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Effects of sodium reduction scenarios on fermentation and quality of sauerkraut

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ABSTRACT

Health authorities advocate the reduction of sodium intake because of its negative impact on health. The effect of sodium reduction on the natural fermentation in sauerkraut was investigated in terms of quality and safety. In addition to 15 g kg^{-1} NaCl control [A], two alternative sodium reduction scenarios were tested: [B] salt reduction (9 g kg^{-1} NaCl), and [C] 40% partial sodium replacement (9 g kg^{-1} NaCl, 4.5 g kg^{-1} KCl, 0.75 g kg^{-1} $MgCl_2$, and 0.75 g kg^{-1} $CaCl_2$). Microbiological safety was similarly assured in all samples, associated with low pH values (3.4–3.7) indicating an adequate lactic acid fermentation. PCR-DGGE and cloning revealed differences in microbial flora between treatments during first weeks of fermentation. *Lactococcus lactis* and *Leuconostoc mesenteroides* dominated [A], [B] and [C], while [C] showed additional abundance of *Lactobacillus paraplantarum* and *Lactobacillus curvatus*. Treatment [B] did not meet industrial criteria for good quality; texture was too soft compared with [A] and [C]. Sensory evaluation showed that both [A] and [C] were equally acceptable as judged by an industrial panel for aroma, taste and texture. Partially replacing sodium salt can successfully maintain high product quality, and thus offers a promising approach to substantially reduce sodium in sauerkraut fermentation.

1. Introduction

Dietary sodium is associated with elevated blood pressure (Doyle & Glass, 2010) which is an important risk factor in cardiovascular disease. Therefore, international and national health organizations together with consumer organizations and the industry are advocating the reduction of sodium salt in food (Dötsch et al., 2009).

The WHO published new guidelines on the intake of sodium salt. In addition WHO now sets guidelines for the use of sodium salt, mineral salt and potassium salt by children for the first time, showing the importance of reduction of sodium intake (WHO, 2012). Sodium chloride salt is used as a traditional preservative in a variety of food products including many fermented products (Kilcast & den Ridder, 2008). In sauerkraut, fermented white cabbage (*Brassica oleracea*), as much as 22.5 g kg^{-1} NaCl may be used (Adams & Moss, 1995). The amount used depends on fermentation temperature and market preference. However, in both the USA and the Netherlands, less NaCl (approximately15 g kg^{-1}) is used.

Fermentation of sauerkraut involves natural microbiota, dominated by lactic acid bacteria that evolve in succession (Pederson, 1975). Gradually the fermenting microflora will be dominated by *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus sake*, *Lactobacillus brevis*, and *Lactobacillus curvatus* (Nout & Rombouts, 2000). Later studies have revealed an additional presence of among others, *Lactobacillus paraplantarum*, *Lactobacillus coryniformis* and *Weissella* sp. (Plengvidhya, Breidt, Lu, & Fleming, 2007). Due to the activity of the lactic acid bacteria, the pH usually decreases to 3.4–3.7 (Plengvidhya et al., 2007). These acidic conditions allow the preservation of ascorbic acid, resulting in vitamin C contents of approximately 15 mg 100 g^{-1} product (Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007).

NaCl in fermented cabbage has been ascribed several functions such as a preservative, inhibitor of endogenous pectolytic enzymes to prevent texture softening, and increasing the osmotic pressure that results in the extraction of the cabbage juice (Man, 2008). The latter contains substrates that are required for fermentation, and selective substances such as allyl-thiocyanates that inhibit undesirable Gram-negative bacteria. Cabbage juice also provides the liquid for submersion of the cabbage, thereby contributing to anaerobic conditions (Steinkraus, 1983).

An attempt to make salt-free sauerkraut was undertaken by Owades (1991), by replacing salt by an osmotically equivalent

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solution of ethanol and organic acids. Although some fermentation of cabbage was achieved, the process was unsuitable for producing commercially acceptable sauerkraut, especially regarding its texture and taste.

Viander, Mäki, and Palva (2003) and Wiander and Ryhänen (2005) showed that sauerkraut juice made at a low salt concentration of 5 g kg^{-1} of a mineral salt mixture (consisting of 57% NaCl, 28% KCl, 12% $MgSO_4$, 2% Lysine.HCl, and 1% SiO_2) obtained a good sensory evaluation. However, low salt sauerkraut containing only about 5 g kg^{-1} NaCl, marketed in the Netherlands is mushy, i.e. lacks the desired crispy bite, and its taste is odd and needs to be masked by additives such as spices.

In addition, solely reducing salt content in fermented products such as sauerkraut could have consequences for food safety. As is well-known, NaCl has an important function in food preservation and safety (Doyle et al., 2010). Its ionic presence achieves a reduction of the water activity (A_w). Recent outbreaks of *Escherichia coli* O157 food infections such as the one associated with salt preserved cabbage in Japan (Westlake, 2012), are facilitated in foods with a reduced salt content. Approaches with salt mixes of reduced Na-content may be helpful to combine the advantages of maintaining reduced A_w for preservation and food safety, and of reducing Na-intake by consumers.

In this study we aimed to study the effect of reduced sodium or reduced salt concentrations on the fermentation outcome and texture of sauerkraut, and investigate the possibilities to reduce NaCl while fulfilling commercial quality criteria. Our aim was to achieve a 40% reduction of sodium in sauerkraut and still obtain a crispy texture and normal fermentation outcomes, particularly microbiota, acidity, flavour, and vitamin C retention. Since a simple lowering of the NaCl concentration results in lower osmotic pressure, we aimed at distinguishing the individual effects of reducing sodium, and of osmotic pressure. For that purpose we compared a 15 g kg^{-1} NaCl control with a salt-reduced (9 g kg^{-1} NaCl) and a salt-replaced (15 g kg^{-1} mineral salts mixture giving approximately equal osmotic pressure as the control) fermentation.

2. Materials and methods

2.1. Plant material

White cabbage was obtained from Kramer and Sons B.V., Zuid-Scharwoude, the Netherlands. We used the variety “Atria”, with a dense crop, popular for commercial sauerkraut making. Atria represents the largest harvests for sauerkraut. The cabbages were harvested in October, during the sauerkraut production season.

2.2. Fermenter design

Custom made fermentation vessels were used for sauerkraut production. These fermenters were made of non-transparent PVC measuring 1.5 m in height and 25 cm in diameter, similar as the design of Steinbuch (1971). A drainage port was located at 90 cm from the bottom. A stainless steel cylindrical sampling device (2 cm diameter) was designed in a way to sample deep in the fermenter without disturbing the cabbage mass.

2.3. Sauerkraut production

Fresh cabbage was de-leafed and mechanically de-cored. Cabbage was cut into 1 mm thick shreds. Three pilot 100 kg scale fermentations were carried out in duplicate, [A] a control with 15 g kg^{-1} NaCl, [B] 40% sodium reduction by applying 9 g kg^{-1} NaCl, and [C] 40% sodium reduction by applying 15 g kg^{-1} of a salt-replacer mix -isotonic to the control- consisting of 9 g kg^{-1} NaCl, 4.5 g kg^{-1} KCl, 0.75 g kg^{-1} $MgCl_2$, and 0.75 g kg^{-1} $CaCl_2$. The rationale behind the composition of the salt replacer mixture is based on the salty taste of KCl and $CaCl_2$, the favourable osmotic effect of $MgCl_2$ and the firmness enhancing effect of $CaCl_2$. Sauerkraut was fermented at ambient temperatures as practiced in the factory, at 12 °C during the first 6 weeks, followed by a storage period of 6 weeks at 9 °C.

2.4. Sampling

Sampling during the fermentation period was done frequently at the start because significant changes occur, and less frequent towards the end when a stable situation is established. Samples were taken after 2, 4, 9, and 37 days. Consistency measurements and sensory analyses were done after 6 weeks i.e. end of fermentation, and after an additional storage period of 6 weeks i.e. 12 weeks in total. The latter sampling was done to simulate the delay between completion of fermentation and retailing.

2.5. Microbiological analysis

A 10 g sample was mixed with 90 ml sterile PPS (peptone physiological saline, 1 g L^{-1} neutralized bacterial peptone and 8.5 g L^{-1} NaCl) in a sterile stomacher bag and homogenized (Stomacher® 400 Circulator, Seward) at normal speed for 1 min. The homogenate was then diluted with PPS and appropriate decimal dilutions were plated, either poured or spiral plated. Aerobic mesophilic micro-organisms were enumerated as Total Viable Count (TVC) using plate count agar (PCA, Oxoid CM0325), incubated at 30 °C for 72 h. Lactic Acid Bacteria (LAB) were enumerated in de Man, Rogosa and Sharpe (MRS) broth (Merck VM 986641) mixed with 15 g L^{-1} bacteriological agar (Oxoid LP0011), supplemented with 2 g L^{-1} Delvocid (50% natamycin, DSM, the Netherlands). Plates were incubated under micro-aerobic conditions (air evacuated to 300 mbar with an Anaxomat WS9000, Mart, and filled with 80% N_2, 10% CO_2 and 10% H_2 gas mixture leaving a final concentration of 6% O_2) at 30 °C for 72 h. Enterobacteriaceae were enumerated in pour plates of violet red bile glucose (VRBG) medium (Oxoid CM0485) with an overlay of the same medium and incubated at 37 °C for 24 h. Yeasts and moulds were enumerated on oxytetracycline glucose yeast extract (OGYE) agar (Oxoid CM0545B) supplemented with chloramphenicol supplement (Oxoid SR0078E) at 25 °C for 5 days.

2.6. DNA extraction and PCR-DGGE

Aliquots of 20 g of sauerkraut were mixed with 20 ml distilled water and homogenized using an Ultra-Turrax® T25 basic (Ika® Werke). Total genomic DNA was extracted using a fast-DNA spin kit for soil (MP Biomedical) according to the manufacturer’s instructions. Microbial genomic DNA isolated from sauerkraut was directly used to amplify the V6-V8 region of the bacterial 16S rRNA gene, using primers described by Nübel et al. (1996). PCR-DGGE was performed according to Lima et al. (2012).

2.7. DNA clone library construction, sequencing and annotation of DGGE fingerprints

Microbial genomic DNA isolated from different stages of fermentation was amplified with 16S rRNA gene universal primers. Clone libraries were made which were later used to annotate DGGE fingerprints, as described by Lima et al. (2012). A marker consisting of a mixture of amplicons obtained from the sauerkraut was included to facilitate gel normalization. Gels were silver stained according to the method of Sanguinetti, Neto, and Simpson (1994),