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

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ABSTRACT

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

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

1. Introduction

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

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

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

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

2. Material and Methods

2.1. Materials

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

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 ºC 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 ºC) 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 ºC 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).

2.6. Determination of ABG

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

2.7. Determination of vitamin C

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

2.8. Determination of 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 and stirring for 1 h at room temperature. Extracts were filtered using Whatman No.1 paper. The ORAC-FL value was determined as described by Dávalos, Gómez-Cordovés, & Bartolomé (2004).

2.9. Determination of anti-inflammatory activity

2.9.1. Cell culture and extracts preparation

Murine macrophages RAW 264.7 from American Type Culture Collection (ATCC, Manassas, VA, USA) were cultured in DMEM containing penicillin/streptomycin 5mL/500mL DMEM), and fetal bovine serum (50 mL/500mL DMEM) at 37 ºC in 5% CO_2 atmosphere. Extracts were prepared by homogenization of 500 mg freeze-dried sample in 20 mL acetone:water solution (1:1) using an UltraTurrax homogenizer T-25 Digital (Ika Werke GMBH & Co., Staufen, Germany), and centrifuged at 3,024 *x g* for 7 min at 5 °C. Supernatant was collected, and the pellet was extracted twice with 10 mL of acetone. Further, supernatants were

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

2.9.2. Cell viability assay

The cell proliferation assay was conducted using the CellTiter 96 Aqueous One Solution Proliferation assay kit following manufacturer´s instructions (Promega Biotech Iberica, Madrid, Spain). Briefly, 5 x 10^4 cells/well were seeded in a 96-well plate. The cells were allowed to grow in DMEM for 24 h at 37 ºC in 5% CO_2. After 24 h incubation, they were treated with different concentrations of raw and fermented cabbage extracts (0-150 μg/mL) for 24 h. After treatment, growth medium was replaced by 100 μL fresh DMEM and 20 μL cell titer solution was added to each well. The plate was incubated for 2 h at 37 ºC and the absorbance measured at 490 nm. The percentage of viable cells was calculated with respect to control (cells treated with vehicle: 0.1mL/100mL DMSO) as follows: $A_{treatment\ 490\ nm}/A_{control\ 490\ nm}$ x 100 = % cell viability).

2.9.3. Measurement of nitric oxide (NO)

Briefly, 5 x 10^4 cells/well were seeded in a 96-well plate and allowed to grow in DMEM for 24 h at 37 ºC in 5% CO_2. The cells were treated with 1 μg/mL LPS from *Escherichia coli* O55:B5 with or without different concentrations of raw and sauerkraut extracts (0-150 μg/mL), for 24 h. Medium was collected after treatment and NO production analyzed. Nitrite accumulation, and indicator of NO synthesis, was measured in the culture medium by Griess reaction (Green, Wagner, Glogowski, Skipper, Wishnok, & Tannenbaum, 1982). Briefly, 100 μL of DMEM were plated in 96-well plate and an equal amount of Griess reagent. The plate was incubated for 5 min and the absorbance measured at 550 nm in a microplate reader (Biotek, Winooski, VT, USA). The amount of NO was calculated using a sodium nitrite standard curve (concentration range 0-115 μmol/L of nitrite). NO production inhibitory potency (IC_{50}) is defined

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

2.10. Sensory analysis

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

2.11. Statistical analysis

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

3. Results and Discussion

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

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

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

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

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

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

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

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

Antioxidant activity of sauerkraut was measured using ORAC-FL assay which is closely related to biological functions of chain-breaking antioxidants against peroxyl radicals (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). Peroxyl radicals contribute to oxidative stress by lipid peroxidation of cell membranes and low-density lipoproteins (LDL) (Betteridge, 2000). Therefore, peroxyl radical absorption capacity of foods would be relevant to fight against oxidative stress and related disorders. ORAC value of raw cabbage (74.8 μmol Trolox/g d.w.) was within the range of values reported by the USDA database (USDA, 2010) (Table 3). Cabbage fermentation led to a noticeable increase (≥ 90%) of ORAC values (Fig. 2) which is consistent with findings observed by Kusznierícz et al. (2010). White cabbage antioxidant 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 μg extract/mL) compared to LP (IC_{50} = 109.2 μg
246 xtract/mL) and LPM (IC_{50} = 105.4 μg extract/mL) sauerkraut extracts (Figure 3C). These results
247 could be due to differences in the GLS hydrolysis products profile among fermented products.

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

3.5. Sensory quality of fermented cabbage by different starter culture

Raw cabbage flavour in commercial and LM sauerkraut was rated lower ($P<0.05$) than LP and LPM (Table 4; Fig. 4). Low scores of this quality attribute are accepted better by consumers because raw cabbage-like flavour is associated with immature sauerkraut (Holzapfel, Schillinger, & Buckenhüskes, 2003). Kraut sulphur flavour is a quality attribute of properly fermented cabbage which is attributed to sulphur compounds derived from S-methyl-cysteine sulfoxide and some GLS (Johanningsmeier et al. 2005). Experimental and commercial sauerkrauts exhibited scores around the middle point of the hedonic scale (4.8-5.9), however, slightly lower values ($P\leq0.05$) were obtained in LP and LM fermented cabbage (Table 4; Fig. 4). Regarding acid flavour, LP and LM sauerkrauts had a mild intensity (4.4 and 5.5, respectively) that was lower compared to LPM and commercial sauerkrauts ($P<0.05$). A mild-acid flavour is a desirable quality attribute than a strong acidity, according to Viander, Maki, & Palva (2003). As it might be expected, the intensity of salty taste was higher ($P<0.05$) in commercial (5.7-6.1) than in experimental sauerkrauts (3.4-4.4) (Table 4; Fig. 4) due to its higher NaCl concentration. Nowadays, consumer preferences are moving towards mild saltiness as it provides better sensorial quality and helps to prevent hypertension (Holzapfel, 2003). Moreover, color was highly variable among commercial samples while slight differences were observed among experimental sauerkrauts. Commercial sauerkrauts exhibited a lighter color than experimental sauerkraut lots (light yellow). The firmness of experimental fermentations (6.8-7.5) was higher ($P<0.05$) than commercial sauerkrauts (4.5-5.9) (Table 4; Fig. 4) which could be explained by a higher NaCl concentration in commercial ones, as suggested by Viander et al. (2003). Regarding the overall acceptability, similar (LM vs. commercial sauerkraut A) or higher values (LP and

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

4. Conclusions

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

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Figure Captions

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

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

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

Fig. 4. Diagram of sensorial evaluation of commercial and experimental sauerkrauts. Mean values of three replicates are presented. ♦, Raw cabbage flavour; ■, Kraut sulfur flavour; ▲, Acid 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}
Sauerkraut						
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 (µmol/100g d.w.)	**Vitamin C (mg/100g d.w.)**	**ORAC (µmol Trolox/g d.w.)**	**Water (g/100 g d.w.)**
Raw cabbage	16.43±1.76[a]	329.45±8.95[c]	74.78±0.28[a]	91.6
Sauerkraut				
LP	175.31±3.00[b]	251.31±7.89[a]	143.12±8.2[b]	92.3
LM	204.80±7.12[c]	249.91±6.59[a]	164.04±4.02[c]	92.2
LPM	178.08±4.73[b]	234.37±8.76[b]	142.15±1.39[b]	92.2

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

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
Commercial sauerkrauts							
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}
Experimental sauerkrauts							
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.

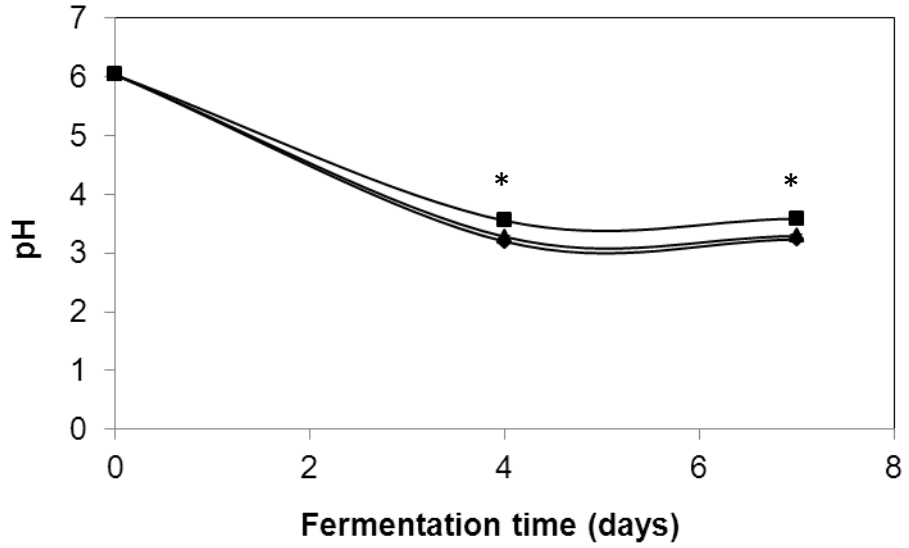


Fig 2.

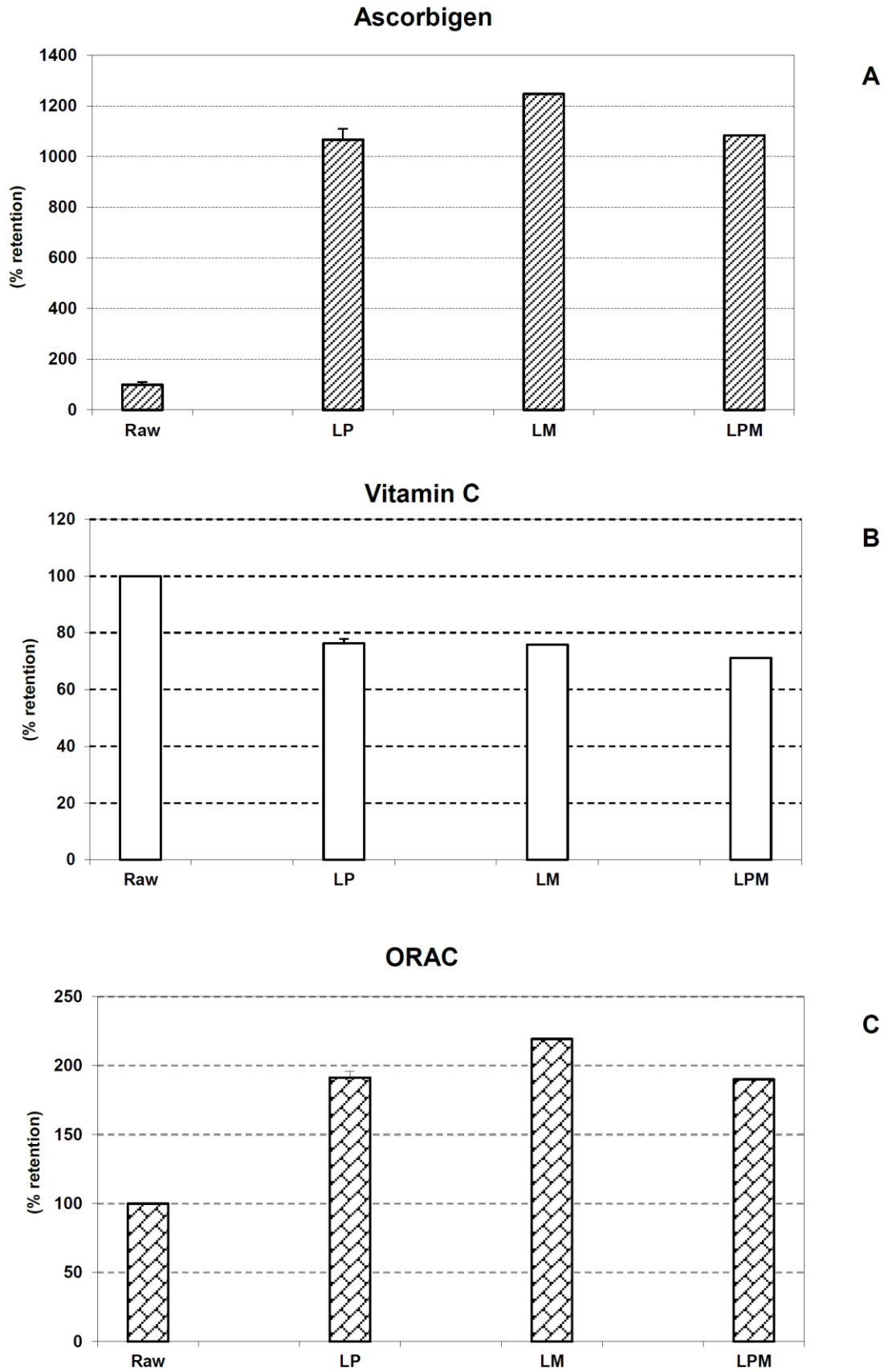

Ascorbigen
A
1400
1200
1000
800
600
400
200
0
(% retention)
Raw
LP
LM
LPM
Vitamin C
B
120
100
80
60
40
20
0
(% retention)
Raw
LP
LM
LPM
ORAC
C
250
200
150
100
50
0
(% retention)
Raw
LP
LM
LPM

Fig. 3.

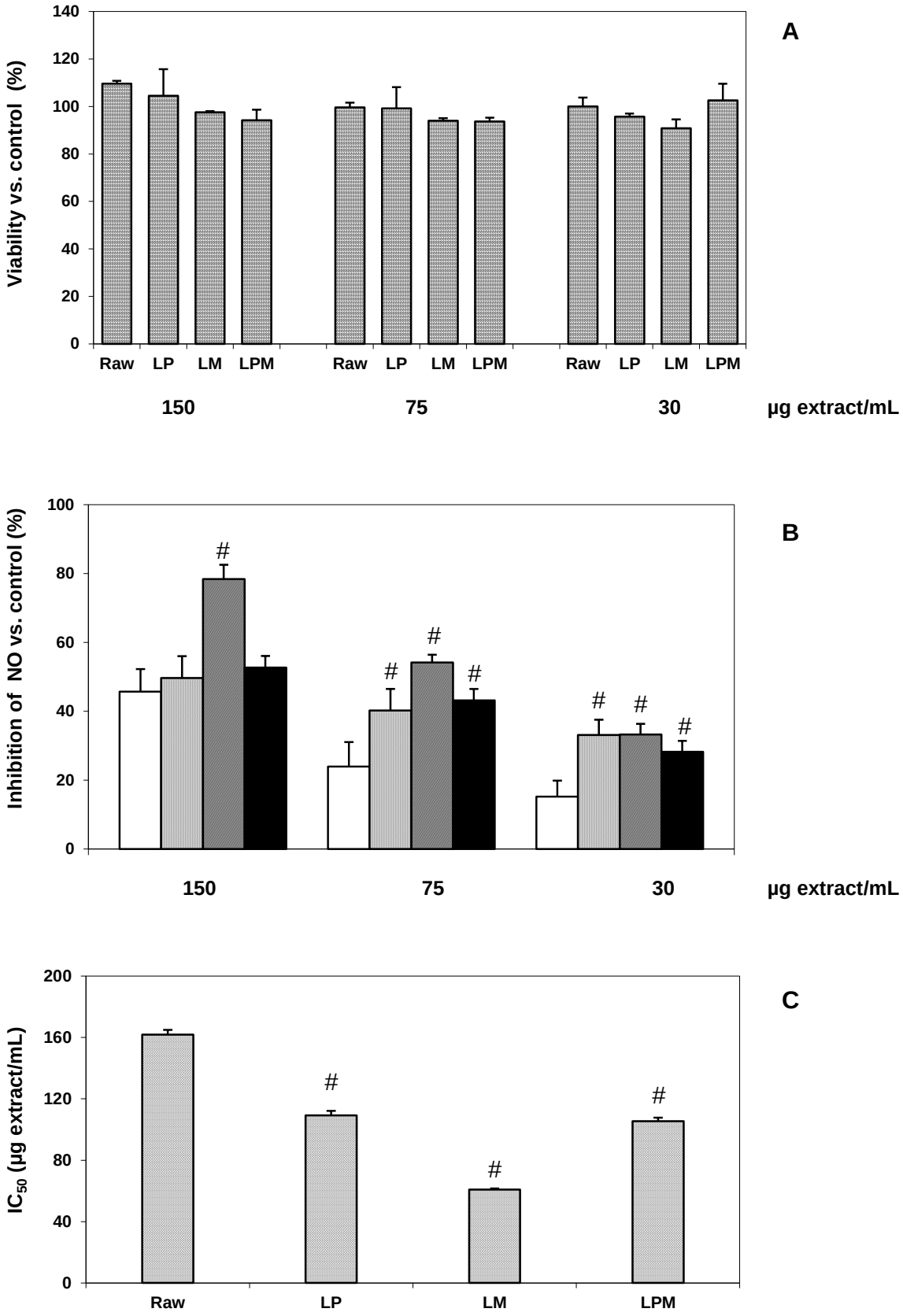
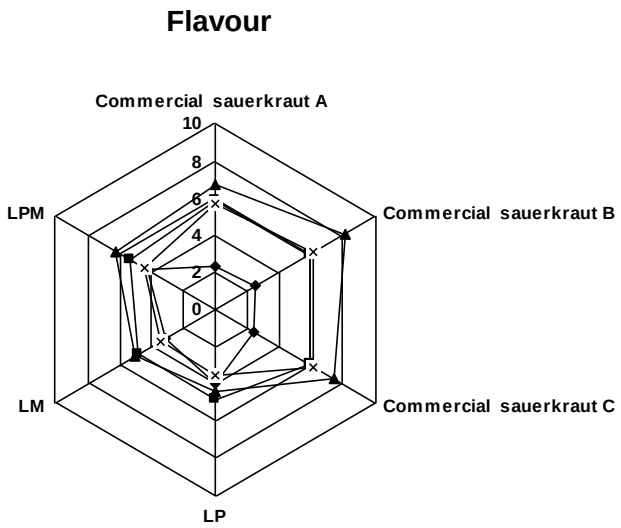
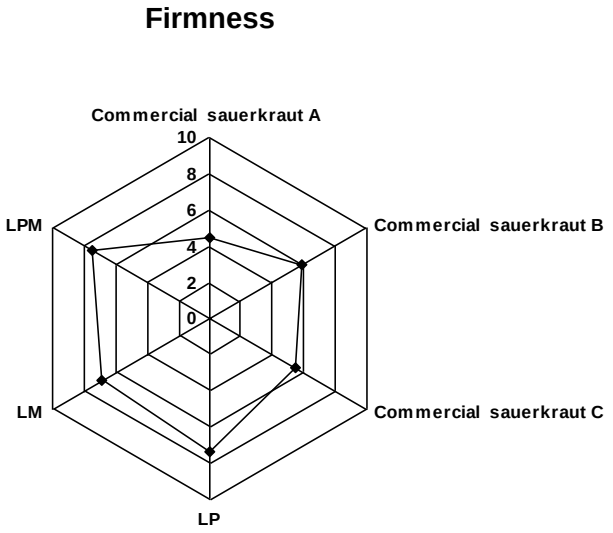
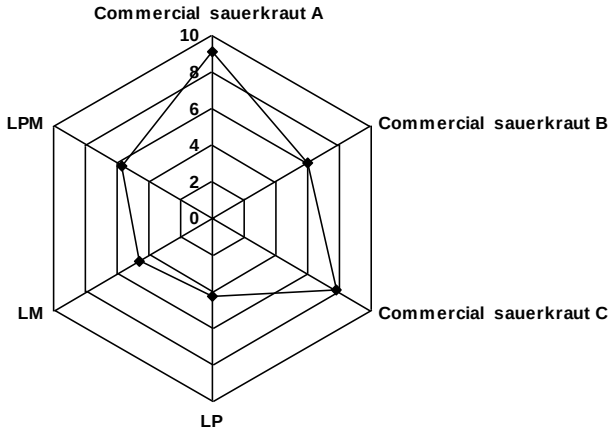
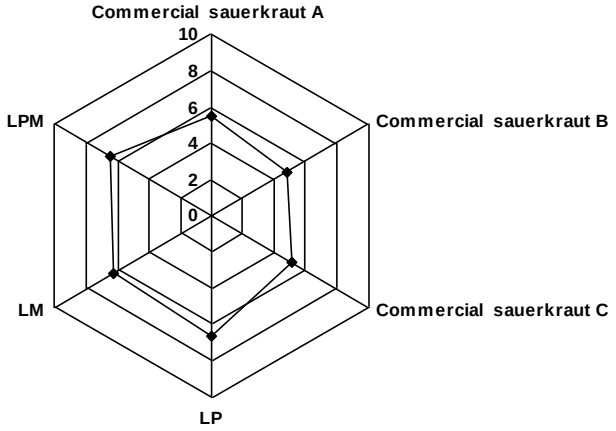

A
Viability vs. control (%)
140
120
100
80
60
40
20
0
Raw LP LM LPM Raw LP LM LPM Raw LP LM LPM
150
75
30
µg extract/mL
B
Inhibition of NO vs. control (%)
100
80
60
40
20
0
#
150
75
30
µg extract/mL
C
IC50 (µg extract/mL)
200
160
120
80
40
0
#
Raw
LP
LM
LPM

Fig. 4.


Flavour
Commercial sauerkraut A
Commercial sauerkraut B
Commercial sauerkraut C
LP
LM
LPM
10
8
6
4
2
0


Firmness
Commercial sauerkraut A
Commercial sauerkraut B
Commercial sauerkraut C
LP
LM
LPM
10
8
6
4
2
0


Color
Commercial sauerkraut A
Commercial sauerkraut B
Commercial sauerkraut C
LP
LM
LPM
10
8
6
4
2
0


Overall acceptability
Commercial sauerkraut A
Commercial sauerkraut B
Commercial sauerkraut C
LP
LM
LPM
10
8
6
4
2
0