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Effect of storage on the content of indole-glucosinolate breakdown products and vitamin C of sauerkrauts treated by high hydrostatic pressure

Elena Peñas, Rocío I. Limón, Concepción Vidal-Valverde, Juana Frias*

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

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ABSTRACT

The effect of refrigerated storage for three months on the content of indole glucosinolate (GLS) breakdown products (ascorbigen –ABG–, indole-3-carbinol –I3C– and indole-3-acetonitrile –I3ACN–) and vitamin C in sauerkrauts treated by high hydrostatic pressure (HHP) was investigated. Sauerkrauts were produced either by spontaneous fermentation (NF) or by using a mixed-starter culture (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*) (PMF) at 0.5 g/100 g and 1.5 g/100 g NaCl concentrations and they were pressurized in order to prolong their shelf life. HHP-sauerkrauts were a good source of vitamin C (143–161 mg/100 g d.m.) and ABG was the main indole GLS derivative (37–65 μmol/100 g d.m), followed by I3C (5–17 μmol/100 g d.m) and I3ACN (1.5–3 μmol/100 g d.m). NF-HHP sauerkrauts presented higher I3C and I3AC and lower vitamin C content than PMF-HHP sauerkrauts. Refrigerated storage led to a gradual decrease of ABG and vitamin C (losses of 33–67% and 96–98%, respectively, after 3 months) while slight changes of I3C and I3ACN were observed.

1. Introduction

Brassicaceous crops are among the most grown vegetables worldwide and over the last decades their consumption has been associated with health benefits (Bjorkman et al., 2011). These benefits are largely attributed to their high content of antioxidant compounds and glucosinolates (GLS) breakdown products formed by the myrosinase action when the plant cells are broken (Kusznierewicz et al., 2008). In cabbage, glucobrassicin is one of the predominant GLS, and once it is fermented, bioactive indole glucobrassicin derivatives such as ascorbigen (indol-3-ylmethyl-ascorbate, ABG), indole-3-carbinol (I3C) and indole-3-acetonitrile (I3ACN) are some of the most important GLS hydrolysis products found in sauerkrauts (Ciska & Pathak, 2004; Martinez-Villaluenga et al., 2009; Peñas, Frias, Sidro, & Vidal-Valverde, 2010).

Several studies have demonstrated the anticarcinogenic effect of ABG due to its ability to induce activation of xenobiotic-metabolizing enzymes and apoptosis of tumoral cells. In addition, ABG exerts a protective action against DNA and decreases the oestrogen pool, thereby reducing the possibility of generating genotoxic compounds (Sepkovic et al., 1994; Sparnins, Venegas, & Wattenberg, 1982) and conferring protection to the skin against oxidative stress (Wagner et al., 2008). I3C is a potent anticarcinogen in mammals by induction of enzymes involved in the carcinogen metabolism, inhibition of steroid hormone binding, scavenging of electrophiles and protection against oxidative damage (Takahashi, Dashwood, Bjeldanes, Williams, & Bailey, 1995). This compound has been shown to inhibit the proliferation of cancer cells from different human tissues *in* vitro (Kim et al., 2006; Sarkar & Li, 2004) at a concentration of 30–100 μM. Regarding I3ACN, it has been shown to inhibit chemical-induced neoplasia in rodents (Wattenberg & Loub, 1978) and to increase the activity of glutathione-S transferase, which has the capacity to detoxify chemical carcinogens (Sparnins et al., 1982). On the other hand, sauerakraut contains a high concentration of vitamin C, a potent antioxidant which may exert its action directly to scavenge free radical species, by metabolyzing peroxides to non-radical products and by chelating metal ions to prevent generation of oxidizing species (Duthie, Ma, Ross, & Collins, 1996). Due to their health promoting properties, the increase of these bioactive compounds in sauerkrauts may have a beneficial impact on the consumer's health.

Sauerkraut is a popular white cabbage fermented product in Central and Eastern Europe and, after its production it is usually kept in domestic refrigerators or it is pasteurized until consumption. Recently, high hydrostatic pressure (HHP) has been successfully applied to minimize the microbial load of sauerkraut, improving its microbiological quality and extending its shelf-life (Peñas, Frias, Gomez, & Vidal-Valverde, 2010). HHP is a non-thermal technology that satisfies the demand for minimally processed products, particularly avoiding the need of antimicrobial

* Corresponding author. Tel.: +34 912587510; fax: +34 915644873.
E-mail address: frias@ictan.csic.es (J. Frias).

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agents (Mújica-Paz, Valdez-Fragoso, Tonello-Samson, Welti-Chanes, & Torres, 2011). To the best of our knowledge, HHP may be a valuable processing alternative to lengthen to shelf-life of sauerkrauts maintaining their health promoting properties. Although the content of GLS breakdown products is high at the end of the fermentation period, there are no data documenting the amount of GLS derivatives and vitamin C after HHP and after further refrigerated storage. Therefore, the objective of the present work was to determine the content of indole GLS breakdown products and vitamin C in HHP-treated sauerkrauts produced either by natural or induced fermentation with different salt concentrations and to follow their content for 1, 2 and 3 months at 4 °C.

2. Materials and methods

2.1. Starter culture preparation

Lactobacillus plantarum (CECT 748) and *Leuconostoc mesenteroides* (CECT 219) strains were provided by the Spanish Type Culture Collection (CECT, Valencia, Spain) and multiplied following the procedure indicated by Peñas, Frias, Sidro, et al. (2010). A starter culture containing equal proportions of both strains was inoculated at approximately 10^6 colony-forming units/g of cabbage.

2.2. Cabbage fermentation process

Fresh white cabbages (*Brassica oleracea* L. var. *capitata* cv. Bronco) grown in the Eastern region of Spain (Levante) were provided by Bejo Iberica S. L. (Madrid, Spain). The edible cabbage parts were shredded into strips (~2 mm) using a domestic shredder (Moka Express, Barcelona, Spain). Different batches with two concentrations of NaCl (1.5 and 0.5 g/100 g) were prepared. Cabbage and brine were then transferred to autoclaved polyethylene vessels (8 L) and were tightly pressed together to remove air. Then, two types of fermentations were performed: natural fermentation using the indigenous microbiota naturally present in raw white cabbage, and induced fermentation using the mixed starter culture of *L. plantarum & L. mesenteroides* previously prepared. Each type of fermentation was run in 3 parallel batches (4 kg per batch) at room temperature (22–25 °C) for 7 days.

2.3. High hydrostatic pressure processing

Several lots of approximately 25 g of natural and inoculated sauerkraut were vacuum-packed and pressurized at 300 MPa at 40 °C for 10 min in a discontinuous high pressure machine (ACB GEC, Alsthom, Nantes, France) according to Peñas, Frias, Gomez, et al. (2010). After the HHP treatment, pressurized naturally produced sauerkrauts (NF-HHP) or inoculated with *L. plantarum* & *L. mesenteroides* (1:1) sauerkrauts (PMF-HHP) were immediately opened, freeze-dried and analysed to quantify the content of indole GLS degradation products and vitamin C. Simultaneously, pressurized packed sauerkrauts were stored for 1, 2 and 3 months at 4 °C. Afterwards, bags were opened, freeze-dried, and analysed to determine the content of indole GLS degradation products and vitamin C. The treatment was carried out in triplicate.

2.4. Chemical analysis

2.4.1. Analysis of indole GLS hydrolysis compounds

The content of ABG, I3C and I3ACN in HHP-treated sauerkrauts and after storage for 3 months at 4 °C was quantified as in Peñas et al. (2012). Quantification was performed by HPLC using an Alliance Separation Module 2695 (Waters, Milford, USA), a Photodiode Array detector 996 at 280 nm (Waters, Milford, USA) and a computer running the Empower 2 chromatographic software (Waters). 20 μL of sample were injected into an ODS-2 column 150 × 4.6 mm i.d., 5 μm size column (Waters) at 30 °C. The chromatogram was developed at a flow rate of 1.2 mL/min using a gradient of mobile phase A (0.1 M ammonium acetate pH 5.7 containing 10% acetonitrile) and mobile phase B (0.1 M ammonium acetate, pH 5.7 containing 80% acetonitrile) as follows: linear gradient of 100% A-100% B for 25 min, isocratic 100% B for 5 min, linear gradient of 100% B-100% A for 5 min and, finally, equilibrate for 5 min.

Standard I3C and I3ACN (Sigma–Aldrich, Steinheim, Germany) were used to identify these compounds in sauerkraut. Standard ABG was synthesized according to Kiss and Neukom (1966) with the modifications described by Peñas, Frias, Sidro, et al. (2010). The purity of standard ABG was determined by HPLC and it was frozen under nitrogen and protected from light.

Calibration curves were made with the standard compounds, then plotted and adjusted by using the method of least squares. The regression coefficients of ABG, I3C and I3ACN curves were greater than 0.990.

2.4.2. Determination of vitamin C

The determination of vitamin C content in HHP-sauerkrauts before and after refrigeration for 3 months was performed by capillary electrophoresis using a fused silica capillary TSP075375 (47 cm × 75 μm) purchased from Composite Metal Services LTD (The Chase, Hallow, Worcester, UK). A P/ACE system 2050 (Beckman Instruments, Fullerton, CA, USA) equipped with UV detection at 254 nm was used for the analysis (Frias, Miranda, Doblado, & Vidal-Valverde, 2005). Ascorbic acid was quantified from a calibration curve built with the pure ascorbic acid standard (Fluka) and with a response factor relative to the internal standard; the regression coefficients were greater than 0.990.

2.5. Statistical analysis

Data were expressed as means of three experiments. 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

HHP technology satisfies the demand for minimally processed products and can provide quality superiority over products obtained by conventional technologies.

Since glucobrassicin is the most abundant indole GLS found in raw white cabbage cv. Bronco (Peñas, Frias, Martinez-Villaluenga, & Vidal-Valverde, 2011), the content of their main breakdown products, ABG, I3C and I3AC, were quantified in pressurized sauerkrauts (NF-HHP and PMF-HHP) and stored for 1, 2 and 3 months (Tables 1 and 2). Fig. 1 represents the effect of storage in those indole GLS derivatives expressed as retention percentage.

Table 1 shows the main indole GLS breakdown products found in the 0.5 and 1.5% NaCl NF-HHP sauerkrauts and their content storage. ABG was the most abundant indole GLS degradation compound (65 μmol/100 g d.m.), followed by I3C (17 μmol/100 g d.m.), while I3ACN was present in the lowest amount (3 μmol/100 g d.m.). The presence of these three compounds in just fermented cabbages is in the range reported previously (Ciska, Verkerk, & Honke, 2009; Peñas et al., 2012). In NF-HHP sauerkrauts produced with the highest NaCl level, the content of ABG (37 μmol/100 g d.m.) and I3C (13 μmol/100 g d.m.) was lower than in those obtained with the lowest salt concentration, while no difference was observed between the two NF-HHP sauerkrauts for I3ACN