at approximately 10^6 colony-forming units (cfu)/g of cabbage.

2.3. Fermentation process. A random representative selection of cabbage heads was chosen and their edible part was shredded to about 2 mm thickness using a domestic shredder (Moka Express, Barcelona, Spain). 5 g/kg NaCl was added into shredded cabbage and mixed thoroughly. Cabbage and brine were transferred into sterile fermentation vessels (8 L), (Nalge Nunc. International, Rochester, NY) and pressed thoroughly to remove air bubbles. Shredded and salted cabbage was spontaneously fermented by the indigenous cabbage microbiota or by *L. mesenteroides* inoculation. Fermentation was carried out in triplicate at room temperature for 7 days.

2.4. Storage conditions. Immediately after fermentation, three samples of sauerkraut corresponding to each fermentation replicate were placed in sterile capped glass vessels (0.5 L). The vessels were filled by thoroughly pressing down to ~0.3 cm from their upper edges simulating the packaging and storage in traditional household sauerkraut production. Then, the samples were stored at 4 ºC for 1, 2, and 3 months (conventional storage). Another three replicated samples were placed in sterile capped glass vessels equipped with a silicone septum on the lid, flushed with high purity N_2 for 5 min and stored at 4 ºC for up to 3 months (N_2-enriched atmosphere storage). The O_2 concentration in these vessels was below 0.5 g/kg, measured by an O_2 detector (Oxybaby 6, Witt, Santander, Spain). Three sauerkrauts replicates were immediately analysed and were considered as unstored sauerkrauts.

2.5. Microbiological analyses. Microbiological analyses were performed in sauerkrauts stored for up to 3 months under conventional or N_2-enriched atmosphere. Five grams of each sample were aseptically diluted in buffered peptone water (Scharlau Chemie, Spain) in a sterile Stomacher bag and homogenised for 1 min in a Stomacher blender (IUL Masticator, Barcelona, Spain). Further serial dilutions were made for plating. The pour plate technique was employed to determine the microbial counts. Total aerobic mesophilic bacteria were enumerated on Tryptone Soya Agar (TSA) after incubation at 30 ºC for 72 h; total anaerobic mesophilic bacteria on TSA after incubation on anaerobic conditions at 30 ºC for 72 h; total and faecal coliforms on Violet Red Bile Agar (VRBA) containing lactose as carbohydrate source, after incubation at 37 ºC and 44 ºC, respectively, for 24 h; moulds and yeasts on Sabouraud-Chloramphenicol Agar, after incubation at 23 ºC for 96 h; and lactic acid bacteria (LAB) on MRS Agar after incubation in anaerobic conditions at 30 ºC for 48 h.

2.6. Vitamin C content. Determination of vitamin C in stored sauerkrauts was performed by capillary electrophoresis as described in Frias, Miranda, Doblado, & Vidal-Valverde (2005).

2.7. Content of GLS breakdown products. The ABG content in fermented cabbages stored in the described conditions was quantified as described by Peñas et al. (2010). The content of isothiocyanates and nitriles formed by GLS hydrolysis in sauerkrauts was determined as in Tolonen et al. (2002) with slight modifications. Briefly, 0.2 g of freeze-dried samples were extracted in 3 mL of methylene chloride by agitation for 4 h at room temperature. After centrifugation (484 x *g* for 10 min), 50 µL of chlorathalonil (0.2 g/L) were added as an internal standard to 1 mL of sample supernatant. All samples were extracted in triplicates. The separation and quantification of these GLS breakdown products were carried out by PE Clarus 500 GC-MS (Perkin-Elmer, Shelton, CT, USA) using splitless injection (1 µL, split-on time 1.40 min) to a double gooseneck liner. PE Elite-5MS (30 m x 0.25 mm i.d., film thickness 0.25 µm) was used as the analytical column with helium as carrier gas (1.0 mL/min). The analysis was performed isothermally at oven temperature of 110 ºC (22 min). The injector was set at 250 ºC and the GC-MS transfer to 260 ºC. MS was employed at scan mode 40-550 m/e. Quantification of IB, IBN and SFN was performed using the calibration

curve of hexyl isothiocyanate (Sigma Aldrich), because of the lack of commercial standards, while the quantification of AC and AITC was done using authentic standards. The identification of IB and IBN was based on the NIST MS-library.

2.9. Antioxidant activity. The antioxidant activity, measured as Oxygen Radical Absorbance Capacity by fluorescence (ORAC-FL), was determined in potassium phosphate buffer (pH 7.0) extracts by suspension of 1 g of freeze-dried sample in 10 mL of extraction buffer, stirring (1 h at room temperature) and filtration through Whatman No.1 filter paper. ORAC-FL values were determined as described by Martinez-Villaluenga, Peñas, Sidro, Ullate, Frias, Vidal-Valverde (2012).

2.10. Statistical analysis. Data were expressed as mean±standard deviation of three independent determinations for each replicated sample. One-way analysis of variance (ANOVA) using the least-squared difference (LSD) test was performed to determine whether there were significant ($P \leq 0.05$) differences between groups. STATGRAPHICS 5.0 software (Statistical Grapahics Corp, Rockville, MD, USA) for Windows was used for the calculations.

3. Results and discussion

3.1. Microbial quality of stored sauerkrauts. Figures 1 and 2 depict the microbial counts of naturally and *L. mesenteroides* fermented cabbages during refrigerated storage under conventional or N_2-enriched atmospheres for 3 months. Spontaneously fermented cabbages presented high populations of aerobic mesophilic bacteria (~6.8 log CFU/g), anaerobic mesophilic bacteria (~6.8 log CFU/g) and LAB (6.7 log CFU/g) (Figure 1), whilst populations of total and faecal coliforms as well as moulds/yeasts were below the detection limit (1 log CFU/g). These results are in agreement with those previously observed in spontaneously fermented cauliflower (Paramithiotis, Hondrodimou, & Drosinos, 2010) and Chinese sauerkrauts (Xiong, Guan, Song, Hao, & Xie, 2012).

A gradual and significant ($P \leq 0.05$) increase of aerobic and anaerobic mesophilic bacteria and LAB were observed during conventional refrigerated storage for 3 months. At the end of the storage period, an increase of 1.3, 1.1 and 1.2 log CFU/g in aerobic mesophilic bacteria, anaerobic

mesophilic bacteria and LAB, respectively, were found in stored sauerkraut. These results indicate that storage at atmospheric conditions was not able to inhibit the growth of these bacterial groups.

In contrast, refrigerated storage in N_2-enriched atmosphere for 1 month caused a significant decrease ($P \leq 0.05$) of all microbial groups in sauerkraut (reductions of 2-4 log CFU/g). A small rise of these microbial populations was observed after 2 and 3 months of storage ($P \leq 0.05$), but microbial counts were much lower than those observed in conventionally stored sauerkrauts. After 3 months, sauerkrauts stored in N_2 atmospheres showed populations of aerobic mesophilic bacteria, anaerobic mesophilic bacteria and LAB, between 3-5 log CFU/g lower than those conventionally stored. It is well known that N_2 has an inhibitory effect on aerobic bacterial growth (Velu, Bakar, Mahyudin, Saari, & Zaman 2013), thus explaining the reduction of aerobic mesophilic bacteria population observed in N_2-stored sauerkraut. The LAB counts in sauerkrauts stored in these conditions were higher than those of aerobic bacteria, since LAB are aerotolerant anaerobic bacteria and their growth is favoured at low O_2 concentrations, situation also observed for anaerobic mesophilic bacteria. Nevertheless, the growth of both bacterial groups was significantly lower than that observed in sauerkraut stored under conventional conditions, suggesting a negative influence of N_2 in the proliferation of these bacteria. There is limited information on the effect of N_2 storage on the microbial status of fresh vegetables. In this sense, Char et al. (2012) reported that storage of arugula leaves in N_2 atmosphere for 8 days at 5 ºC after sanitisation with NaClO was effective in controlling the growth of total aerobic mesophilic bacteria, results in agreement with those obtained in the present work during longer storage period. On the other hand, Koseki and Itoh (2002) found that N_2 gas packaging did not significantly affect the growth of total aerobic bacteria and coliforms in fresh-cut vegetables (lettuce and cabbage) at 1, 5 and 10 ºC for 5 days. These results differ from our findings, probably due to the shorter storage time and different plant material used by these authors.

Figure 2 illustrates the microbial status of cabbages fermented by *L. mesenteroides* and stored for 3 months. The evolution of microbial populations in induced-fermented cabbages during storage at conventional or modified atmospheres showed a similar trend than that observed in

198 spontaneously obtained sauerkraut. At the end of the storage, the counts for all microbial groups in
199 *L. mesenteroides* sauerkratus stored in N_2 atmosphere were between 3 and 5 log CFU/g lower than
200 in those stored at conventional conditions and between 2-4 log CFU lower than in unstored
201 sauerkraut. These findings suggest that the use of N_2-enriched atmospheres during refrigerated
202 storage could be a practical and economical approach to improve the microbial quality of
203 sauerkrauts and to extend their shelf-life.
204 *3.2. ABG and vitamin C contents in stored sauerkrauts.* ABG and vitamin C contents of natural
205 sauerkrauts during storage are summarised in Table 1. Spontaneously fermented cabbage presented
206 high ABG concentration (18.58 μmol/100g fresh weight, f.w.), but its content suffered a gradual
207 and significant decrease during refrigerated storage in conventional conditions. The first month of
208 storage did not lead to large losses of ABG (retention percentage of 92%), but losses of about 17%
209 and 31%, respectively, were observed during the second and the third storage months. Our results
210 differ from those reported by Ciska and Pathak (2004) who did not observe changes in ABG content
211 during conventional storage of sauerkraut at 5 ºC for 17 weeks. These differences could be
212 attributed to the different O_2 concentration present in the vessels used during storage. During
213 storage of naturally fermented sauerkraut in N_2 atmospheres for 2 months, no significant differences
214 in ABG levels were observed, when compared with conventional storage. Surprisingly, a significant
215 ($P \leq 0.05$) lower ABG concentration was found in N_2-stored sauerkraut (retention percentage of
216 63%) than in that conventionally stored (retention percentage of 69 %) after 3 months. ABG is an
217 unstable compound that can be degraded by oxidation and, therefore, it would be expected higher
218 losses of this compound during storage in conventional conditions. The low concentration of O_2 in
219 the vessels during conventional storage (since sauerkraut was strongly pressed for O_2 removal)
220 could explain the high retentions of ABG during conventional storage. Nevertheless, it is difficult to
221 provide an explicit explanation for the larger diminution of this compound during the third month of
222 storage in N_2-enriched atmospheres. It could be speculated that N_2 favours the decomposition of

223 ABG in other compounds such as I3C. The elucidation of this phenomenon would require
224 quantifying the concentration of I3C and that of all potential products of I3C condensation.
225 The content of vitamin C in natural fermented cabbage was rather high (20.60 mg/100 g
226 f.w), level that dropped significantly ($P \leq 0.05$) during conventional storage, and retentions of 77%,
227 50% and 35% were observed after 1, 2 and 3 months, respectively. No significant differences
228 ($P \leq 0.05$) in vitamin C concentrations were observed in sauerkrauts stored under N_2 atmospheres. It
229 is well known that ascorbic acid is very stable at acidic pH, but the presence of O_2 causes losses of
230 this vitamin by oxidation. The low concentration of O_2 in the vessels stored at conventional
231 conditions, as explained above, could explain the similar reductions of vitamin C content observed
232 in both types of storage.
233 Table 2 shows the content of ABG and vitamin C in *L. mesenteroides* sauerkrauts after
234 storage in conventional and N_2-enriched atmospheres. As in the case of naturally fermented
235 cabbages, ABG content declined significantly ($P \leq 0.05$) during conventional storage and losses of
236 23% were found after 3 months. Significant ($P \leq 0.05$) higher reductions of this compound were
237 observed when sauerkraut was stored at high N_2 concentrations. However, no significant differences
238 ($P \leq 0.05$) in vitamin C levels were found between both types of storage. Lower vitamin C losses in
239 vegetables stored under modified atmosphere conditions in comparison with conventional storage
240 has been previously reported (Gil, Ferreres, & Tomas-Barberan, 1999; Kader, 2009). These
241 observations correspond to atmospheres with enhanced CO_2 and reduced O_2 contents. However,
242 losses of vitamin C were reported in fresh-cut red chard baby leaves after storage in N_2-enriched
243 atmosphere at 5 ºC for 8 days (Tomás-Callejas, Boluda, Robles, Artés, & Artés-Hernández, 2011).
244 Furthermore, Moreira, Roura, & Del Valle (2003) found that the use of N_2 fertilizers at high rates
245 led to a decrease of vitamin C levels in Swiss Chard. No negative influence of N_2 on vitamin C
246 content has been observed, however, in the present work.
247 *3.3. Content of glucosinolate breakdown products (isothiocyanates and nitriles) in stored*
248 *sauerkrauts.* Tables 3 and 4 collect the concentration of GLS hydrolysis compounds in sauerkrauts

obtained naturally or by *L. mesenteroides* inoculation during storage for 3 months. AC was the major GLS derivative found in spontaneously fermented cabbage (5.8 µmol/100 g f.w), followed by AITC (3.9 µmol/100 g f.w.), IBN (3.6 µmol/100 g f.w) and IB (3.1 µmol/100 g f.w) (Table 3). SFN was the GLS breakdown product present in the lowest concentration (2.7 µmol/100 g f.w.) in these sauerkrauts. High levels of AC and AITC, which are sinigrin derivatives, were expected in sauerkrauts obtained from cabbage cv. Megaton, since sinigrin is the major GLS compound present in this cultivar (Peñas, Frias, Martínez-Villaluenga, & Vidal-Valverde, 2011). All these GLS derivatives were previously identified in spontaneously fermented cabbages (Ciska & Pathak, 2004; Tolonen, Taipale, Viander, Pihlava, Korhonen, & Ryhänen, 2002) although the proportion between the GLS breakdown products reported by these authors was different. These differences can be attributed to the variation in the GLS composition of the cabbages used in each study, which is dependent on the cultivar. Differences in endogenous myrosinase activity and microbial populations between different cultivars can also contribute to the differences in the composition of GLS derivatives observed, as previously reported (Peñas et al., 2011, 2012).

During conventional refrigerated storage, different tendency in the evolution of the GLS derivatives analysed was observed (Table 3). IB, AC and SFN declined gradually and losses of about 18 %, 4 % and 17 %, respectively, were noted after 3 months. However, IBN and AITC were stable during all the storage period. N_2-storage led to significant reductions ($P \leq 0.05$) on the concentration of IB, IBN and AITC when compared with conventional storage (Table 3), whilst no significant differences ($P \leq 0.05$) in AC and SFN contents were found at the end of the storage period between both types of storage.

L. mesenteroides sauerkrauts (Table 4) showed similar or slightly lower GLS derivatives content than naturally fermented cabbages, results in accordance with those reported by Tolonen et al. (2002). During storage in conventional conditions, no significant changes ($P \geq 0.05$) on the concentration of these compounds were observed, with the exception of IB that decreased in the second month and SFN that declined at the end of the storage period. Similar contents of IB, IBN

and SFN to those found in conventional stored sauerkrauts were observed during storage under modified atmospheres for 3 months. However, the concentration of AC was significantly ($P \leq 0.05$) higher in fermented cabbages stored under N_2 during the first 2 months than in those conventionally stored, whilst the level of AITC was significantly ($P \leq 0.05$) lower in the former.

Howard, Jeffery, Matthew, Wallig, & Klein (1997) observed losses of 55.3% and 95.5% of SFN and IBN concentrations, respectively, in broccoli stored in conventional atmospheres for 21 days at 4 ºC. These reductions are larger than those noted in the present study in stored sauerkrauts, but the results reported by these authors are not directly comparable with our results since the composition of the vegetable matrix differs. The concentration of GLS hydrolysis compounds found in this work in conventionally stored sauerkrauts was considerably higher than those reported by Ciska and Pathak (2004) in spontaneously obtained sauerkrauts stored at 5 ºC for 17 weeks. The differences between both studies can be explained not only by the different content of GLS degradation products in sauerkrauts before storage, but also by the different chemical and microbial stability of these compounds in the distinct acidic environments present in sauerkrauts analysed in each work.

Several authors have studied the effect of CA storage and modified atmosphere packaging on GLS concentration in *Brassica* vegetables (Rangkadilok et al., 2002; Toivonen & Forney, 2004), and they have not found a clear tendency in the evolution of such compounds during storage since their contents depended on the gas composition and storage conditions. However, there is scarce information in the literature on the influence of CA storage and modified atmosphere packaging on the concentration of GLS derivatives in *Brassica* vegetables. One study have reported that the concentration of volatile isothiocyanates declined during the storage of cabbage in CA (2.5 % O_2 and 5 % CO_2) for periods from 38 to 172 days followed by refrigeration at 1 ºC to the 214th day (Berard, & Chong, 1984). To the best of our knowledge, this is the first study reporting the influence of N_2-enriched atmospheres on the content of several phytochemicals of sauerkraut. Our results indicate that similar contents of GLS degradation compounds were found in *L.*

mesenteroides sauerkrauts stored at 4 ºC in the presence of air or N_2 enriched atmospheres, suggesting that the use of N_2 did not negatively affect the stability of the identified GLS-derived compounds. At the end of the storage period in N_2 atmospheres, retention percentages ranging from 89 to 95% were observed for all GLS breakdown products analysed. These results are of great importance since these compounds have been previously shown to have anticarcinogenic properties. AITC can potentially inhibit bladder cancer development (Savio, da Silva, de Camargo, & Salvadori, 2014), whilst SFN has shown antiproliferative activity and induction of mitochondrial apoptosis in melanoma cells (Rudolf, Cervinka, & Rudolf, 2014). IB has been shown to inhibit the proliferation of human glioblastoma and neuroblastoma cells through the induction of cell apoptosis at low concentrations of 2.5 μM (Jadhav, Ezhilarasan, Vaughn, Berhow, & Mohanam, 2007; Jadhav, Vaughn, Berhow, & Mohanam, 2007). It has been reported than the consumption of 38 mg/kg (equivalent to 0.6 μmol/kg body weight) of IBN by rats enhanced the activity of glutathione reductase that is involved in the protection against oxidative stress (Staak, Kingston, Waillig, & Jeffery, 1998). Moreover, Zhao et al (2001) found that a weekly intake of ITCs above 53 μmol reduced the risk of lung cancer. Taking into account the contents of GLS breakdown products observed in N_2-stored sauerkrauts for 3 months, it could be concluded that a daily consumption of 50-100 g of sauerkraut would provide effective doses of GLS degradation products to exert health-promoting effects.

3.4. Antioxidant activity in stored sauerkrauts. Table 5 shows the ORAC-FL values obtained for spontaneously or *L. mesenteroides* fermented cabbages during refrigerated storage. No significant differences in the antioxidant activity were found between natural (11.2 μmol Trolox/g f.w.) and *L. mesenteroides* sauerkrauts (12.8 μmol Trolox/g f.w.). These sauerkrauts presented higher antioxidant activity than that reported for raw white cabbage (Ciska, Karamac, & Kosinska, 2005; Kusznierewicz, Bartoszek, Wolska, Drzewiecki, Gorinstein, & Namiesnik, 2008; Martinez-Villaluenga et al., 2012). Several authors have also observed an increased antioxidant activity in spontaneously fermented white and Chinese cabbages (Kusznierewicz et al., 2008; Sun, Chou, &

Yu, 2009). ORAC-FL assay measures the chain-breaking action of "traditional" antioxidants (ascorbic acid, α-tocopherol, β-carotene and flavonoids) against peroxyl radicals (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). The high antioxidant activity of sauerkrauts can be attributed, on one hand, to the ability of LAB to hydrolyse polyphenols, compounds that are present in cabbage at high concentration (Lee, Boyce and Breadmore, 2011), into other simpler and more antioxidant ones. On the other hand, ABG and other GLS breakdown derivatives formed during fermentation can contribute to the overall antioxidant activity of sauerkraut, since they have showed free radical scavenging activity (Wagner & Rimbach, 2008; Cabello-Hurtado, Gicquel, & Esnault, 2012). In addition, shredding of cabbage before fermentation could be partially responsible for the initial increase of antioxidant activity as it has been shown by Reyes, Villareal and Cisneros-Zeballos (2007) after wounding of white cabbage tissues.

No significant ($P \leq 0.05$) changes of antioxidant activity were observed during the storage of naturally obtained sauerkraut both at conventional and N_2-enriched atmospheres. In contrast, conventional storage of *L. mesenteroides* sauerkrauts led to a gradual and significant ($P \leq 0.05$) reduction of ORAC values, and losses of about 25% were observed after 3 months. Conversely, the antioxidant activity remained unchanged during the storage in N_2-enriched atmospheres, suggesting that the use of N_2 is an efficient approach for maintaining the antioxidant activity of stored sauerkrauts. Antioxidant activity is a valuable attribute for marketing the potential health benefits of *L. mesenteroides* sauerkrauts. Kusznierewicz et al. (2010) have indicated that phytochemicals of white cabbage, both raw and processed, at doses expected during normal daily consumption, may prevent oxidative damage to biomolecules. These authors also found that fermentation increased 3 to 4-fold the antioxidant activity of cabbage. Our results together with those observed by Kusznierewicz et al. (2010) suggest that the consumption of stored sauerkrauts could provide potential health benefits.

4. Conclusions

Refrigerated storage of sauerkraut in conventional conditions for 3 months increased the populations of LAB and aerobic/anaerobic mesophilic bacteria and reduced the contents of several bioactive compounds. The use of N_2-enriched atmospheres during refrigerated storage reduced the counts of these bacterial groups (2-4 log CFU/g) in sauerkraut. Moreover, at the end of the storage period, N_2 stored sauerkrauts presented counts for all the bacterial groups studied 3-5 log CFU/g lower than those conventionally stored. *L. mesenteroides* fermented cabbages stored in N_2-enriched atmospheres for 3 months presented larger antioxidant activity than those stored in conventional conditions and contained high levels of vitamin C, ABG, and other GLS breakdown compounds. The application of N_2 atmosphere during sauerkraut storage is a promising and cost-effective approach to improve the microbial quality of this product. This storage method allows to preserve the antioxidant activity of sauerkraut and to retain high concentrations of cabbage phytochemicals.

Acknowledgements

This work was supported by the Spanish Commission of Science and Technology (CICYT), project number AGL2007-63462. Elena Peñas is indebted to a José Castillejo mobility grant funded by the Ministry of Science and Innovation and to a JAE-doc grant funded by CSIC and ESF.

References

Berard, L. S., & Chong, C. (1984). Influence of storage on glucosinolate fluctuations in cabbage. *Acta Horticulturae*, *157*, 2003-2010.

Björkman, M., Klingen, I., Birch, A. N. E., Bones, A. M., Bruce, T. J. A., Johansen, T. J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L. E., & Stewart, D. (2011). Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. *Phytochemistry*, *72*, 538-556.

Cabello-Hurtado, F., Gicquel, M., & Esnault, M-A. (2012). Evaluation of the antioxidant potencial of cauliflower (Brassica oleracea) from a glucosinolate content perspective. *Food Chemistry*, *132*, 1003-1009.

Char, C., Silveira, A. C., Inestroza-Lizardo, C., Hinojosa, A., Machuca, A., & Escalona, V. H. (2012). Effect of noble gas-enriched atmospheres on the overall quallity of ready-to-eat arugula salads. *Postharvest Biology and Technology*, *73*, 50-55.

Ciska, E., & Pathak, D. R. (2004). Glucosinolate derivatives in stored fermeted cabbage. *Journal of Agricultural and Food Chemistry, 52*, 7938–7943.

Ciska, E., Karamac, M., & Kosinska, A. (2005). Antioxidant activity of extracts of white cabbage and sauerkraut. *Polish Journal of Food and Nutrition Sciences, 14*, 367-373.

Dinkova-Kostova, A. T., & Kostov, R. V. (2012). Glucosinolates and isothiocyanates in health and disease. *Trends in Molecular Medicine*, *18*, 337-347.

Gil, M. I., Ferreres, F., & Tomas-Barberan, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry*, *47*, 2213-2217.

Guo, Y., Gao, Z., Li, L., Wang, Y., Zhao, H., Hu, M., & Zhang, Z. (2013). Effect of controlled atmosphers with varying O_2/CO_2 levels of the postharvest senescence and quality of broccoli (*Brassica oleracea* L. var. *italica*) florets. *European Food Research and Technology*, *237*, 943-950.

Frias, J., Miranda, L. M., Doblado, R., & Vidal-Valverde, C. (2005). Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. multolupa. *Food Chemistry*, *92*, 211–220.

Holzapfel, W., Schillinger, U., & Buckenhüskes, H. J. (2003). Sauerkraut. In E. R Farnworth, *Handbook of fermented functional foods* (pp. 343-359). Boca Raton, F.L: CRC Press.

Howard, L. A., Jeffery, E. H., Wallig, M. A., & Klein, B. P. (1997). Retention of phytochemicals in fresh and processed broccoli. *Journal of Food Science*, *62*, 1098-1104.

Jadhav, U., Ezhilarasan, R., Vaughn, S. F., Berhow, M. A., & Mohanam, S. (2007). Dietary isothiocyanate iberin inhibits growth and induces apoptosis in human glioblastoma cells. *Journal of Pharmacological Sciences*, *103*, 247-251.

Jadhav, U., Vaughn, S. F., Berhow, M. A., & Mohanam, S. (2007). Iberin induces cell cycle arrest and apoptosis in human neuroblastoma cells. *International Journal of Molecular Medicine*, *19*, 353-361.

Jahgangir, M. J., Kim, H. K., Choi, Y.H., & Verpoorte, R. (2009). Health-affecting compounds in *Brassicaceae*. *Comprehensive Reviews in Food Science and Food Safety*, *8*, 31-43.

Jaiswal, A. K., Raiauria, G., Abu-Ghannam, N., & Gupta, S. (2011). Phenolic composition, antioxidant capacity and antibacterial activity of Brassica vegetables. *Natural Product Communication*, *6*, 1299-1304.

Kader, A. A. (2009). Effects in nutritional quality. In modified and controlled atmospheres for the storage, transportation and packaging of horticultural commodities (E. M. Yahia ed.) CRC Press, Boca Raton, Florida, pp. 111-118.

Koseki, S., & Itoh, K. (2013). Effect of nitrogen gas packaging on the quality and microbial growth of fresh-cut vegetables under low temperatures. *Journal of Food Protection*, *65*, 326-332.

Kusznierewicz, B, Bartoszek, A, Wolska, L, Drzewiecki, J, Gorinstein, S, & Namiesnik, J. (2008). Partial characterization of white cabbage (*Brassica oleracea* var. capitata f. alba) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. *LWT-Food Science and Technology*, *41*, 1-9.

Kusznierewicz, B, Lewandowska, J., Kruszyna, A., Piasek, A., Smiechowska, A., Namiesnik, J., & Bartoszek, A. (2010). The antioxidative properties of white cabbage (*Brassica oleracea* var. *Capitata* F. *Alba*) fresh and submitted to culinary processing. *Journal of Food Biochemistry*, *34*, 262-285.

Lee, I. S., Boyce. M. C., & Breadmore, M. C. (2011). A rapid quantitative determination of phenolic acids in *Brassica Oleracea* by capillary zone electrophoresis. *Food Chemistry, 127*, 797-801.

Martínez-Villaluenga, C., Peñas, E., Sidro, B., Ullate, M., Frias, J., & Vidal-Valverde, C. (2012). White cabbage fermentation improves ascorbigen content, antioxidant and nitric oxide production inhibitory activity in LPS-induced macrophages. *LWT-Food Science and Technology*, *46*, 77-83.

Moreira, M. R., Roura, S. I., & del Valle, C. E. (2003). Quality of Swiss chard produced by conventional and organic methods. *LWT-Food Science and Technology*, *36*, 135-141.

Ou, B., Huang, D., Hampsch-Woodil, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry*, *50*, 3122-3128.

Paramithiotis, S., Hondrodimou, O. L., & Drosinos, E. H. (2010). Development of the microbial community during spontaneous cauliflower fermentation. *Food Research International*, *43*, 1098-1103.

Peñas, E., Frias, J., Sidro, B., & Vidal-Valverde, C. (2010). 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. *Journal of Agricultural and Food Chemistry*, *58*, 3549-3557.

Peñas, E., Frias, J., Martínez-Villaluenga, C., & Vidal-Valverde, C. (2011). Bioactive compounds, myrosinase activity and antioxidant capacity of white cabbages grown in different locations of Spain. *Journal of Agricultural and Food Chemistry*, *59*, 3772-3779.

448 Peñas, E., Pihlava J-M., Vidal-Valverde, C., & Frias, J. (2012). Influence of fermentation conditions
449 of Brassica oleracea L. var. capitata on the volatile glucosinolate hydrolysis compounds of
450 sauerkrauts. *LWT- Food Science and Technol*ogy, *48*, 16-23.
451 Rangkadilok, N., Tomkins, B., Nicolas, N. M., Permier, R. R., Bennett, R. N., Eagling, D. R., &
452 Taylor, P. W. (2002). The effect of post-harvest and packaging treatments on glucoraphanin
453 concentration in broccoli (*Brassica oleracea* var. *italica*). *Journal of Agricultural and Food*
454 *Chemistry*, *50*, 7386-7391.
455 Reyes, L. F., Villareal, J. E., & Cisneros-Zevallos, L. (2007). The increase in antioxidant capacity
456 alter wounding depends on the type of fruit or vegetable tissue. *Food Chemistry, 101*, 1254-
457 1264.
458 Rudolf, K., Cervinka, M., & Rudolf, E. (2014). Sulforaphane-induced apoptosis involves p53 and
459 p38 in melanoma cells. *Apoptosis*, *19*, 734-747.
460 Savio, A. L., da Silva, G.N., de Camargo, E. A., & Salvadori, D. M. (2014). Cell cycle kinetics,
461 apoptosis rates, DNA damage and *TP53* gene expression in bladder cancer cells treated with
462 allyl isothiocyanate (mustard essential oil). *Mutation Research, 762*, 40-46.
463 Singh, J, Upadhyay, A. K., Bahadur, A., Singh, B., Singh, K. P., &
464 Rai, M. (2006). Antioxidant phytochemicals in cabbage (Brassica oleracae L. var. capitata).
465 *Scientia Horticulturae, 108*, 233–237.
466 Staack, R., Kingston, S., Wallig, M. A., & Jeffery, E. H. (1998). A comparison of the individual and
467 collective effects of four glucosinolate breakdown products from Brussels sprouts on
468 induction of detoxification enzymes. *Toxicology and Applied Pharmacology*, *149*, 17-23.
469 Stephensen, P. U., Bonnesen, C., Bjeldanes, L. F., & Vang, O. (1999). Modulation of cytochrome
470 P4501A1 activity by ascorbigen in murine hepatoma cells. *Biochemical Pharmacology, 58*,
471 1145–1153.
472 Sun, Y-P., Chou, C-C., & Yu, R-C. (2009). Antioxidant activity of lactic-fermented Chinese
473 cabbage. *Food Chemistry*, *115*, 912-917.
474 Tolonen, M., Taipale, M., Viander, B., Pihlava, J. M., Korhonen, H., & Ryhanen, E. L. (2002).
475 Plant-derived biomolecules in fermented cabbage. *Journal of Agricultural and Food*
476 *Chemistry*, *50*, 6798-6803.
477 Tomás-Callejas, A., Boluda, M., Robles, P. A., Artés, F. (2011). Innovative active modified
478 atmosphere packaging improves overall quality of fresh-cut red chard baby leaves. *LWT-*
479 *Food Science and Technology*, *44*, 1422-1428.
480 Toivonen, P. M. A., & Forney, C. (2004). In K. C. Gross, C. Y. Wang, & M. Saltveit, *The*
481 *commercial storage of fruits, vegetables and florist and nursery stock*. Agriculture

Handbook 66. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD.

Velu, S., Bakar, F. A., Mahyudin, N. A., Saari, N., & Zaman, M. Z. (2013). Effect of modified atmosphere packaging on microbial flora changes in fishery products. *International Food Research Journal*, *20*, 17-26.

Wagner, A. E., Rimbach, G. (2009) Ascorbigen: chemistry, occurence, and biologic properties. *Clinics in Dermatology*, *27*, 217-224.

Xiong, T., Guan, Q., Song, S., Hao, M., & Xie, M. (2012). Dynamic changes on lactic acid bacteria flora during Chinese sauerkraut fermentation. *Food Control*, *26*, 178-181.

Yang, H., Zhou, C., Wu, F., Cheng, J. (2010). Effect of nitric oxide on browning and lignification of peeled bamboo shoots. *Postharvest Biology and Technology*, *57*, 72–76.

Zhao, B., Seow, A., Lee, E. J. D., Poh, W.-T., Teh, M., Wang, Y. .T, Tan, W. C., Yu, M. C., Lee, H. P. (2001). Dietary isothiocyanates, glutathione S-transferase-M1, -T1 polymorphisms and lungc ancer risk among Chinese women in Singapore. *Cancer Epidemiology, Biomarkers & Prevention*, 10, 1063-1067.

Figure captions

Figure 1. Microbiological status of natural fermented cabbage during storage for 3 months at 4 ºC in conventional (▲) and N_2-enriched atmospheres (□). Results are the mean of three independent experiments (n=3)

Figure 2. Microbiological status of cabbage fermented with *L. mesenteroides* during storage for 3 months at 4 ºC in conventional (▲) and N_2-enriched (□) atmospheres. Results are the mean of three independent experiments (n=3)