

White cabbage fermentation improves ascorbigen content, antioxidant and nitric oxide production inhibitory activity in LPS-induced macrophages

C. Martínez-Villaluenga, E. Peñas, B. Sidro, M. Ullate, J. Frias, C. Vidal-Valverde*

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

Author's email address:

C. Martinez-Villaluenga: c.villaluenga@ictan.csic.es

E. Peñas: elenape@ifi.csic.es

B. Sidro: bsidro@ictan.csic.es

J. Frias: frias@ictan.csic.es

M. Ullate: mullate@ifi.csic.es

C. Vidal-Valverde: cvidal@ictan.csic.es

*Corresponding author contact information:

Phone: + 34 915622900 Ext. 241

Fax: + 34 915644873

1 **ABSTRACT**

2 Consumption of foods rich in dietary antioxidants and anti-inflammatory compounds is becoming
3 a key strategy to lower oxidative stress and inflammation. The objective of this work was to study
4 the effect of fermentation and starter culture on ascorbigen (ABG) and vitamin C content, as well
5 as antioxidant and anti-inflammatory properties of white cabbage (*Brassica oleracea* var.
6 *capitata* cv. Megaton). *Lactobacillus plantarum* CECT 748 (LP), *Leuconostoc mesenteroides*
7 *CECT 219* (LM) or a mixed culture of both strains at 1:1 ratio (LPM) were used as starter
8 cultures in sauerkraut manufacture. Microbiological and sensorial quality of sauerkraut was also
9 examined. White cabbage fermentation increased (P<0.05) ABG content (up to 12-fold), oxygen
10 radical absorbance capacity (ORAC) values (up to 2-fold) and NO production inhibitory potency
11 (up to 2.6-fold). Vitamin C content slightly decreased (P<0.05) up to 1.4-fold during
12 fermentation. LM sauerkraut showed the highest (P<0.05) ABG concentration (204.8
13 $\mu\text{moles}/100\text{g d.w.}$), ORAC values (164.0 $\mu\text{moles Trolox/g d.w.}$) and NO inhibitory potency (IC_{50}
14 = 60.8 $\mu\text{g extract/mL}$). The microbiological quality of LM, LP and LPM sauerkrauts was
15 satisfactory. Experimental sauerkrauts showed higher overall acceptability (P<0.05) compared to
16 commercial products. Consequently, selection of starter culture is of great importance in the
17 manufacture of sauerkraut with improved content of bioactive compounds and health-promoting
18 potential.

19
20 **Keywords:** starter culture, sauerkraut, ascorbigen, antioxidant activity, anti-inflammatory activity

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24

25 1. Introduction

26 Oxidative stress is linked to inflammation playing together an important role in the
27 pathogenesis of cancer (Maeda & Omata, 2008), cardiovascular diseases (Montecucco, Pende,
28 Quercioli, & Mach, 2011), type 2 diabetes and obesity (Sell & Eckel, 2009). Oxidative stress is
29 an imbalance between production of reactive oxygen species and antioxidant defenses
30 (Betteridge, 2000). The redox stress triggers the activation of immune cells which release pro-
31 inflammatory cytokines, reactive oxygen and nitrogen species causing damage to biological
32 molecules and inducing imbalances in physiological and pathological pathways (Lonkar &
33 Dedon, 2011). Epidemiological and *in vivo* studies have provide evidence that dietary intake of
34 antioxidant and anti-inflammatory compounds is a key strategy for health promotion by lowering
35 oxidative stress and inflammation (Watz, 2008).

36 Sauerkraut is an important dietary ingredient in Central Europe that results from the lactic
37 acid fermentation of shredded and brined white cabbage. During fermentation of white cabbage,
38 glucosinolates (GLS) undergo complete hydrolysis to form an array of health-promoting products
39 (Ciska & Pathak 2004). The main GLS hydrolysis product in fermented cabbage is ascorbigen
40 (ABG) which is formed by the enzymatic hydrolysis of glucobrassicin to indol-3-carbinol (I3C)
41 and its subsequent reaction with L-ascorbic acid (Hrncirik, Valusek, & Velisek, 1998). Regarding
42 health-promoting properties, fermented cabbage has a high antioxidant potential (Kusznierewicz
43 et al., 2010) as it is rich in antioxidants such as vitamin C and ABG (Wagner et al., 2008a).
44 Moreover, GLS hydrolysis products in sauerkraut such as ABG, I3C, sulforaphane (SF), allyl
45 isothiocyanate (AITC), butyl isothiocyanate (BITC) and phenylethyl isothiocyanate (PITC)
46 (Tolonen et al., 2002; Ciska & Pathak, 2004; Peñas, Pihlava, Frias, & Vidal-Valverde, 2011)
47 have shown to be effective in the attenuation of oxidative stress by up-regulation of antioxidant
48 and phase 2 enzymes gene expression (Ernst et al., 2011; Guerrero-Beltrán, Calderón-Oliver,
49 Pedraza-Chaverri, & Chirino, 2010; Wagner et al. 2008b). Sauerkraut also provides a pool of

50 anti-inflammatory compounds such as SF (Lin, Wu, Wu, Khor, Wang, & Kong, 2008), and I3C
51 (Cho et al. 2008). Consequently, it seems that bioactives in sauerkraut have a promising potential
52 in the fight of oxidative stress and inflammation, however, the potential anti-inflammatory
53 activity of sauerkraut has not been reported yet.

54 GLS hydrolysis products profile in sauerkraut is determined by factors such as individual
55 GLS content in the raw material, NaCl concentration in the brine and starter culture used (Peñas
56 et al., 2011a) which would affect to the health-promoting properties of sauerkraut. Therefore,
57 those factors require control to obtain a final product with high content of bioactives and a
58 desirable potential from the human health point of view. Thus, the objective of this research was
59 to determine the influence of fermentation and starter culture on the ABG and vitamin C content,
60 and the antioxidant and anti-inflammatory activity of white cabbage.

61

62 **2. Material and Methods**

63 *2.1. Materials*

64 White cabbages (*Brassica oleracea* L. var. *capitata* cv. Megaton) grown in the North
65 region of Spain (Calahorra, La Rioja) during winter season 2009 were provided by Bejo Iberica
66 S. L. (Madrid, Spain). Cabbage cv. Megaton was selected based on its high glucobrassicin
67 content (Peñas, Frias, Martínez-Villaluenga & Vidal-Valverde, 2011). Lactic acid bacteria
68 (LAB) strains, *Lactobacillus plantarum* CECT 748 and *Leuconostoc mesenteroides* CECT 219,
69 were obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). Murine
70 macrophages RAW 264.7, Dubelcco's modified Eagle Medium (DMEM), fetal bovine serum
71 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Unless
72 otherwise specified, all chemicals were purchased from Sigma (Dorset, UK).

73

74 2.2. *Starter culture preparation*

75 LAB cultures were inoculated (1%) in MRS broth (Difco Laboratories, Detroit, MI,
76 USA) and incubated at 30 °C for 16 h. Cells were harvested by centrifugation at 8,000 g for 10
77 min, washed twice and resuspended in sterile saline solution (0.9g NaCl/100 mL water). This
78 cell suspension (9 log cfu/mL) was used as starter culture for white cabbage fermentation.

79

80 2.3. *Sauerkraut manufacture*

81 Outer leaves and central core were removed from cabbage heads that were further
82 shredded into 2 mm thick strips using a domestic shredder (Moka Express, Barcelona, Spain).
83 Starter cultures were inoculated at 6 log cfu/g and 0.5 g NaCl/100 g cabbage was added.
84 Cabbage was mixed thoroughly, placed in sterile polyethylene vessels (8 L) and tightly pressed
85 to exclude air. Three fermentation trials were performed in triplicate using different starter
86 cultures: *L. plantarum* (LP), *L. mesenteroides* (LM) and a mixed starter culture containing *L.*
87 *plantarum* and *L. mesenteroides* at 1:1 ratio (LPM). Each fermentation trial was performed in
88 triplicate (4 Kg per batch) at room temperature (22-25 °C) for 7 days. On the third day, cabbage
89 was pricked to remove releasing gases. Raw cabbage and sauerkrauts were freeze-dried, milled
90 and stored under vacuum at -20 °C until their analysis.

91

92 2.4. *Determination of pH*

93 Brine from each fermentation batch (2 mL) was collected at 0, 3 and 7 days and pH was
94 measured using a pH meter Basic 20 (Crison, Barcelona, Spain).

95

96 2.5. *Microbiological counts*

97 Microbiological analyses were performed in triplicate on raw and fermented cabbages as
98 described by Peñas, Frias, Sidro, & Vidal-Valverde (2010).

99 2.6. *Determination of ABG*

100 The content of ABG was determined in raw cabbage and sauerkrauts as described in Peñas
101 et al. (2010).

102

103 2.7. *Determination of vitamin C*

104 The quantification of vitamin C was performed by capillary electrophoresis (CE) in raw
105 cabbage and sauerkrauts (Frias, Miranda, Doblado, & Vidal-Valverde, 2005).

106

107 2.8. *Determination of antioxidant activity*

108 The antioxidant activity, measured as Oxygen Radical Absorbance Capacity by
109 fluorescence (ORAC-FL) was determined in potassium phosphate buffer (pH 7.0) extracts by
110 suspension of 1 g of freeze-dried sample in 10 mL of extraction buffer and stirring for 1 h at
111 room temperature. Extracts were filtered using Whatman No.1 paper. The ORAC-FL value was
112 determined as described by Dávalos, Gómez-Cordovés, & Bartolomé (2004).

113

114 2.9. *Determination of anti-inflammatory activity*

115 2.9.1. *Cell culture and extracts preparation*

116 Murine macrophages RAW 264.7 from American Type Culture Collection (ATCC,
117 Manassas, VA, USA) were cultured in DMEM containing penicillin/streptomycin 5mL/500mL
118 DMEM), and fetal bovine serum (50 mL/500mL DMEM) at 37 °C in 5% CO₂ atmosphere.
119 Extracts were prepared by homogenization of 500 mg freeze-dried sample in 20 mL
120 acetone:water solution (1:1) using an UltraTurrax homogenizer T-25 Digital (Ika Werke GMBH
121 & Co., Staufen, Germany), and centrifuged at 3,024 $x g$ for 7 min at 5 °C. Supernatant was
122 collected, and the pellet was extracted twice with 10 mL of acetone. Further, supernatants were

123 combined, filtered using Whatman No. 1 paper and evaporated to dryness. Finally, the residue
124 was resuspended in dimethylsulfoxide (DMSO) at 0.1mL in 100mL distilled water.

125

126 2.9.2. *Cell viability assay*

127 The cell proliferation assay was conducted using the CellTiter 96 Aqueous One Solution
128 Proliferation assay kit following manufacturer's instructions (Promega Biotech Iberica, Madrid,
129 Spain). Briefly, 5×10^4 cells/well were seeded in a 96-well plate. The cells were allowed to grow
130 in DMEM for 24 h at 37 °C in 5% CO₂. After 24 h incubation, they were treated with different
131 concentrations of raw and fermented cabbage extracts (0-150 µg/mL) for 24 h. After treatment,
132 growth medium was replaced by 100 µL fresh DMEM and 20 µL cell titer solution was added to
133 each well. The plate was incubated for 2 h at 37 °C and the absorbance measured at 490 nm. The
134 percentage of viable cells was calculated with respect to control (cells treated with vehicle:
135 0.1mL/100mL DMSO) as follows: $A_{\text{treatment } 490 \text{ nm}}/A_{\text{control } 490 \text{ nm}} \times 100 = \% \text{ cell viability}$).

136

137 2.9.3. *Measurement of nitric oxide (NO)*

138 Briefly, 5×10^4 cells/well were seeded in a 96-well plate and allowed to grow in DMEM
139 for 24 h at 37 °C in 5% CO₂. The cells were treated with 1 µg/mL LPS from *Escherichia coli*
140 O55:B5 with or without different concentrations of raw and sauerkraut extracts (0-150 µg/mL),
141 for 24 h. Medium was collected after treatment and NO production analyzed. Nitrite
142 accumulation, and indicator of NO synthesis, was measured in the culture medium by Griess
143 reaction (Green, Wagner, Glogowski, Skipper, Wishnok, & Tannenbaum, 1982). Briefly, 100 µL
144 of DMEM were plated in 96-well plate and an equal amount of Griess reagent. The plate was
145 incubated for 5 min and the absorbance measured at 550 nm in a microplate reader (Biotek,
146 Winooski, VT, USA). The amount of NO was calculated using a sodium nitrite standard curve
147 (concentration range 0-115 µmol/L of nitrite). NO production inhibitory potency (IC₅₀) is defined

148 as the concentration of extract ($\mu\text{g extract/mL}$) that inhibited 50% of the NO production in non-
149 treated cells. IC_{50} values were determined by dose–response curves in which the range of
150 concentrations was distributed in a logarithmic scale and using the non-linear regression
151 sigmoidal curve fit function in GraphPad Prism 4.00 (Graphpad Software Inc., San Diego, CA,
152 USA).

153

154 *2.10. Sensory analysis*

155 Sensory analyses of each fermentation trial were carried out by ten panellists from Institute
156 of Food Science, Technology and Nutrition (ICTAN-CSIC), selected on the basis of their
157 sauerkraut acceptance and ability to distinguish the basic scale tastes. Acceptance of experimental
158 sauerkrauts was compared to different commercial sauerkrauts purchased in local markets. A
159 structure hedonic scale of 10 points was used for evaluation of flavour, firmness and colour
160 attributes as well as the overall acceptability of sauerkraut (Table 1), as described by
161 Johanningsmeier, McFeeters, Fleming, & Thompson (2007). Samples were randomly presented
162 to each panellist at each testing section. Water and unsalted crackers were provided to panellists
163 for palate cleansing in between samples.

164

165 *2.11. Statistical analysis*

166 Data are expressed as means \pm standard deviations of three replicates. One-way analysis of
167 variance (ANOVA) using the least-squared difference (LSD) test to determine whether there
168 were significant ($P < 0.05$) differences between groups and multiple correlations were performed.
169 Statgraphics 5.0 software (Statistical Graphics Corporation, Rockville, MD, USA) and Statistica
170 5.1 Program (Statsoft, Tulsa, OK 74104 USA) for Windows were used.

171

172

173 **3. Results and Discussion**

174 *3.1. Effect of fermentation and starter culture on pH and microbiological quality of cabbage*

175 White cabbage brine pH values (6.03) decreased below 4 after 4 days of fermentation and
176 it remained constant until the end of fermentation (Fig. 1). Brine pH was slightly higher ($P < 0.05$)
177 in LM fermented cabbage (3.55 and 3.58) compared to LP (3.20 and 3.23) and LPM (3.28 and
178 3.29) after 4 and 7 days of fermentation, respectively, which was correlated ($r = 0.75$) with LAB
179 counts (Table 2). Therefore, lower pH values in LM sauerkraut could be explained by lower LAB
180 counts compared to LP and LPM. Conversely, Tolonen et al. (2004) observed lower pH values in
181 LM than LP brine sauerkrauts. This is likely due to differences such as type of strain and
182 inoculum concentration.

183 Microbial profile of raw cabbage was constituted by aerobic mesophilic bacteria (5.19 log
184 cfu/g f.w.), followed by anaerobic bacteria (4.38 log cfu/g f.w.), low counts of LAB (2.42 log
185 cfu/g f.w.) and total coliforms (1.17 log cfu/g f.w.). Furthermore, faecal coliforms, moulds and
186 yeasts were < 1 log cfu/g f.w. Fermentation increased ($P < 0.05$) aerobic and anaerobic mesophilic
187 bacteria, and LAB counts up to 3 log cfu/g f.w. and 5 log cfu/g f.w., respectively; however, total
188 and faecal coliforms, moulds and yeasts were not detected (Table 2). These results indicate a
189 satisfactory microbial quality of LP, LM, and LPM sauerkrauts according to the guidelines for
190 ready-to-eat foods reported by the Public Health Leadership Society (PHLS, 2000).

191

192 *3.2. Effect of fermentation and starter culture on ABG and vitamin C content in cabbage.*

193 Raw cabbage exhibited a low content of ABG (16.4 $\mu\text{mol}/100\text{g d.w.}$) (Table 3) and
194 fermentation led to a sharp increment (Fig. 2). The type of starter culture had a significant on
195 ABG concentration in sauerkraut ranging from 175.3 to 204.8 $\mu\text{moles}/100\text{g d.w.}$ (Table 3). LM
196 sauerkraut showed the highest ($P < 0.05$) ABG compared to LP and LPM, results in agreement
197 with Tolonen et al. (2004) who suggested that starter cultures possess different levels of

198 myrosinase-like activity, enzyme involved in the ABG formation. This statement is supported by
199 previous research where was demonstrated the ability of LAB to degrade GLS *in vitro* (Nugon-
200 Baudon, Rabot, Wal, & Szytit, 1990). ABG content in LM sauerkraut was 2-fold higher than
201 sauerkrauts from white cabbage cv. Bronco and cv. Taler (Peñas et al. 2010; Martinez-
202 Villaluenga et al. 2009), differences which could be attributed to higher myrosinase activity
203 found in cv. Megaton (Peñas et al. 2011b).

204 Vitamin C content (Table 3) in raw white cabbage (329.5 mg/100g d.w.) was within the
205 range reported in the literature (Podsedek, 2007). During cabbage fermentation, vitamin C
206 content decreased between 24% and 29% depending on starter culture (Fig. 2), in consistency
207 with our previous studies (Peñas et al., 2010; Martinez-Villaluenga et al., 2009). Losses observed
208 in vitamin C could be attributed to the participation of ascorbic acid in ABG formation which
209 may reach up to 10% (Hrncirik, Valusek, & Velisek, 2001). In addition, oxidation of ascorbic
210 acid during sauerkraut manufacture could also take place (Klieber & Frankin, 2000).

211

212 3.3. *Effect of fermentation and starter culture on antioxidant activity of cabbage.*

213 Antioxidant activity of sauerkraut was measured using ORAC-FL assay which is closely
214 related to biological functions of chain-breaking antioxidants against peroxy radicals (Ou,
215 Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). Peroxy radicals contribute to oxidative
216 stress by lipid peroxidation of cell membranes and low-density lipoproteins (LDL) (Betteridge,
217 2000). Therefore, peroxy radical absorption capacity of foods would be relevant to fight against
218 oxidative stress and related disorders. ORAC value of raw cabbage (74.8 $\mu\text{mol Trolox/g d.w.}$)
219 was within the range of values reported by the USDA database (USDA, 2010) (Table 3).
220 Cabbage fermentation led to a noticeable increase ($\geq 90\%$) of ORAC values (Fig. 2) which is
221 consistent with findings observed by Kusznierycz et al. (2010). White cabbage antioxidant
222 activity measured by ORAC is attributed to vitamin C and polyphenols (Sikora, Cieslik,

223 Leszczynska, Filipiak-Florkiewicz, & Pisulewski, 2008). In addition, Wagner et al. (2008a)
224 showed that ABG acts as a moderate free radical scavenger *in vitro* and as a potent inhibitor of
225 chemical-induced lipid peroxidation in human keratinocytes. Antioxidant activity differed
226 significantly among the fermented products (LP, LM and LPM). LM sauerkraut showed the
227 highest antioxidant capacity ($P < 0.05$) which may also be attributed to its high concentration of
228 ABG (Table 3).

229
230 3.4. *Effect of fermentation and starter culture on NO production inhibitory activity of cabbage*
231 *in LPS-induced macrophages.*

232 Viability of macrophages treated with raw cabbage and sauerkraut (LP, LM and LPM)
233 extracts did not significantly ($P > 0.05$) differ from that of non-treated cells. Raw cabbage, LP, LM
234 and LPM sauerkraut extracts suppressed ($P < 0.05$) NO production in a dose-dependent manner
235 (Fig 3B). Interestingly, NO production inhibitory potency improved after white cabbage
236 fermentation (Fig. 3C) which could be related to the formation of compounds exerting such
237 biological activity. White cabbage fermentation led to formation of ABG (Table 2) which have
238 shown a moderate NO production inhibitory activity in LPS-induced mouse peritoneal
239 macrophages (Peñas et al., 2011c). Fermentation of cabbage cv. Megaton give rise to the
240 production of iberin, iberin nitrile, allyl cyanide, AITC and SF (Peñas et al., 2011a). Among these
241 GLS hydrolysis products, SF and AITC have proven anti-inflammatory properties (Brandenburg,
242 Kipp, Lucius, Pufe, & Wruck, 2010; Han, Park, Um, Kim, & Jeong, 2011).

243 Starter culture had an impact in the NO production inhibitory activity of sauerkraut extracts in
244 cultured LPS-induced macrophages. LM sauerkraut extract exhibited higher ($P < 0.05$) NO
245 production inhibitory activity ($IC_{50} = 60.89 \mu\text{g extract/mL}$) compared to LP ($IC_{50} = 109.2 \mu\text{g}$
246 extract/mL) and LPM ($IC_{50} = 105.4 \mu\text{g extract/mL}$) sauerkraut extracts (Figure 3C). These results
247 could be due to differences in the GLS hydrolysis products profile among fermented products.

248 For instance, LM sauerkraut exhibited a remarkable higher ABG (Table 2) and SF concentration
249 (Peñas et al., 2011a) compared to LP and LPM sauerkrauts.

250

251 3.5. *Sensory quality of fermented cabbage by different starter culture*

252 Raw cabbage flavour in commercial and LM sauerkraut was rated lower ($P < 0.05$) than LP
253 and LPM (Table 4; Fig. 4). Low scores of this quality attribute are accepted better by consumers
254 because raw cabbage-like flavour is associated with immature sauerkraut (Holzapfel, Schillinger,
255 & Buckenhüskes, 2003). Kraut sulphur flavour is a quality attribute of properly fermented
256 cabbage which is attributed to sulphur compounds derived from S-methyl-cysteine sulfoxide and
257 some GLS (Johanningsmeier et al. 2005). Experimental and commercial sauerkrauts exhibited
258 scores around the middle point of the hedonic scale (4.8-5.9), however, slightly lower values
259 ($P \leq 0.05$) were obtained in LP and LM fermented cabbage (Table 4; Fig. 4). Regarding acid
260 flavour, LP and LM sauerkrauts had a mild intensity (4.4 and 5.5, respectively) that was lower
261 compared to LPM and commercial sauerkrauts ($P < 0.05$). A mild-acid flavour is a desirable
262 quality attribute than a strong acidity, according to Viander, Maki, & Palva (2003). As it might be
263 expected, the intensity of salty taste was higher ($P < 0.05$) in commercial (5.7-6.1) than in
264 experimental sauerkrauts (3.4-4.4) (Table 4; Fig. 4) due to its higher NaCl concentration.
265 Nowadays, consumer preferences are moving towards mild saltiness as it provides better
266 sensorial quality and helps to prevent hypertension (Holzapfel, 2003). Moreover, color was
267 highly variable among commercial samples while slight differences were observed among
268 experimental sauerkrauts. Commercial sauerkrauts exhibited a lighter color than experimental
269 sauerkraut lots (light yellow). The firmness of experimental fermentations (6.8-7.5) was higher
270 ($P < 0.05$) than commercial sauerkrauts (4.5-5.9) (Table 4; Fig. 4) which could be explained by a
271 higher NaCl concentration in commercial ones, as suggested by Viander et al. (2003). Regarding
272 the overall acceptability, similar (LM vs. commercial sauerkraut A) or higher values (LP and

273 LPM vs. commercial sauerkraut B and C) were observed (Table 4; Fig. 4). In addition, overall
274 acceptability was not significantly ($P>0.05$) different among the experimental fermented
275 products. Higher ratings of experimental sauerkrauts could be attributed to their mild acidity, low
276 saltiness and higher firmness which are attributes associated to a better acceptability by the
277 consumers (Johanningsmeier et al. 2005).

278

279 **4. Conclusions**

280 Fermentation increased ABG content, antioxidant and NO inhibitory activity of white
281 cabbage and the type of starter had a noticeable impact on their health-promoting attributes. Thus,
282 the utilization of *L. mesenteroides* as starter culture should be advised for the production of
283 sauerkraut with improved ABG content, antioxidant capacity and NO production inhibitory
284 activities. Although these results warrant further *in vivo* studies, the presented *in vitro* data
285 suggest the potential of sauerkraut to attenuate oxidative stress and inflammation.

286

287 **Acknowledgements**

288 This work was supported by the Spanish Commission of Science and Technology
289 (CICYT), Project number AGL2007-62044. E. P. is indebted to a JAE-doc grant funded by
290 CSIC.

291

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401

402 **Figure Captions**

403 **Fig. 1.** Change of pH during induced cabbage fermentation by different starter cultures. Mean
404 values of three replicates are presented. *Significantly different between LM to LP and LPM
405 fermented cabbage ($P < 0.05$). ♦, LP; ■, LM; ▲, LPM.

406 **Fig. 2.** Relative content of ABG, vitamin C acid and ORAC in sauerkraut fermented by different
407 starter cultures (expressed as % of their values vs. raw cabbage). Mean values of three replicates
408 are presented. Bars indicate standard error of means.

409 **Fig. 3. A)** Cell viability (expressed as % vs. non-treated cells); **B)** NO production inhibitory
410 activity (expressed as % vs. non-treated cells) □, Raw; ◻, LP; ◼, LM; ■, LPM; and **C)** NO
411 inhibitory potency (IC_{50}) of extracts from raw cabbage and sauerkrauts fermented by different
412 starter cultures in LPS-activated macrophages RAW 264.7. Mean values of three replicates are
413 presented. Bars indicate standard error of means. #Significantly different from treated cells
414 respect to raw cabbage extract ($P < 0.05$).

415 **Fig. 4.** Diagram of sensorial evaluation of commercial and experimental sauerkrauts. Mean
416 values of three replicates are presented. ♦, Raw cabbage flavour; ■, Kraut sulfur flavour; ▲, Acid
417 flavour; x, Saltiness.

Table 1

Defined attributes for sensorial analysis of sauerkraut using a structure hedonic scale

Attributes	Definition	Hedonic scale
Raw cabbage flavour	Green and vegetative flavour of raw cabbage	0 = not detectable to 10 = very strong
Sulphur flavour	Sulphur note of properly fermented sauerkraut	0 = not detectable to 10 = very strong
Acid flavour	Sour taste associated with organic acids	0 = not detectable to 10 = very strong
Saltiness	Basic taste associated with sodium chloride	0 = not detectable to 10 = very strong
Firmness	Effort required for masticating the sample	0 = very soft to 10 = very firm
Colour	Graduated scale from green to creamy colour	0 = green to 10 = creamy
Overall acceptability	Overall qualification	0 = not acceptable to 10 = excellent acceptability

Table 2

Microbiological quality of raw cabbage and sauerkrauts fermented with different starter cultures

	Aerobic mesophilic bacteria	Anaerobic mesophilic bacteria	Lactic acid bacteria	Total Coliforms	Faecal Coliforms	Yeasts and moulds
Raw cabbage	5.19±0.11 ^a	4.38± 0.11 ^a	2.42± 0.11 ^a	1.17± 0.18 ^b	<1 ^a	<1 ^a
<i>Sauerkraut</i>						
LP	7.95±0.07 ^c	7.76±0.11 ^b	7.93±0.10 ^c	<1 ^a	<1 ^a	<1 ^a
LM	7.71± 0.13 ^b	7.64± 0.16 ^c	7.74± 0.13 ^b	<1 ^a	<1 ^a	<1 ^a
LPM	8.10± 0.05 ^d	8.06± 0.08 ^d	8.04±0.07 ^d	<1 ^a	<1 ^a	<1 ^a

Data are expressed as mean (expressed as log cfu/g fresh weight) ± standard deviation of three independent experiments. The same superscript in the same column indicates no significant difference (P<0.05).

Table 3

Effect of fermentation by different starter cultures on ascorbigen and vitamin C content and antioxidant activity of white cabbage (*B. oleracea* var. *capitata* cv. Megaton)

	Ascorbigen ($\mu\text{mol}/100\text{g d.w.}$)	Vitamin C ($\text{mg}/100\text{g d.w.}$)	ORAC ($\mu\text{mol Trolox}/\text{g d.w.}$)	Water ($\text{g}/100\text{g d.w.}$)
Raw cabbage	16.43 \pm 1.76 ^a	329.45 \pm 8.95 ^c	74.78 \pm 0.28 ^a	91.6
<i>Sauerkraut</i>				
LP	175.31 \pm 3.00 ^b	251.31 \pm 7.89 ^a	143.12 \pm 8.2 ^b	92.3
LM	204.80 \pm 7.12 ^c	249.91 \pm 6.59 ^a	164.04 \pm 4.02 ^c	92.2
LPM	178.08 \pm 4.73 ^b	234.37 \pm 8.76 ^b	142.15 \pm 1.39 ^b	92.2

Data are expressed as mean \pm standard deviation of three independent experiments. The same superscript in the same column indicates no significant difference ($P < 0.05$).

Table 4

Acceptance of commercial and experimental sauerkrauts obtained by white cabbage (*B. oleracea* var. *capitata* cv. Megaton) fermentation with different starter cultures

	Raw cabbage flavour	Sulphur flavour	Acid flavour	Saltiness	Firmness	Color	Overall acceptability
<i>Commercial sauerkrauts</i>							
A	2.3±2.5 ^a	5.9±1.8 ^c	6.7±1.6 ^b	5.7±1.7 ^b	4.5±1.7 ^a	9.1±1.8 ^e	5.5±1.5 ^{ab}
B	2.5±2.6 ^a	5.9±2.0 ^c	8.0±1.3 ^c	6.1±2.0 ^b	5.9±1.6 ^b	6.0±2.4 ^c	4.8±2.0 ^a
C	2.4±2.5 ^a	5.9±2.4 ^c	7.4±1.3 ^c	6.1±2.0 ^b	5.4±1.7 ^b	7.8±2.7 ^d	5.1±1.3 ^a
<i>Experimental sauerkrauts</i>							
LP	4.0±2.6 ^b	4.8±2.0 ^a	4.4±1.5 ^a	3.5±1.5 ^a	7.4±0.9 ^c	4.3±1.5 ^a	6.6±1.3 ^c
LM	3.1±3.0 ^{ab}	4.8±2.1 ^{ab}	5.0±1.6 ^a	3.4±1.4 ^a	6.8±1.4 ^c	4.6±1.3 ^{ab}	6.3±1.9 ^{bc}
LPM	4.2±2.2 ^b	5.3±0.8 ^{abc}	6.2±1.3 ^b	4.4±1.0 ^a	7.5±1.3 ^c	5.7±1.1 ^{bc}	6.5±1.4 ^c

Data are expressed as mean ± standard deviation of three independent experiments. The same superscript in the same column indicates no significant difference (P<0.05).

Fig. 1.

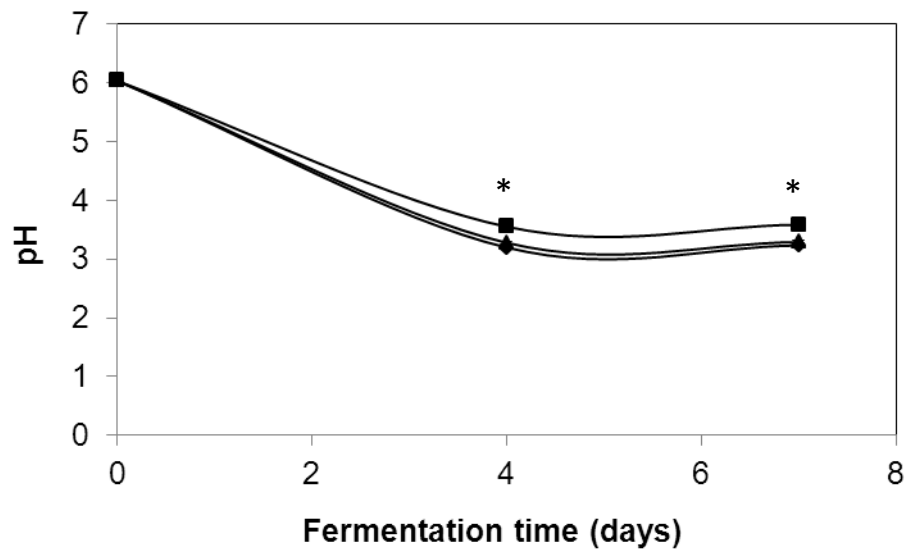


Fig 2.

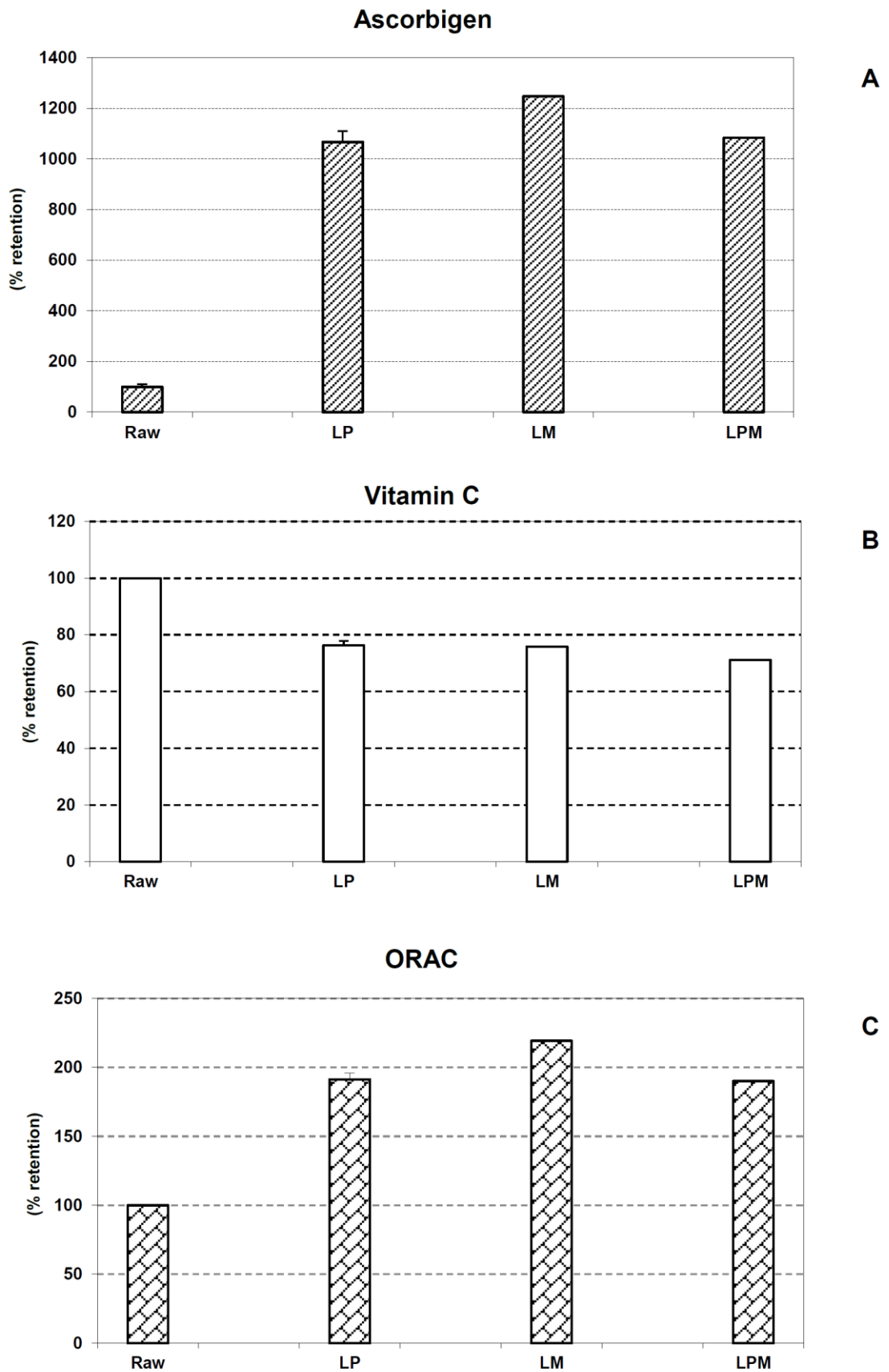


Fig. 3.

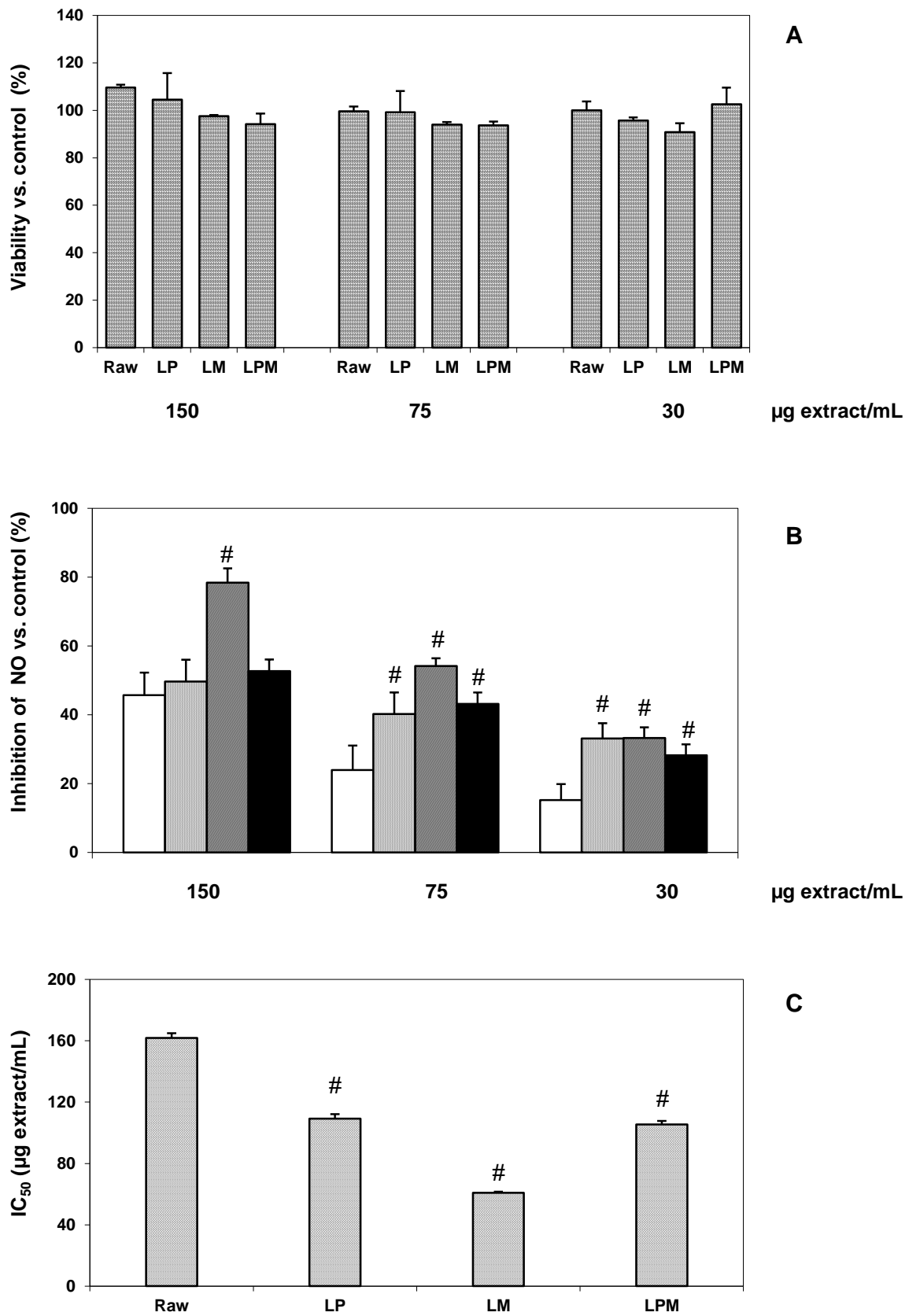


Fig. 4.

