Influence of fermentation conditions of *Brassica oleracea* L. var. *capitata* on the volatile glucosinolate hydrolysis compounds of sauerkrauts

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ABSTRACT

The influence of fermentation conditions on the volatile glucosinolate (GLS) hydrolysis products in two different white cabbage cultivars (Bronco and Megaton) was studied. Natural and induced fermentation using *L. plantarum*, *L. mesenteroides* or a mixed starter culture of both microorganisms were performed. Cabbage cv. Bronco was fermented at a concentration of 0.5% and 1.5% NaCl while cv. Megaton was fermented only at 0.5% NaCl. Four commercial sauerkrauts were also analysed in order to compare with the experimental products. No volatile GLS hydrolysis products were detected in raw cabbages. Fermentation caused the appearance of iberin (IB), iberin nitrile (IBN), allyl cyanide (AC), allyl isothiocyanate (AITC) and sulforaphane (SFN) in experimental sauerkrauts, while only IB, IBN and SFN were detected in the commercial ones. Megaton sauerkrauts presented higher volatile GLS derivative content than those from cv. Bronco. The content of these compounds was affected by the starter culture and the salt concentration and it was in the range of those reported as having beneficial effect. Hence, sauerkraut can be considered as a health-promoting food and its intake is highly advised for disease prevention.

Keywords: white cabbage, fermentation, volatile glucosinolates hydrolysis products.

1. **Introduction**

The increased incidence of cancer in the general population has directed much research attention towards the search of compounds having an efficient suppressive effect against cancer. Considerable epidemiological evidence shows that diets rich in *Brassica* vegetables reduce the risk of several types of cancer (Kristal & Lampe, 2002; Wang, Giovannuci, Hunter, Neuberg, Su, & Christiani, 2004). The chemopreventive benefits of these vegetables are attributed to their relatively high glucosinolate content (Fahey, Zalcmann & Talalay, 2001; Keum, Jeong & Kong, 2005). Glucosinolates (GLS) are sulfur-containing secondary metabolites, whose nature and level vary in different plant species and also between cultivars, plant individuals, developmental stage, and the part of the plant examined due to factors such as genetics, environment and nutrient availability (Fenwick, Heaney & Mullin, 1983; Rosa, 1997; Kushad et al., 1999; Brown, Tokulhisa, Reichelt, Gershenzon, 2003). Sinigrin, glucoiberin and glucobrassicin are the most abundant GLS present in white cabbages, but small amounts of progoitrin, glucoraphanin, gluconapin, 4-methoxyglucobrassicin and neoglucobrassicin can be also detected (Peñas et al., 2011a). GLS are inactive in the intact vegetable but upon cellular disruption they are hydrolysed by the endogenous enzyme myrosinase (thioglucoside glucohydrolyase E.C. 3.2.3.1.) to a broad range of bioactive breakdown products including volatile compounds such as isothiocyanates, nitriles, thiocyanates and other less volatile or non-volatile compounds such as oxazolidinethiones and indoles (Mithen, 2001). The formation of these compounds will depend on the substrate, pH conditions, availability of ferrous ions, the level and activity of myrosinase and the presence of some specific protein cofactors such as the epithiospecifier protein (Ludikhuyze, Rodrigo & Hendrickx, 2000; Bones & Rossiter, 2006). Some GLS derivatives, particularly isothiocyanates, nitriles and indoles, have attracted attention as potential chemopreventive agents against certain types of cancer (Verhoeven, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997; Jahangir, Kim, Choi, & Verpoorte, 2009). In this context, sulforaphane (SFN), an isothiocyanate derived from the GLS glucoraphanin, is a potent inducer of phase 2 detoxification enzymes such as quinone reductase (QR), UDP-glucoronosyl transferases (UGTs) and glutathione transferases (GSTs), thereby blocking the action of potential carcinogens (Fahey & Talalay, 1999; Brooks, Paton, & Vidanes, 2001). In addition, SFN (1-isothiocyanate-4(methylsulfinyl)-butane) prevents carcinogen activation through inhibition of cytochrome P450, appears to inhibit angiogenesis (Jackson, Singletary, & Venema, 2007), and exerts anti-inflammatory effects in cancer cells mediated through the inhibition of cyclooxygenase-2 expression (Woo & Kwon, 2007). SFN also inhibits the activity of histone deacetylase, and the inhibitors of this enzyme possess chemotherapeutic potential (Myzak, Karplus, Chung, & Dashwood, 2004). Allyl isothiocyanate (AITC, 3-isothiocyanate-1-propene), a volatile breakdown product derived from sinigrin, the predominant GLS found in cabbage, has been also widely studied since it induces cell death in both colorectal cells and prostate cancer cells (Xiao et al., 2003; Smith, Lund, Parker, Clarke, & Johnson, 2004) and inhibits the proliferation of various types of human cancer cells (Okulicz, 2010). In addition, AITC presents antimicrobial properties (Lin, Preston & Wei, 2000) and inhibits the production of nitric oxide (NO) and the expression of inducible nitric oxide synthase (iNOS) that are involved in inflammation and cancer (Ippoushi, Takeuchi, & Azuma, 2010). Numerous studies performed on rodents have shown that AITC and SFN are effective inhibitors of chemically induced tumors in several organs, including the bladder, colon, esophagus, mamary glands, pancreas and stomach (Conaway, Yang, & Chung, 2002; Zhang, 2004). Furthermore, in agreement with the rodent results, several recent epidemiological studies performed in humans have shown that dietary intake of isothyocyanates correlates strongly with reduced susceptibility to cancers at different organs, including breast, lung and colon (Zhao et al., 2001; Seow, Yuan, Sun, Berg, Lee, & Yu, 2002; Ambrosone, McCann, Freudenheim, Marshall, Zhang, & Shields, 2004). Iberin (IB, 1-isothiocyanate-3(methylsulfinyl)-propane) derived from glucoiberin decomposition, has been less studied than SFN and AITC, but it has an interesting anticarcinogenic potential because it is able to inhibit the proliferation of human glioblastoma and neuroblastoma cells through the induction of cell apoptosis (Jadhav, Ezhilarasan, Vaughn, Berhow, & Mohanam, 2007a; Jadhav, Vaughn, Berhow, & Mohanam, 2007b). Apart from the volatile compounds described above, which are the most characterized GLS derivatives, there are many other compounds that are present in lower quantities and may also contribute to the anti-carcinogenic properties of *Brassica* vegetables, such as iberin nitrile (IBN, 4-(methylsulfinyl)-butane nitrile) and allyl cyanide (AC, 3-butenenitrile) (Fahey et al., 2001).

Due to their proved anticarcinogenic properties, the enhancement of the concentration of these health-promoting compounds in specific vegetable-based foods could be a very cost-effective way of cancer prevention. Fermentation could be a valuable technological process to achieve this purpose, since it favours the hydrolysis of GLS to several potentially beneficial breakdown products (Tolonen, Taipale, Viander, Pihlava, Korhonen, & Ryhänen, 2002; Ciska & Pathak, 2004, Peñas et al. 2011b). It has been previously reported that fermentation of cabbage enhanced the formation of a potent chemopreventive agent, ascorbigen, a compound which results from the reaction of indole-3-carbinol, derived from glucobrassicin, and vitamin C in sauerkraut (Martinez-Villaluenga et al., 2009; Peñas, Frias, Sidro, & Vidal-Valverde, 2010). However, to our knowledge, there is no literature information on the effect of different fermentation conditions on the content of volatile compounds derived from GLS hydrolysis. In view of the above, the objective of this paper was to evaluate the influence of fermentation conditions on the content and profile of volatile GLS breakdown products in two different cabbage cultivars (cv. Bronco and cv. Megaton) and to compare with four commercial sauerkrauts.

2. Material and methods

2.1. Plant material

Two different white cabbage (*Brassica oleracea* L. var. *capitata*) cultivars were used in the present work: cv. Bronco, grown in the winter of 2008 in the Eastern region of Spain (Levante) and cv. Megaton grown in the winter of 2009 in the Northern region of Spain (La Rioja). These cultivars were selected among five different Spanish cultivars, based on their highest glucobrassicin content (Peñas et al. 2011a). Fresh cabbages were provided by Bejo Iberica S. L. (Madrid, Spain) and they were fermented upon reception.

Samples of four commercial sauerkrauts (A, B, C and D) were purchased in a local supermarket, and included in the present work.

2.2. Starter culture preparation

L. plantarum (CECT 748) and *L. mesenteroides* (CECT 219) strains were supplied by the Spanish Type Culture Collection (CECT, Valencia, Spain). They were multiplied twice in MRS broth (Difco Laboratories, Detroit, Mich.) and incubated overnight at 30 ºC. After centrifugation at 6429 *xg*, for 10 min, the cells were harvested and then washed twice in a sterile saline solution. Starter cultures were inoculated at approximately 10^6 colony-forming units (cfu)/g of cabbage. Three different starter cultures were separately used in the fermentation process: *L. plantarum, L. mesenteroides* and a mixed starter culture containing equal proportions of both strains.

2.3. Sauerkraut production

Fresh cabbage heads were prepared by removing the outer leaves and coring the heads. The edible part of cabbages was then shredded into strips of about 2 mm thick using a domestic shredder (Moka Express, Barcelona, Spain). Afterwards, salt was added onto shredded cabbage and mixed vigorously. Two NaCl levels (1.5g/100g and 0.5g/100g; w/w) were assayed for cv. Bronco while for cv. Megaton only the concentration of 0.5 g/100g NaCl was studied. Subsequently, cabbage and brine were transferred to autoclaved polyethylene vessels (8 L) and pressed together to exclude air so that the subsequent lactic acid fermentation takes place. Fermentations were performed spontaneously (NF) by the indigenous microbiota present on raw cabbage or by using three different starter cultures (induced fermentation): *L. plantarum* (LP), *L. mesenteroides* (LM) or a mixed culture of both microorganisms (1:1) (LPM). Each type of fermentation was performed in 3 parallel batches (4 Kg per batch) at room temperature (22-25 ºC) for 7 days. On the third day, cabbage was pricked to remove releasing gases.

Experimental raw and fermented cabbages, as well as commercial sauerkrauts, were freeze-dried, milled and stored at -20 ºC until further analysis.

2.4. Determination of water content

Moisture of raw cabbage and sauerkrauts was determined according to AOAC (1990) to constant weight.

2.5. Analysis of volatile GLS breakdown products

The content of volatile GLS degradation products in sauerkraut products was determined as in Tolonen et al. (2002) with some modifications. Briefly, 200 mg of freeze-dried material was extracted using methylene chloride (3 ml) in a screw cap test tube by agitation for 4 hours at room temperature. After centrifugation at 484 *xg* for 10 min, 50 μL of chlorathalonil (200 μg/ml) were added as an internal standard to 1 ml of the sample supernatant. All samples were prepared in triplicates.

The separation and quantification of volatile GLS breakdown products were performed by PE Clarus 500 GC-MS (Perkin-Elmer, Shelton, CT, USA) using splitless injection (1 μl, split-on time 1.40 min) to a double gooseneck liner. PE Elite-5MS (30 m x 0.25 mm i.d., film thickness 0.25 μm) was used as the analytical column with helium as a carrier gas (1.0 ml/min). The analysis was done isothermally at oven temperature of 110 ºC (22 min). The injector was set at 250 ºC and the GC-MS transfer to 260 ºC. MS was employed at scan mode 40-550 m/e. Quantification of iberin (IB), iberin nitrile (IBN) and sulforaphane (SFN) was made using the calibration curve of hexyl isothiocyanate (Sigma Aldrich), because of the lack of commercial standards, while the quantification of allyl cyanide (AC) and allyl isothiocyanate (AITC) was done using authentic standars. The identification of IB and IBN was based on the NIST MS-library.

2.6. Statistical analysis

Results were compared by one-way analysis of variance (ANOVA) using the least significant differences ($P \leq 0.05$) (Statgraphic 5.0 software, Statistical Graphics Corporation, Rockville, MD, USA).

3. Results and discussion

Table 1 presents the content of volatile GLS breakdown products identified in the four different commercial sauerkrauts. IBN was the most abundant volatile GLS degradation compound in all the commercial samples analysed, and its content ranged from 35 to 38 μmol/100g d.m. IB was found in commercial sauerkrauts A, B and C at concentrations ranged between 28-30 μmol/100g d.m, and sauerkraut A showed significant higher amount of this compound compared with samples B and C. IB was not detected in sauerkraut D. Commercial sauerkrauts A and C also presented SFN (28 and 27 μmol/100g d.m, respectively), whilst this compound was absent in sauerkrauts B and D. AC and AITC, derived both from sinigrin, were not detected in the commercial sauerkrauts studied.

Figures 1-3 give an overview of the concentration of volatile GLS derivatives found in fermented cabbages obtained under experimental conditions in our laboratory from cultivars Bronco and Megaton at different fermentation conditions. Volatile GLS hydrolysis products were not detected in both raw white cabbage cultivars (data not shown), results expected since the hydrolysis of GLS requires the plant tissue damage to release GLS from plant vacuoles and subsequent hydrolysis by myrosinase enzyme (Sun, Liu, Zhao, Yan, Wang, 2010). Fermentation caused the formation of 5 different volatile GLS degradation products (IB, IBN, AC, AICT and SFN), and their concentration depended on the process conditions (Figures 1-3).

IB content ranged between 29-39 μmol/100g d.m in both Bronco and Megaton cultivars (Figure 1). For cv. Bronco, the highest amount of this compound (38.6 μmol/100g d.m) was observed in sauerkrauts produced by LP at 1.5 g/100g NaCl followed by those produced by LM at both salt concentrations (32 μmol/100g d.m), while LPM fermentation led to the lowest IB content (29 μmol/100g d.m). No significant differences ($P \leq 0.05$) between both salt levels were observed, with the exception of LP fermentation, where 1.5 g/100g NaCl led to significantly ($P \leq 0.05$) higher IB content than 0.5 g/100g NaCl. Respect to cv. Megaton, NF produced the highest content of IB (36 μmol/100g d.m), followed by LM fermentation (33 μmol/100g d.m), while those performed by LP and LPM exhibited the lowest content of this compound (29 μmol/100g d.m). Higher amount of IB was observed in cv. Megaton sauerkrauts obtained by NF than in that obtained from cv. Bronco, while no significant differences ($P \leq 0.05$) in the concentration of this compound between both cultivars was observed when the fermentation was performed by the addition of starter cultures.

Respect to IBN, fermented cabbages produced from cv. Bronco presented concentrations between 36 and 39 μmol/100g d.m and sauerkrauts obtained by NF and LP showed the largest amount of this compound (38-39 μmol/100g d.m). The salt level used during fermentation had no significant ($P \leq 0.05$) influence on the IBN formation, regardless of the starter culture used. Sauerkrauts produced from cv. Megaton by NF and LM presented the highest IBN content (41-42 μmol/100g d.m), while those obtained by LP showed the lowest (37μmol/100g d.m.). Significant ($P \leq 0.05$) higher content of IBN was observed in sauerkrauts obtained from cv. Megaton compared with those produced from cv. Bronco at lower NaCl concentration in all fermentations performed, except for that carried out with LP, where cv. Bronco exhibited higher content of this compound than cv. Megaton (Figure 1).

AC was the major volatile GLS hydrolysis product found in experimental sauerkrauts and concentrations in the range of 65-72 μmol/100g d.m were observed after cabbage fermentation under the different conditons assayed (Figure 2). For cv. Bronco sauerkrauts, 1.5% NaCl LP fermentation produced the highest formation of this compound (72 μmol/100g d.m) and the influence of the salt level was different depending on the starter culture used. Thus, the addition of lower NaCl concentration during fermentation enhanced the production of AC in NF while higher NaCl levels favoured its formation in LP and LPM fermentations. However for LM sauerkrauts, no significant differences ($P \leq 0.05$) on the content of this compound were observed between both salt concentrations. Regarding cv. Megaton, LP and LM sauerkrauts presented the highest amount of AC (69-70 μmol/100g d.m) and, moreover, the concentration of this compound was higher in all fermentations performed from this cultivar in comparison with those from cv. Bronco at 0.5 g/100g NaCl. The only exception was NF, which led to significant ($P \leq 0.05$) higher amount of AC in cv. Bronco than in cv. Megaton.

AITC was the second most abundant volatile GLS derivative found in experimental sauerkrauts, and its content ranged from 39 to 49 μmol/100g d.m, as it can be seen in Figure 2. For cv. Bronco sauerkrauts, LM fermentation at 1.5 g/100g NaCl brought about the highest formation of AITC (46 μmol/100g d.m) and the influence of NaCl concentration, as it was observed for AC, was different depending on the type of fermentation. In this sense, the content of AITC increased in NF and decreased in LM fermentation when 0.5 g/100g NaCl was used in comparison with 1.5 g/100g, while the NaCl level did not significantly ($P \leq 0.05$) affect the content of this compound in LP and LPM sauerkratus. For cv. Megaton, LM and LPM sauerkrauts exhibited the largest AITC amount (48-49 μmol/100g d.m) and, besides that, AITC content in Megaton sauerkrauts was significantly ($P \leq 0.05$) higher than that of cv. Bronco fermented cabbages, regardless the fermentation conditions.

AC and AITC are derived from sinigrin, the most abundant GLS found in white cabbage (Peñas, Frias, Martínez-Villaluenga, Vidal-Valverde, 2011a), and for this reason AC and AITC were the major GLS breakdown products found in sauerkrauts manufactured in the present work. However, we observed that AC was formed in higher concentration than AITC, results than can be explained by the pH value of the sauerkrauts obtained in the present work (between 3.27 to 3.67), since Gil and MacLeod (1980) reported that at pH 4-5, isothiocyanate is the predominant compound liberated from sinigrin, while cyanate is the major compound at pH values below 3.7.

The content of SFN on sauerkrauts produced at different conditions is showed in Figure 3. This compound was not detected in NF and LPM sauerkrauts produced from cv. Bronco, while its content ranged from 26 to 32 μmol/100g d.m in the rest of fermentations from both cabbage cultivars. For cv. Bronco fermented cabbages, the largest SFN concentration was observed after LP fermentation at 1.5 g/100g NaCl (28 μmol/100g d.m), and the salt concentration only affected its content in LP sauerkrauts, where the highest salt concentration produced the highest SFN content. Regarding cv. Megaton sauerkrauts, NF led to the highest formation of this compound (32 μmol/100g d.m). The formation of SFN was enhanced in sauerkrauts from cv. Megaton compared with those from cv. Bronco. The only excepcion was LP fermentation, for which not significant ($P \leq 0.05$) differences were observed between both studied sauerkrauts.

In the view of the results obtained, it can be stated that the experimental sauerkrauts obtained by spontaneous or induced fermentation in this study presented similar amounts of IB and IBN than the commercial ones. However, AC and AITC were not found in commercial fermented cabbages while the experimental sauerkrauts presented values in the range of 65-72 μmol/100g d.m., and 39-49 μmol/100 g d.m, respectively. Due to this higher content of AITC, compound with well-known anticarcinogenic properties, experimental sauerkrauts would have presumably higher health-promoting properties than the commercial products analysed in the present work. Regarding SFN, it was found in similar amounts (25-32 μmol/100 g d.m.) in the experimental sauerkrauts and in two of the commercial ones. These results indicate that the differences found in the concentration of volatile GLS derivatives could be due to the different cabbage variety and type and duration of the fermentation processes used for sauerkraut production.

The influence of the cabbage cultivar on the profile and amount of volatile GLS breakdown products is evident when comparing the results obtained for cv. Bronco and Megaton. In this sense, cv. Megaton seems to be a good choice for sauerkraut production, since the formation of most of the volatile GLS degradation products is enhanced in comparison with cv. Bronco. Although lower levels of the GLS precursors (glucoiberin, glucoraphanin and sinigrin) of these compounds were found in cv. Megaton than in cv. Bronco (Peñas et al., 2011a), the highest content of volatile GLS derivatives in cv. Megaton is probably due to their higher endogenous myrosinase activity (Peñas et al., 2011b). In addition, the microbiota present in white cabbage can also affect the formation of GLS-derivatives, since a myrosinase-like activity has been described previously in several lactic acid bacteria (Nugon-Baudon, Rabot, Wal, & Szylit, 1990; Palop, Smiths, & ten Brink, 1995; Cheng, Hashimoto, & Uda, 2004). Then, the different concentration of volatile GLS-derivatives found in both cabbage cultivars can be also attributed to the different myrosinase-like activity present in the endogenous microbiota of each cultivar. Besides that, our group previously studied the content of ascorbigen, a compound derived from glucobrassicin that is considered a potent anticarcinogen, in sauerkrauts produced from cv. Bronco and cv. Megaton grown during winter in the East and North regions of Spain, respectively. We found that fermented cabbage obtained from cv. Megaton presented higher ascorbigen concentration than those produced from cv. Bronco (Peñas et al., 2010; Martínez-Villaluenga et al., 2010). Hence, the manufacture of sauerkrauts from cv. Megaton grown during winter in the North of Spain could be a good strategy to increase the consumer intake of compounds having a beneficial impact for human health.

Regarding the influence of NaCl concentration on the content of volatile GLS-derivatives, no clear tendency was observed. In general, NaCl level had not remarkable effect on the content of volatile GLS derivatives in NF, LM and LPM sauerkrauts, however most of them increased in LP sauerkrauts at the higher salt concentration compared to the lower one. The different influence of salt level on the contents of volatile GLS breakdown products may result from the diverse sensitivity of the bacterial strains used as starter culture to osmotic pressure, as Halász, Baráth, Holzapfel (1999) has previously reported.

There is scarce information in the literature on the content of volatile GLS degradation products on sauerkrauts. The concentration of AC and AITC found in the present work are higher than those reported for Tolonen et al., (2002) in sauerkrauts obtained by natural fermentation or by addition of a *L. mesenteroides-Pediococcus dextrinicus* starter culture. Furthermore, Ciska and Pathak (2004) reported lower concentrations of these compounds in sauerkrauts obtained by natural fermentation stored for 2 weeks. However, these authors did not report the volatile GLS derivative content before storage.

The results of the present work provide evidences that a high concentration of volatile GLS breakdown products can be achieved in the diet by ingesting sauerkraut. In order to exert their biological effect, ITCs must reach effective concentrations on plasma. Ye, Dinkova-Kostova, Wade, Zhang, Shapiro, & Talalay (2002) reported that peak plasma concentrations of 1-2 μM ITC equivalents were achieved after 1 h of ingestion of a single dose of broccoli sprout extract contaning 200 μmol of ITC (77% SFN in its composition). In another study, the SFN concentration in human mammary glands reached 60 μM 1h after ingestion of a single dose of 150 μmol of SFN (Cornblatt et al., 2007). These orally achieved concentrations are within the range in which SFN has anticarcinogenic effects (Conaway et al., 2002; Zhang et al., 2004). On the other hand, Zhao et al., (2001) reported that a weekly intake of ITCs above 53 μmol reduced the risk of lung cancer, to a greater extent in smokers than nonsmokers. Staak, Kingston, Waillig, & Jeffery (1998) found that the ingestion of 38 mg/kg body weight for 7 days (equivalent to 0.6 μmol/kg body weight) by rats increased the activity of glutathione reductase, an enzyme that plays an important role in the protection against oxidative stress. The total content of ITCs in the sauerkrauts found in the present work was above 200 μmol/100 g d.m. (22 μmol/100 g fresh weight), concentration in the range of those reported in the literature as having cancer protective properties. Hence, the weekly consumption of a portion of 200-250 g of sauerkraut would provide the ingestion of an effective ITC dosis to exert a health-promoting effect.

4. Conclusions

On the basis of the results obtained, it can be concluded that the fermentation enhanced the content of volatile GLS breakdown products in cabbage and the magnitude on such increment depended on the white cabbage cultivar used, as well as on the fermentation conditions, such as the starter culture and salt concentration employed. Megaton cultivar, grown during winter in the North of Spain, is a good choice for sauerkraut production since it presented high concentration of the studied GLS derivatives. No clear tendency was observed for the influence of NaCl level on the formation of such volatile compounds on sauerkraut. Although certain amount of the glucosinolate derivatives analysed in the present work can be lost during the freeze-drying and grinding processes, it should be noticed that the content found for all of these compounds is slightly higher than those observed by other authors in fresh products (Tolonen et al., 2002; Ciska & Pathak, 2005) and, moreover, they are in the range of those concentrations previously reported as having anticarcinogenic benefit. Hence, sauerkraut could have a beneficial effect and its consumption should be highly advised for chronic disease prevention.

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Table 1. Volatile GLS hydrolysis compounds of commercial sauerkrauts*.

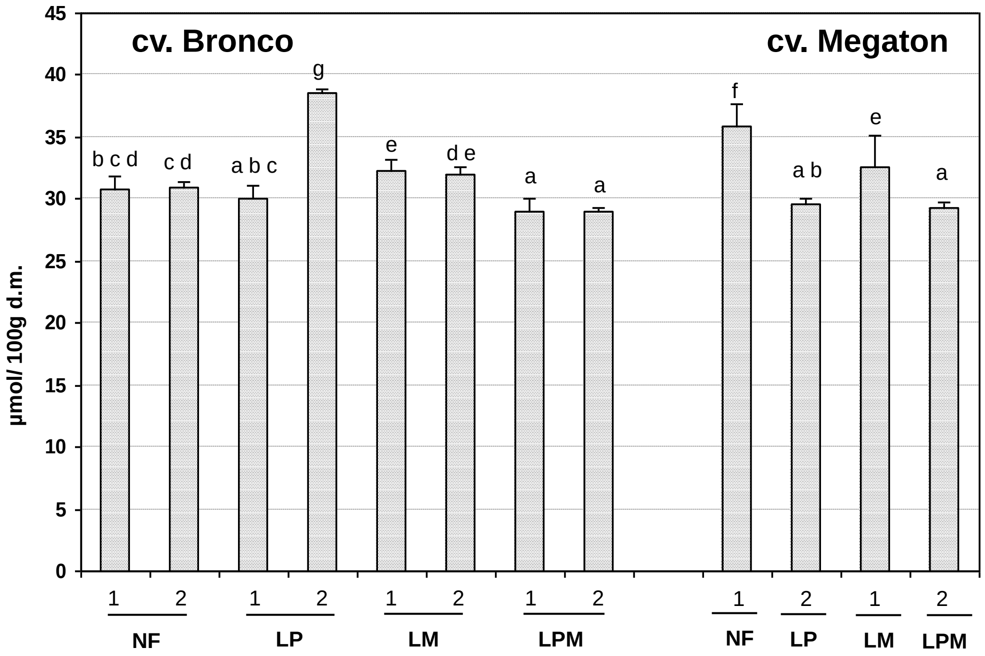
Sauerkrauts	IB	IBN	AC	AITC	SFN	Water (%)
Sample A	29.98±0.41^{c}	37.39±0.40^{b}	ND^{a}	ND^{a}	27.79 ±0.64^{c}	90.5
Sample B	28.44±0.10^{b}	34.95±0.63^{a}	ND^{a}	ND^{a}	ND^{a}	91.7
Sample C	28.71±0.56^{b}	37.70±1.55^{b}	ND^{a}	ND^{a}	26.57±0.83^{b}	89.5
Sample D	ND^{a}	34.65±0.66^{a}	ND^{a}	ND^{a}	ND^{a}	89.2

*) µmol /100g d.m. IB = iberin; IBN = iberin nitrile; AC = allyl cyanide; AITC = allyl isothiocyanate; SFN = sulforaphane.
Mean value ± SD. The same superscript in the same column means no significant difference ($P \leq 0.05$).

Figure 1

Figure 1. Iberin and iberin nitrile content of fermented cabbages

Iberin

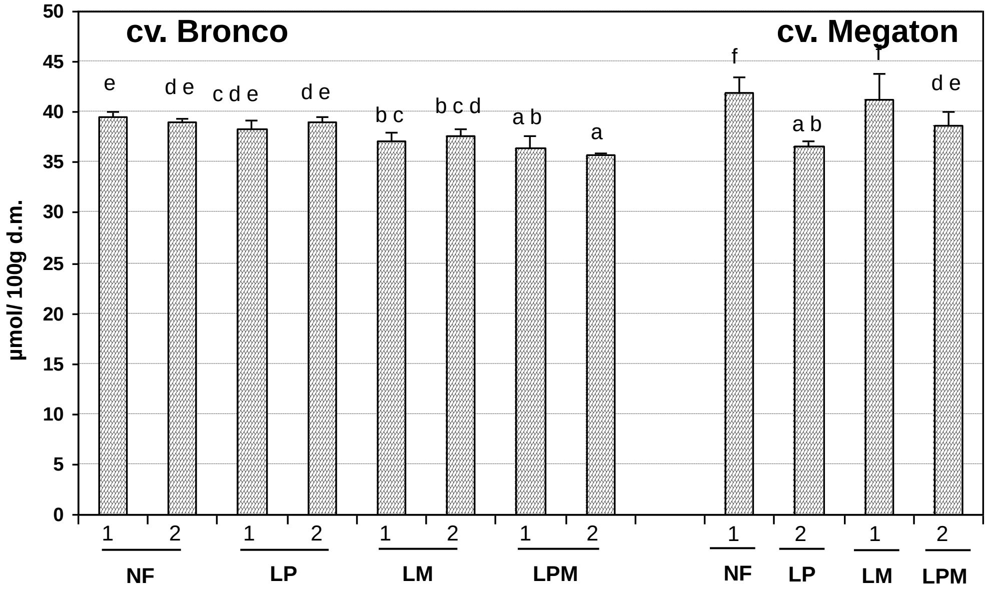


1 = 0.5g/100g NaCl; 2 = 1.5g/100g NaCl

NF: natural fermentation; LP: fermentation with *L. plantarum*; LM: fermentation with *L.mesenteroides;* LPM: fermentation with mixed starter culture

The same letter between bars means not significant difference (P ≤ 0.05)

Iberin nitrile



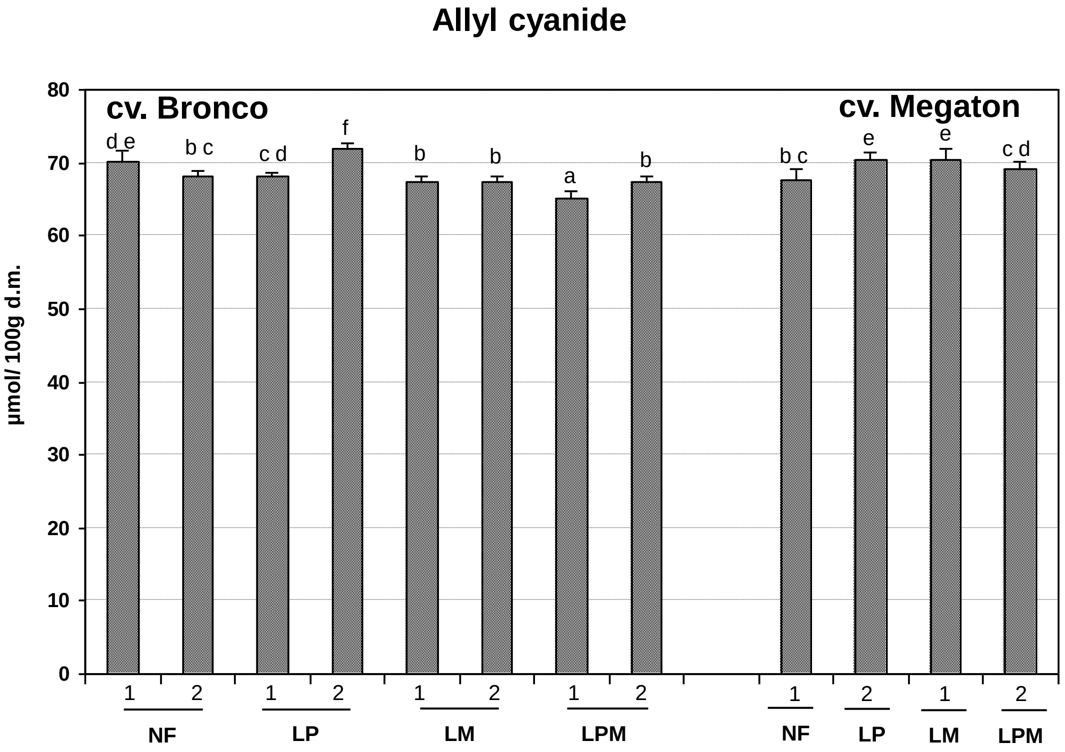
1 = 0.5g/100g NaCl; 2 = 1.5g/100g NaCl

NF: natural fermentation; LP: fermentation with *L. plantarum*; LM: fermentation with *L.mesenteroides;* LPM: fermentation with mixed starter culture

The same letter between bars means not significant difference (P ≤ 0.05)

Figure 2

Figure 2. Allyl cyanide and allyl isothiocyanate content of fermented cabbages

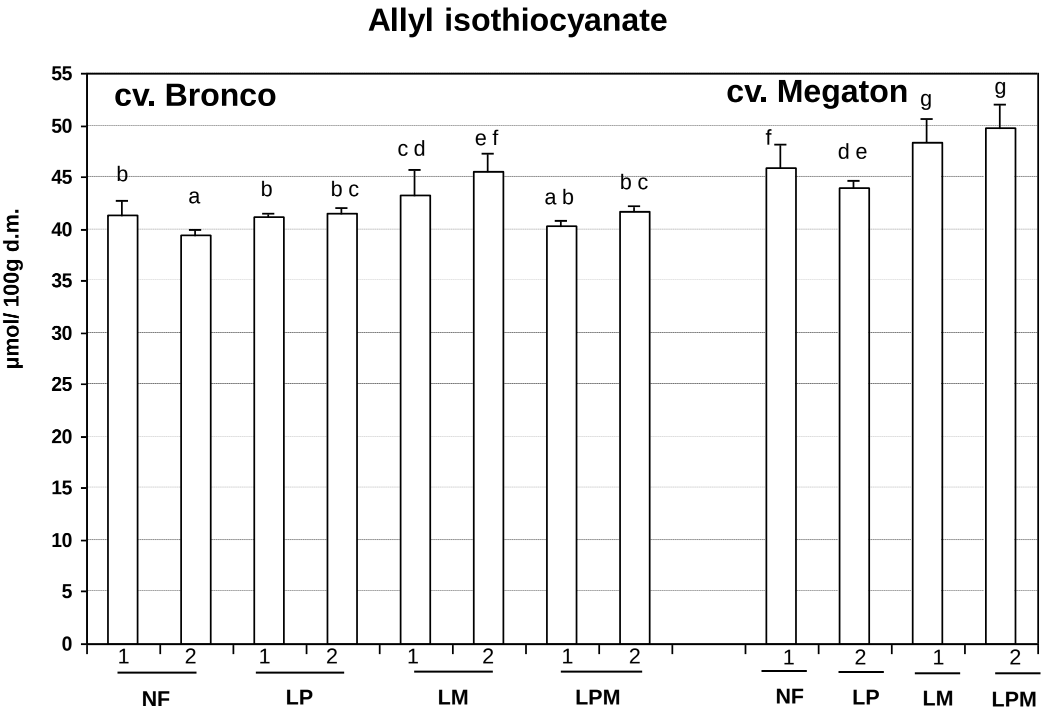


Allyl cyanide

1 = 0.5g/100g NaCl; 2 = 1.5g/100g NaCl

NF: natural fermentation; LP: fermentation with *L. plantarum*; LM: fermentation with *L.mesenteroides;*
LPM: fermentation with mixed starter culture

The same letter between bars means not significant difference (P ≤ 0.05)



Allyl isothiocyanate

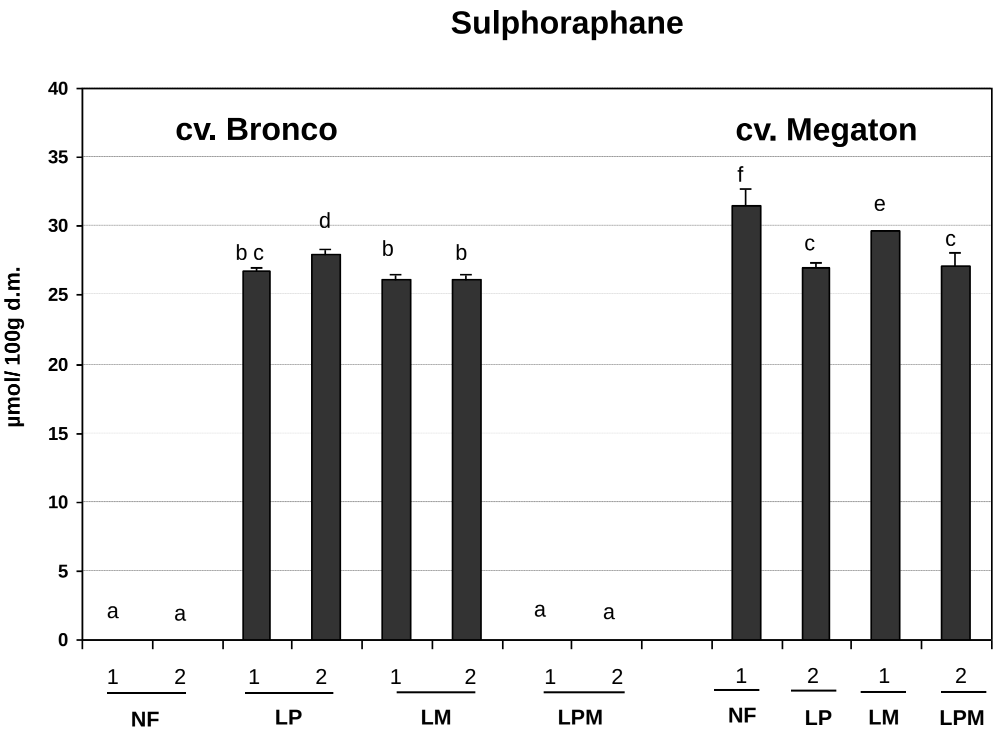
1 = 0.5g/100g NaCl; 2 = 1.5g/100g NaCl

NF: natural fermentation; LP: fermentation with *L. plantarum*; LM: fermentation with *L.mesenteroides;*
LPM: fermentation with mixed starter culture

The same letter between bars means not significant difference (P ≤ 0.05)

Figure 3

Figure 3. Sulphoraphane content of fermented cabbages



1 = 0.5g/100g NaCl; 2 = 1.5g/100g NaCl

NF: natural fermentation; LP: fermentation with *L. plantarum*; LM: fermentation with *L.mesenteroides;* LPM: fermentation with mixed starter culture

The same letter between bars means not significant difference (P ≤ 0.05)