

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

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23 **ABSTRACT**

24 The aim of this work was to investigate the influence of storage at 4 °C in conventional or nitrogen  
25 (N<sub>2</sub>)-enriched atmospheres for 3 months on the microbial status of sauerkraut obtained by natural  
26 fermentation or by *L. mesenteroides* inoculation. The content of vitamin C, glucosinolate  
27 derivatives and the antioxidant activity of stored sauerkrauts were also evaluated. Aerobic/anaerobic  
28 mesophilic bacteria and lactic acid bacteria populations decreased sharply during N<sub>2</sub> storage, whilst  
29 they increased during conventional storage. Ascorbigen and vitamin C levels decreased gradually  
30 during storage and no significant differences were found between both storage types. The  
31 concentration of nitriles and isothiocyanates decreased during storage and, in general, lower content  
32 of these compounds were found in N<sub>2</sub>-stored sauerkrauts. The antioxidant capacity of fermented  
33 cabbages was retained after storage at both conditions, and *L. mesenteroides* sauerkrauts presented  
34 significantly higher antioxidant activity at the end of the storage period when N<sub>2</sub> atmosphere were  
35 used. Thus, the use of N<sub>2</sub>-atmosphere during refrigerated storage is a promising and cost-effective  
36 approach to improve the microbial quality of sauerkraut, and consequently, to extend its shelf-life.  
37 Sauerkrauts stored in these conditions had large antioxidant activity and retained high  
38 phytochemical concentrations.

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

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43 **INTRODUCTION**

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

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

69           Sauerkraut can be stored for long periods since LAB produce acids during fermentation that  
70 inhibit the growth of spoilage microorganisms. However, the high populations of LAB in fermented  
71 cabbage can lead to an excessive acidification of the product, reducing the consumer acceptability,  
72 since European consumers prefer mild acidified products (Holzapfel, Schillinger, & Buckenhüskes,  
73 2003).

74           In the last decades, the use of different modified atmosphere conditions during low-  
75 temperature storage has gained popularity for extending the shelf-life of fresh-cut fruits and  
76 vegetables. Controlled atmosphere (CA) storage involves the alteration of the proportion of normal  
77 atmospheric gases through the storage of the product under atmospheres generally enriched in CO<sub>2</sub>  
78 and with reduced levels of O<sub>2</sub> (Guo et al., 2013). In the last years, the use of non-conventional  
79 atmospheres enriched in Argon (Ar), nitric oxide (NO) or nitrogen (N<sub>2</sub>) has gained interest (Char,  
80 Silveira, Inestroza-Lizardo, Hinojosa, Machuca, & Escalona, 2012; Yang, Zhou, Wu, & Cheng,  
81 2010). The modification of the normal atmosphere allows to extend the shelf-life of the product and  
82 to prevent the development of enzymatic browning reactions. Although CA storage is frequently  
83 used for fresh-cut vegetables, CA storage of fermented cabbage could represent a valuable approach  
84 to extend its shelf-life by improving the microbial quality, and consequently, to avoid the excessive  
85 decrease of sauerkraut pH, phenomenon that could increase the acceptance of the product by  
86 consumers. CA storage of sauerkraut could also prevent the loss by oxidation of bioactive  
87 sauerkraut phytochemicals such as vitamin C and ABG that occurs during conventional refrigerated  
88 storage, as it was previously reported (Peñas et al., 2010).

89           N<sub>2</sub>-enriched atmospheres are commonly industrially applied in beverages to prevent  
90 oxidation (Koseki & Itoh, 2002), but there is little information on their use during vegetables  
91 storage. Therefore, this work was aimed to examine the effect of refrigerated storage in N<sub>2</sub>-enriched  
92 atmospheres for 3 months on sauerkraut, with particular attention on their effects on sauerkraut  
93 microbial quality and on the content of bioactive compounds. Additionally, the influence of N<sub>2</sub>-  
94 storage on the antioxidant activity of sauerkraut was evaluated.

## 95 MATERIALS AND METHODS

96 2.1. *Plant material.* White cabbages (*Brassica oleracea* L. var. *capitata* cv. Megaton) grown in the  
97 North region of Spain (Calahorra, La Rioja) were provided by Bejo Iberica S. L. (Madrid, Spain).

98 2.2. *Starter culture preparation.* *L. mesenteroides* (CECT 219) strain was supplied by the Spanish  
99 Type Culture Collection (CECT, Valencia, Spain) and was inoculated (1%) in MRS broth (Difco  
100 Laboratories, Detroit, MI, USA) and incubated at 30 °C for 16 h. After centrifugation (6429 g, 10  
101 min), cells were harvested and then washed twice in a sterile saline solution. The starter culture  
102 was inoculated at approximately 10<sup>6</sup> colony-forming units (cfu)/g of cabbage.

103 2.3. *Fermentation process.* A random representative selection of cabbage heads was chosen and  
104 their edible part was shredded to about 2 mm thickness using a domestic shredder (Moka Express,  
105 Barcelona, Spain). 5 g/kg NaCl was added into shredded cabbage and mixed thoroughly. Cabbage  
106 and brine were transferred into sterile fermentation vessels (8 L), (Nalge Nunc. International,  
107 Rochester, NY) and pressed thoroughly to remove air bubbles. Shredded and salted cabbage was  
108 spontaneously fermented by the indigenous cabbage microbiota or by *L. mesenteroides* inoculation.  
109 Fermentation was carried out in triplicate at room temperature for 7 days.

110 2.4. *Storage conditions.* Immediately after fermentation, three samples of sauerkraut corresponding  
111 to each fermentation replicate were placed in sterile capped glass vessels (0.5 L). The vessels were  
112 filled by thoroughly pressing down to ~0.3 cm from their upper edges simulating the packaging and  
113 storage in traditional household sauerkraut production. Then, the samples were stored at 4 °C for 1,  
114 2, and 3 months (conventional storage). Another three replicated samples were placed in sterile  
115 capped glass vessels equipped with a silicone septum on the lid, flushed with high purity N<sub>2</sub> for 5  
116 min and stored at 4 °C for up to 3 months (N<sub>2</sub>-enriched atmosphere storage). The O<sub>2</sub> concentration  
117 in these vessels was below 0.5 g/kg, measured by an O<sub>2</sub> detector (Oxybaby 6, Witt, Santander,  
118 Spain). Three sauerkrauts replicates were immediately analysed and were considered as unstored  
119 sauerkrauts.

120 2.5. *Microbiological analyses.* Microbiological analyses were performed in sauerkrauts stored for  
121 up to 3 months under conventional or N<sub>2</sub>-enriched atmosphere. Five grams of each sample were  
122 aseptically diluted in buffered peptone water (Scharlau Chemie, Spain) in a sterile Stomacher bag  
123 and homogenised for 1 min in a Stomacher blender (IUL Masticator, Barcelona, Spain). Further  
124 serial dilutions were made for plating. The pour plate technique was employed to determine the  
125 microbial counts. Total aerobic mesophilic bacteria were enumerated on Tryptone Soya Agar (TSA)  
126 after incubation at 30 °C for 72 h; total anaerobic mesophilic bacteria on TSA after incubation on  
127 anaerobic conditions at 30 °C for 72 h; total and faecal coliforms on Violet Red Bile Agar (VRBA)  
128 containing lactose as carbohydrate source, after incubation at 37 °C and 44 °C, respectively, for 24  
129 h; moulds and yeasts on Sabouraud-Chloramphenicol Agar, after incubation at 23 °C for 96 h; and  
130 lactic acid bacteria (LAB) on MRS Agar after incubation in anaerobic conditions at 30 °C for 48 h.

131 2.6. *Vitamin C content.* Determination of vitamin C in stored sauerkrauts was performed by  
132 capillary electrophoresis as described in Frias, Miranda, Doblado, & Vidal-Valverde (2005).

133 2.7. *Content of GLS breakdown products.* The ABG content in fermented cabbages stored in the  
134 described conditions was quantified as described by Peñas et al. (2010). The content of  
135 isothiocyanates and nitriles formed by GLS hydrolysis in sauerkrauts was determined as in Tolonen  
136 et al. (2002) with slight modifications. Briefly, 0.2 g of freeze-dried samples were extracted in 3 mL  
137 of methylene chloride by agitation for 4 h at room temperature. After centrifugation (484 x g for 10  
138 min), 50 µL of chlorathalonil (0.2 g/L) were added as an internal standard to 1 mL of sample  
139 supernatant. All samples were extracted in triplicates. The separation and quantification of these  
140 GLS breakdown products were carried out by PE Clarus 500 GC-MS (Perkin-Elmer, Shelton, CT,  
141 USA) using splitless injection (1 µL, split-on time 1.40 min) to a double gooseneck liner. PE Elite-  
142 5MS (30 m x 0.25 mm i.d., film thickness 0.25 µm) was used as the analytical column with helium  
143 as carrier gas (1.0 mL/min). The analysis was performed isothermally at oven temperature of 110 °C  
144 (22 min). The injector was set at 250 °C and the GC-MS transfer to 260 °C. MS was employed at  
145 scan mode 40-550 m/e. Quantification of IB, IBN and SFN was performed using the calibration

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

149 *2.9. Antioxidant activity.* The antioxidant activity, measured as Oxygen Radical Absorbance  
150 Capacity by fluorescence (ORAC-FL), was determined in potassium phosphate buffer (pH 7.0)  
151 extracts by suspension of 1 g of freeze-dried sample in 10 mL of extraction buffer, stirring (1 h at  
152 room temperature) and filtration through Whatman No.1 filter paper. ORAC-FL values were  
153 determined as described by Martinez-Villaluenga, Peñas, Sidro, Ullate, Frias, Vidal-Valverde  
154 (2012).

155 *2.10. Statistical analysis.* Data were expressed as mean±standard deviation of three independent  
156 determinations for each replicated sample. One-way analysis of variance (ANOVA) using the least-  
157 squared difference (LSD) test was performed to determine whether there were significant ( $P\leq 0.05$ )  
158 differences between groups. STATGRAPHICS 5.0 software (Statistical Graphics Corp, Rockville,  
159 MD, USA) for Windows was used for the calculations.

### 160 **3. Results and discussion**

161 *3.1. Microbial quality of stored sauerkrauts.* Figures 1 and 2 depict the microbial counts of  
162 naturally and *L. mesenteroides* fermented cabbages during refrigerated storage under conventional  
163 or N<sub>2</sub>-enriched atmospheres for 3 months. Spontaneously fermented cabbages presented high  
164 populations of aerobic mesophilic bacteria (~6.8 log CFU/g), anaerobic mesophilic bacteria (~6.8  
165 log CFU/g) and LAB (6.7 log CFU/g) (Figure 1), whilst populations of total and faecal coliforms as  
166 well as moulds/yeasts were below the detection limit (1 log CFU/g). These results are in agreement  
167 with those previously observed in spontaneously fermented cauliflower (Paramithiotis,  
168 Hondrodinou, & Drosinos, 2010) and Chinese sauerkrauts (Xiong, Guan, Song, Hao, & Xie, 2012).

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

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

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

195 Figure 2 illustrates the microbial status of cabbages fermented by *L. mesenteroides* and  
196 stored for 3 months. The evolution of microbial populations in induced-fermented cabbages during  
197 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<sub>2</sub> 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<sub>2</sub>-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 °C for 17 weeks. These differences could be  
212 attributed to the different O<sub>2</sub> concentration present in the vessels used during storage. During  
213 storage of naturally fermented sauerkraut in N<sub>2</sub> atmospheres for 2 months, no significant differences  
214 in ABG levels were observed, when compared with conventional storage. Surprisingly, a significant  
215 (P≤0.05) lower ABG concentration was found in N<sub>2</sub>-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<sub>2</sub> in  
219 the vessels during conventional storage (since sauerkraut was strongly pressed for O<sub>2</sub> 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<sub>2</sub>-enriched atmospheres. It could be speculated that N<sub>2</sub> 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 °C 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

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

263 During conventional refrigerated storage, different tendency in the evolution of the GLS  
264 derivatives analysed was observed (Table 3). IB, AC and SFN declined gradually and losses of  
265 about 18 %, 4 % and 17 %, respectively, were noted after 3 months. However, IBN and AITC were  
266 stable during all the storage period.  $\text{N}_2$ -storage led to significant reductions ( $P \leq 0.05$ ) on the  
267 concentration of IB, IBN and AITC when compared with conventional storage (Table 3), whilst no  
268 significant differences ( $P \leq 0.05$ ) in AC and SFN contents were found at the end of the storage period  
269 between both types of storage.

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

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

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

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

301 *mesenteroides* sauerkrauts stored at 4 °C in the presence of air or N<sub>2</sub> enriched atmospheres,  
302 suggesting that the use of N<sub>2</sub> did not negatively affect the stability of the identified GLS-derived  
303 compounds. At the end of the storage period in N<sub>2</sub> atmospheres, retention percentages ranging from  
304 89 to 95% were observed for all GLS breakdown products analysed. These results are of great  
305 importance since these compounds have been previously shown to have anticarcinogenic properties.  
306 AITC can potentially inhibit bladder cancer development (Savio, da Silva, de Camargo, &  
307 Salvadori, 2014), whilst SFN has shown antiproliferative activity and induction of mitochondrial  
308 apoptosis in melanoma cells (Rudolf, Cervinka, & Rudolf, 2014). IB has been shown to inhibit the  
309 proliferation of human glioblastoma and neuroblastoma cells through the induction of cell apoptosis  
310 at low concentrations of 2.5 µM (Jadhav, Ezhilarasan, Vaughn, Berhow, & Mohanam, 2007;  
311 Jadhav, Vaughn, Berhow, & Mohanam, 2007). It has been reported that the consumption of 38  
312 mg/kg (equivalent to 0.6 µmol/kg body weight) of IBN by rats enhanced the activity of glutathione  
313 reductase that is involved in the protection against oxidative stress (Staak, Kingston, Waillig, &  
314 Jeffery, 1998). Moreover, Zhao et al (2001) found that a weekly intake of ITCs above 53 µmol  
315 reduced the risk of lung cancer. Taking into account the contents of GLS breakdown products  
316 observed in N<sub>2</sub>-stored sauerkrauts for 3 months, it could be concluded that a daily consumption of  
317 50-100 g of sauerkraut would provide effective doses of GLS degradation products to exert health-  
318 promoting effects.

319 *3.4. Antioxidant activity in stored sauerkrauts.* Table 5 shows the ORAC-FL values obtained for  
320 spontaneously or *L. mesenteroides* fermented cabbages during refrigerated storage. No significant  
321 differences in the antioxidant activity were found between natural (11.2 µmol Trolox/g f.w.) and *L.*  
322 *mesenteroides* sauerkrauts (12.8 µmol Trolox/g f.w.). These sauerkrauts presented higher  
323 antioxidant activity than that reported for raw white cabbage (Ciska, Karamac, & Kosinska, 2005;  
324 Kusznierevicz, Bartoszek, Wolska, Drzewiecki, Gorinstein, & Namiesnik, 2008; Martinez-  
325 Villaluenga et al., 2012). Several authors have also observed an increased antioxidant activity in  
326 spontaneously fermented white and Chinese cabbages (Kusznierevicz et al., 2008; Sun, Chou, &

327 Yu, 2009). ORAC-FL assay measures the chain-breaking action of “traditional” antioxidants  
328 (ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene and flavonoids) against peroxy radicals (Ou, Huang,  
329 Hampsch-Woodill, Flanagan, & Deemer, 2002). The high antioxidant activity of sauerkrauts can be  
330 attributed, on one hand, to the ability of LAB to hydrolyse polyphenols, compounds that are present  
331 in cabbage at high concentration (Lee, Boyce and Breadmore, 2011), into other simpler and more  
332 antioxidant ones. On the other hand, ABG and other GLS breakdown derivatives formed during  
333 fermentation can contribute to the overall antioxidant activity of sauerkraut, since they have showed  
334 free radical scavenging activity (Wagner & Rimbach, 2008; Cabello-Hurtado, Gicquel, & Esnault,  
335 2012). In addition, shredding of cabbage before fermentation could be partially responsible for the  
336 initial increase of antioxidant activity as it has been shown by Reyes, Villareal and Cisneros-  
337 Zeballos (2007) after wounding of white cabbage tissues.

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

351

#### 352 **4. Conclusions**

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

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- 497

498 **Figure captions**

499 **Figure 1.** Microbiological status of natural fermented cabbage during storage for 3 months at 4 °C in  
500 conventional (▲ ) and N<sub>2</sub>-enriched atmospheres ( □ ). Results are the mean of three independent  
501 experiments (n=3)

502 **Figure 2.** Microbiological status of cabbage fermented with *L. mesenteroides* during storage for 3  
503 months at 4 °C in conventional (▲ ) and N<sub>2</sub>-enriched (□) atmospheres. Results are the mean of three  
504 independent experiments (n=3)

505