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

E. Peñas1, C. Martínez-Villaluenga1, J-M. Pihlava2, J. Frias1*

1 Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Juan de la Cierva 3, 28006, Madrid, Spain.

2 MTT Agrifood Research Finland, Biotechnology and Food Research, 31600 Jokioinen, Finland

*Corresponding author

Telephone number: +34 912587510
Fax number: +34 91 5644853
E-mail: frias@ictan.csic.es
ABSTRACT

The aim of this work was to investigate the influence of storage at 4 °C in conventional or nitrogen (N₂)-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 N₂ 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 N₂-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 N₂ atmosphere were used. Thus, the use of N₂-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, Sirdro, & 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)-propene) and iberin nitrile (IBN, 4-(methylsulfinyl)-butane nitrile) derived from glucoberin (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 N₂ for 5 min and stored at 4 ºC for up to 3 months (N₂-enriched atmosphere storage). The O₂ concentration in these vessels was below 0.5 g/kg, measured by an O₂ 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 N₂-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≤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 N₂-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≤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 N₂-enriched atmosphere for 1 month caused a significant decrease (P≤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≤0.05), but microbial counts were much lower than those observed in conventionally stored sauerkrauts. After 3 months, sauerkrauts stored in N₂ 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 N₂ 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 N₂-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 O₂ 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 N₂ in the proliferation of these bacteria. There is limited information on the effect of N₂ storage on the microbial status of fresh vegetables. In this sense, Char et al. (2012) reported that storage of arugula leaves in N₂ 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 N₂ 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
spontaneously obtained sauerkraut. At the end of the storage, the counts for all microbial groups in
*L. mesenteroides* sauerkrauts stored in N\textsubscript{2} atmosphere were between 3 and 5 log CFU/g lower than
in those stored at conventional conditions and between 2-4 log CFU lower than in unstored
sauerkraut. These findings suggest that the use of N\textsubscript{2}-enriched atmospheres during refrigerated
storage could be a practical and economical approach to improve the microbial quality of
sauerkrauts and to extend their shelf-life.

3.2. *ABG and vitamin C contents in stored sauerkrauts.* ABG and vitamin C contents of natural
sauerkrauts during storage are summarised in Table 1. Spontaneously fermented cabbage presented
high ABG concentration (18.58 µmol/100g fresh weight, f.w.), but its content suffered a gradual
and significant decrease during refrigerated storage in conventional conditions. The first month of
storage did not lead to large losses of ABG (retention percentage of 92%), but losses of about 17%
and 31%, respectively, were observed during the second and the third storage months. Our results
differ from those reported by Ciska and Pathak (2004) who did not observe changes in ABG content
during conventional storage of sauerkraut at 5 ºC for 17 weeks. These differences could be
attributed to the different O\textsubscript{2} concentration present in the vessels used during storage. During
storage of naturally fermented sauerkraut in N\textsubscript{2} atmospheres for 2 months, no significant differences
in ABG levels were observed, when compared with conventional storage. Surprisingly, a significant
(P≤0.05) lower ABG concentration was found in N\textsubscript{2}-stored sauerkraut (retention percentage of
63%) than in that conventionally stored (retention percentage of 69 %) after 3 months. ABG is an
unstable compound that can be degraded by oxidation and, therefore, it would be expected higher
losses of this compound during storage in conventional conditions. The low concentration of O\textsubscript{2} in
the vessels during conventional storage (since sauerkraut was strongly pressed for O\textsubscript{2} removal)
could explain the high retentions of ABG during conventional storage. Nevertheless, it is difficult to
provide an explicit explanation for the larger diminution of this compound during the third month of
storage in N\textsubscript{2}-enriched atmospheres. It could be speculated that N\textsubscript{2} favours the decomposition of
ABG in other compounds such as I3C. The elucidation of this phenomenon would require quantifying the concentration of I3C and that of all potential products of I3C condensation.

The content of vitamin C in natural fermented cabbage was rather high (20.60 mg/100 g f.w), level that dropped significantly (P≤0.05) during conventional storage, and retentions of 77%, 50% and 35% were observed after 1, 2 and 3 months, respectively. No significant differences (P≤0.05) in vitamin C concentrations were observed in sauerkrauts stored under N₂ atmospheres. It is well known that ascorbic acid is very stable at acidic pH, but the presence of O₂ causes losses of this vitamin by oxidation. The low concentration of O₂ in the vessels stored at conventional conditions, as explained above, could explain the similar reductions of vitamin C content observed in both types of storage.

Table 2 shows the content of ABG and vitamin C in L. mesenteroides sauerkrauts after storage in conventional and N₂-enriched atmospheres. As in the case of naturally fermented cabbages, ABG content declined significantly (P≤0.05) during conventional storage and losses of 23% were found after 3 months. Significant (P≤0.05) higher reductions of this compound were observed when sauerkraut was stored at high N₂ concentrations. However, no significant differences (P≤0.05) in vitamin C levels were found between both types of storage. Lower vitamin C losses in vegetables stored under modified atmosphere conditions in comparison with conventional storage has been previously reported (Gil, Ferreres, & Tomas-Barberan, 1999; Kader, 2009). These observations correspond to atmospheres with enhanced CO₂ and reduced O₂ contents. However, losses of vitamin C were reported in fresh-cut red chard baby leaves after storage in N₂-enriched atmosphere at 5 °C for 8 days (Tomás-Callejas, Boluda, Robles, Artés, & Artés-Hernández, 2011). Furthermore, Moreira, Roura, & Del Valle (2003) found that the use of N₂ fertilizers at high rates led to a decrease of vitamin C levels in Swiss Chard. No negative influence of N₂ on vitamin C content has been observed, however, in the present work.

3.3. Content of glucosinolate breakdown products (isothiocyanates and nitriles) in stored 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, Firas, 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. N2-storage led to significant reductions (P≤0.05) on the concentration of IB, IBN and AITC when compared with conventional storage (Table 3), whilst no significant differences (P≤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≥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 \( \text{N}_2 \) 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 °C. 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 °C 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 \textit{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 \textit{Brassica} vegetables. One study have reported that the concentration of volatile isothiocyanates declined during the storage of cabbage in CA (2.5 % \text{O}_2 and 5 % \text{CO}_2) for periods from 38 to 172 days followed by refrigeration at 1 °C to the 214\textsuperscript{th} day (Berard, & Chong, 1984). To the best of our knowledge, this is the first study reporting the influence of \text{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 \textit{L.}}
mesenteroides sauerkrauts stored at 4 °C in the presence of air or N₂ enriched atmospheres, suggesting that the use of N₂ did not negatively affect the stability of the identified GLS-derived compounds. At the end of the storage period in N₂ 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 N₂-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, &
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≤0.05) changes of antioxidant activity were observed during the storage of naturally obtained sauerkraut both at conventional and N₂-enriched atmospheres. In contrast, conventional storage of L. mesenteroides sauerkrauts led to a gradual and significant (P≤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 N₂-enriched atmospheres, suggesting that the use of N₂ 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


Figure captions

Figure 1. Microbiological status of natural fermented cabbage during storage for 3 months at 4 °C in conventional (▲) and N₂-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 °C in conventional (▲) and N₂-enriched (□) atmospheres. Results are the mean of three independent experiments (n=3).