Food Chemistry 168 (2015) 471–477

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The optimisation of traditional fermentation process of white cabbage (in relation to biogenic amines and polyamines content and microbiological profile)

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ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form 10 July 2014
Accepted 12 July 2014
Available online 23 July 2014

Keywords:
Fermented whole cabbage heads
Biogenic amines and polyamines
Microbiological profile
Response Surface Methodology

ABSTRACT

White cabbage heads cultivar "Futoški" and hybrid "Bravo" were investigated during fermentation process, for 50 days, at different temperature regimes (16–18; 18–20; 20–22 °C) and salt concentrations 1, 1.5 and 2%. The quantity of biogenic amines (tryptamine, phenylethylamine, putrescine, cadaverine, histamine, serotonine, tyramine, spermidine and spermine), as well as microbiological profile (lactic acid bacteria, total number of microorganisms, yeasts and moulds and *Enterobacteriaceae*) have been determined during fermentation. The optimum processing conditions were determined by Response Surface Method, coupled with Fuzzy Synthetic Evaluation algorithm. The optimal process parameters, regarding low biogenic amines and polyamines content, for "*Futoški*" cabbage was: salt concentration of 2%, at 18 °C, and for hybrid "*Bravo*": salt concentration of 1%, at 20 °C.

1. Introduction

Traditional food is an important element of cultural heritage worldwide. Foods that are typical of certain region or area have their own peculiar characteristics that arise from the use of local ingredients and production techniques, which are deeply rooted in tradition and linked to the specific geographic area. Traditionally fermented white cabbage, a product obtained by the spontaneous lactic acid fermentation of salted and shredded cabbage is one of the best known traditional foods. Fermented cabbage is very important foodstuff because it is rich in minerals, vitamin C, dietary fibers, and phytochemicals, with beneficial effect on human health (Chu, Sun, Wu, & Liu, 2002; Jahangir, Kim, Choi, & Verpoorte, 2009; Martinez-Villaluenga et al., 2009; Podsędek, 2007; Verhoeven, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997). Fermented cabbages also contain high levels of glucosinolate hydrolysis products, which present important anticarcinogenic activity (Bonnesen, Eggleston, & Hayes, 2001; Martinez-Villaluenga et al., 2009; Verhoeven et al., 1997). Spontaneously fermented cabbage as source of autochthonous functional starter cultures (Beganović et al., 2014) traditionally produced in the Balkans, including Serbia, using whole heads and fermentation takes longer time (several months) and represents a higher risk, especially for the survival of pathogens, like *Listeria monocytogenes* and *Escherichia coli* (Niksic et al., 2005). Fermentation is a means for preventing cabbage deterioration and extending its shelf life, since the organic acids released by lactic acid bacteria inhibit the growth of undesirable microorganisms (Xiong, Guan, Song, Hao, & Xie, 2012). The succession of growth of particular lactic acid bacteria (LAB) species and their metabolic activities are responsible for the quality and safety of the traditionally fermented white cabbage (Malinowska-Pan'czyk, 2012). However, high microbial populations may produce measurable detrimental metabolites such as biogenic amines and polyamines (Peñas, Frias, Sidro, & Vidal-Valverde, 2010).

Biogenic amines and polyamines are basic nitrogenous compounds that are formed mainly by microbial decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. Biogenic amines and polyamines in food and beverages are formed by microbial amino acid decarboxylase activity (Silla-Santos, 2001). As the microbial spoilage of food may be accompanied with the increased production of decarboxylases, the presence of biogenic amines and polyamines might serve as a useful indicator of food spoilage (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Most important biogenic amines and polyamines occurring in foods are histamine (HI), putrescine (PUT), cadaverine (CAD), tyramine (TY), tryptamine (TR), phenylethylamine (PHE), spermine (SPM) and spermidine (SPD) (Shalaby, 1996). Many authors investigate biogenic amines and polyamines

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http://dx.doi.org/10.1016/j.foodchem.2014.07.068
0308-8146/

content in traditionally fermented, shredded white cabbage, average values of 174, 146, and 50 mg/kg have been reported for TY, PUT, and CAD, respectively, in household-prepared and commercial sauerkraut from the Czech Republic and Austria, with the lowest concentrations in the household-prepared product (Kalač, Špička, Křížek, Steidlová, & Pelikánová, 1999). Fermented whole cabbage heads are very important commercial and artisan's product because it is deeply ingrained in culinary habits of people, and it is highly specific product in Serbia and Western Balkan's region, so it is very important to investigate safety aspects of this traditional process and to optimise it. Also, there is no much information about fermented whole cabbage regarding microbiology, biogenic amines and polyamines content. In Serbia farmers cultivate hybrids because of their higher yield; compact head, uniform quality and resistance to diseases, but traditional varieties are still highly prized because of their taste, tradition and suitability for fermentation. Traditional varieties are characterised by loose heads suitable for spontaneous fermentation, since brine diffuses easier inside the heads (Cvetković, Bardić, Jokanović, & Mastilović, 2008). Fermentation of shredded cabbage is faster then in whole cabbage because shredding releases carbohydrates and more acid was produced (Tamang & Kailasapathy, 2010).

Response Surface Methodology (RSM) is used as an effective tool for optimising a variety of processes (Koprivica, Pezo, Ćurčić, Lević, & Šuput, 2013). The main advantage of RSM is reduced number of experimental runs that provide sufficient information for statistically valid results. The RSM equations describe effects of the test variables on the observed responses, determine test variables interrelationships and represent the combined effect of all test variables in the observed responses, enabling the experimenter to make efficient exploration of the process to find the workable optimums.

The main aim of the present work was to investigate the differences between two cultivars of white cabbage, "*Futoški*" and hybrid "*Bravo*" during fermentation, in relation to biogenic amines and polyamines content and microbiological profile. Current study intends to investigate the effects of salt concentration, time and temperature, and it is focused on finding the appropriate mathematical model for biogenic amines and polyamines content and microbiological profile, during spontaneous fermentation of white cabbage.

2. Materials and methods

This paper deals with the fermented whole cabbage heads of hybrid "*Bravo*" and traditional cultivar "*Futoški*".

2.1. Plant material

White cabbage heads, cultivar "*Futoški*" and hybrid "*Bravo*" were harvested from parcels in Futog district, northern Serbia (Province of Vojvodina). They are late fall varieties.

2.2. Fermentation trials

Cultivar "*Futoški*", and hybrid "*Bravo*" cabbage have been subjected to spontaneous fermentation process. Cabbage heads with approximate diameter of 170 mm for "*Futoški*" and 140 for "*Bravo*" were prepared by removing the 3–4 outer leaves. Fermentation was performed in 50 dm^3 plastic barrels, each containing approximately 25 kg of tightly packed cabbage heads. NaCl solution was applied on cabbage heads and all together was pressed tightly and covered with a plastic film. The NaCl was used in concentrations of 1, 1.5 and 2% (w/w), and the temperature was maintained at the following intervals, 16–18, 18–20 and 20–22 °C in accordance with traditional way of whole cabbage heads fermentation. Fermentations trials were performed with three repetitions.

2.3. Microbiological analysis

The microbial profile of traditionally fermented white cabbage was investigated at 0, 3, 6, 10, 15, 21, 28, 40 and 50th day of fermentation by total number of microorganisms (TN), (E. ISO, 2003) yeasts and molds (YM) (ISO., 2008), *Enterobacteriaceae* (*Enth.*) (H. ISO, 2004) and the lactic acid bacteria (LAB) number was determined by incubation (30 °C, 72 h) of inoculated Man, Rogosa and Sharpe (MRS) agar (LabM, United Kingdom) containing 0.02% sodium azide.

2.4. Biogenic amines and polyamines determination

TR, PHE, PUT, CAD, HI, TY, SPD and SPM were determined following the high-performance liquid chromatography. Briefly, 2.00 g of each sample were weighted and put into test tube. Appropriate amount of internal standard (1,7-diaminoheptane) was added and sample was homogenised with 20 ml of 0.1 M HCl. Further extraction and derivatization were done according to (Peñas et al., 2010). HPLC analysis was performed according (Tasić et al., 2012) by using a liquid chromatography (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser, an auto sampler and a thermo stated column compartment, on an Agilent, Eclipse XDB-C18, 1.8 mm, 4.6–50 mm column. Recoveries were over the 82% for all the amines and detection limits of the amines were determined to be 0.10 mg/kg for PUT and SPD, 0.17 mg/kg for CAD and TY, 0.25 mg/kg for TR, PHE and HI and 0.50 mg/kg for SPM.

2.5. Statistical analyses

A descriptive statistical analysis for all the obtained results was performed. All measurements were performed with three repetitions. Evaluation of analysis of variance (ANOVA) and Principal Component Analysis (PCA) of the obtained results was performed using StatSoft Statistica 10.0® software. Significant differences were calculated according to post hoc Tukey's HSD ("honestly significant differences") test at $p < 0.05$ significant level, 95% confidence limit. The experimental data used for the study of experimental results were obtained using a 3^3 full factorial experimental design (3 levels-3 parameter), with 27 runs (one for each cultivar), according to RSM, considering three factors: duration of fermentation, processing temperature and salt concentration.

The presence of TR, PHE and SPM were not detected, during the experimental measurements, for both "*Futoški*" cultivar and "*Bravo*" hybrid cabbage heads, and only PUT, CAD, HI, TY and SPD content were modelled. Microbiological profile, according to lactic acid bacteria, total number of microorganisms, yeasts and moulds and *Enterobacteriaceae* were also modelled.

Second order polynomial (SOP) models in the following form were developed to relate responses (Y) and three process variables (X), for each cultivar:

$$Y_k^l = \beta_{k0}^l + \sum_{i=1}^{3}\beta_{ki}^l \cdot X_i + \sum_{i=1}^{3}\beta_{kii}^l \cdot X_i^2 + \sum_{i=1,j=i+1}^{3}\beta_{kij}^l \cdot X_i \cdot X_j, k = 1-9, l = 1-2, \quad (1)$$

where: β_{k0}^l, β_{ki}^l, β_{kii}^l, β_{kij}^l were constant regression coefficients; Y_k^l, either PUT ($k = 1$), CAD ($k = 2$), HI ($k = 3$), TY ($k = 4$) or SPD ($k = 5$) content, LAB ($k = 6$), TN ($k = 7$), YM ($k = 8$) and *Enth.* ($k = 9$) in