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CHEMOMETRIC APPROACH TO OPTIMIZATION OF WHITE CABBAGE FERMENTATION
HEMOMETRIJSKI PRISTUP OPTIMIZACIJE PROCESA FERMENTACIJE BELOG KUPUSA

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ABSTRACT

The aim of the present work is to optimize fermentation process of whole cabbage heads of white cabbage (cultivar „Futoški" and hybrid "Bravo" were considered within this research). Both cabbages were subjected to fermentation process, with addition of 1- 2% NaCl, at temperature 18-22°C. Different organic acids contents (oxalic – OK_1, lactic – OK_2, acetic – OK_3, malic – OK_4, citric – OK_5, succinic – OK_6 and formic– OK_7) within cabbage leaves and brine were monitored during 80 days. Principal component analysis exerted good discrimination capabilities for different samples of „Futoški" and hybrid "Bravo" cabbage heads fermentation process, while standard score helped in optimal process parameters determination.

Key words: *cabbage, fermentation, organic acid, pca, optimization.*

REZIME

Cilj ovog rada je da se optimizuje proces fermentacije belog kupusa u glavicama (populacija "Futoški" beli kupus i hibrid "Bravo" su analizirani). Obe vrste kupusa su podvrgnute procesu fermentacije uz dodatak 1-2% soli, i na temperaturi od 18-22°C. Sastav različitih organskih kiselina (oksalna – OK_1, mlečna – OK_2, sirćetna – OK_3, jabučna – OK_4, limunska – OK_5, ćilibarna – OK_6 i mravlja – OK_7) je određivan u listovima kupusa i nalivu tokom 80 dana fermentacije.

Maksimalna detektovana količina mlečne kiseline u rasolu bila je $11,9 \cdot 10^3$ i $15,7 \cdot 10^3$ mg/L za kupus "Futoški" i hibrid "Bravo", respektivno. Koncentracija mlečne kiselina u listovima bila je $11,2 \cdot 10^3$ mg/kg i $14,2 \cdot 10^3$ mg/kg za "Futoški" i hibrid "Bravo", respektivno. Fermentacija glavice Futoškog" kupusa je završena ranije nego fermentacija kod hibrida "Bravo", ali je kod hibrida "Bravo" bila veća koncentracija organskih kiselina. Primenjena višeparametarska analiza glavnih komponenata (Principal Component Analysis) pokazala je dobre mogućnosti za određivanje grupa među različitim uzorcima glavica sorte „Futoškog" i hibrida "Bravo", u toku procesa fermentacije. Analiza standardnih ocena (Standard score) je korišćena za određivanje optimalnih procesnih parametara.

Ključne reči: *kupus, fermentacija, organska kiselina, pca, optimizacija.*

INTRODUCTION

Lactic acid fermentation, an ancient preservation method, is nowadays especially favored as a "natural" process to increase the shelf-life, with effect on sensorial characteristics of various products *(Halász et al., 1999; Korovičova et al. , 2003; Breidt et al, 2007; Di Cagno et al. 2011)*. Proper cabbage fermentation depends on cabbage variety *(Dobričević et al., 2004; Stamer et al., 1969)*, temperature and on the addition of sodium chloride at the correct concentration *(Pederson & Albury, 1969; Wiander & Ryhänen, 2005; Wiander et al., 2003, Penas et al., 2010; Wolkers-Rooijackers et al., 2013)*. Sauerkraut as shredded salted and fermented cabbage is well reported in the literature *(Johanningsmeier et al., 2007; Johanningsmeier et al., 2005; Lu et al., 2003; Wiander & Palva, 2008; Martinez-Villaluenga et al., 2009)*. The same is not true for the fermentation of whole cabbage heads which is tradition in the region of western Balkan, and the study of its quality is aim of this work.

Chemical changes during fermentation process have been analyzed.

MATERIAL AND METHOD

Fermentation trials

About 60 kg of cultivar "Futoški", and hybrid "Bravo" cabbage have been subjected to spontaneous fermentation process. Cabbage heads were prepared by removing the 3-4 outer leaves. 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), temperature of fermentation was applied in intervals 16-18°C, 18-20°C and 20-22°C. NaCl was purchased from the local market. Oxalic, lactic, acetic, malic, citric, succinic, formic, propionic and butyric acid concentration have been measured during fermentation.

Cabbage heads were taken from the barrel in the given time intervals, blended and frozen (-18°C) for further organic acids analysis as one represetative sample. Brine samples were also taken. pH value was measured directly in cabbage brine with mobile ExStik pH Meter (Extech instruments, U.S.A).

Organic acid analysis:

For organic acid analysis brine samples were defrosted, diluted (1:1), centrifuged for 10 min at 4000 rpm and applied in chromatograph, after filtration through 0.45 µm filter. Cabbage tissue samples were defrosted, choped and homogenized in Ultra-turrax IKA T25 digital, 6000 rpm and diluted with distilled water (ratio 1:1.5). Homogenized cabbage leaves samples along with water were stired on magnetic stirrer, 400 rpm, for 30 minutes, for better extraction, centrifuged for 15 minutes, 4000 rpm and filtered (0,45 µm) before injection into column. Determination of organic acids was carried out by HPLC system on Liquid Chromatograph Agilent Technologies 1200 series, with diode array detector, in column Zorbax SB C18, 3 x 250 mm, according to Jayabalan et al., 2007. Organic acids were detrmined in cabbage tissue after 10 , 20, and 50 days of fermenation, and in the brine on 3rd, 6th, 10th, 15th, 22nd, 37th, 55th and 80th day of fermentation.

Analysis of variance (ANOVA) and Response Surface Methodology (RSM) were performed using StatSoft Statistica, for

Windows, version 10.0 program. Principal component analysis (PCA) has been applied successfully to classify and discriminate the different cultivars of cabbage. Pattern recognition technique has been applied within results descriptors to characterize and differentiate all varieties of samples. In order to describe and optimize the fermentation process, organic acid contents were monitored. The accepted 33 full factorial, central composite experimental design, with 3 levels and 3 parameters in 1 block was applied in calculation. The RSM method was selected to estimate the main effect of the process variables on organic acids content. The independent variables were: duration of fermentation process (X_1) - 0, 10 and 20 days (for cabbage heads), and 0,40 and 80 days for brine; temperature (X_2) - 18, 20 and 22°C; X_3 is the salt concentration (1, 1.5 and 2% w/w), and the dependent variables were the responses: oxalic – OK_1, lactic– OK_2, acetic– OK_3, malic– OK_4, citric– OK_5, succinic– OK_6 and formic– OK_7. The following second order polynomial (SOP) model was fitted to the data. Seven models of the following form were developed to relate four responses (OK) to three process variables (X):

$$OK_k = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} X_i + \sum_{i=1}^{3} \beta_{kii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} X_i X_j ,$$

k=1-7 (1)

where: β_0, β_i, β_{ii}, β_{ij} are constant regression coefficients.

Min-max normalization is one of the most widely used technique to compare various characteristics of complex samples determined using multiple assays, where samples are ranked based on the ratio of raw data and extreme values of the measurement used. Since the units and the scale of the data from various parameters are different, the data in each data set should be transformed into normalized scores, according to following equations:

$$\overline{x}_i = \frac{\max_i x_i - x_i}{\max_i x_i - \min_i x_i}, \quad \forall i \tag{2}$$

where: x_i represents the raw data. Above written formula was used for standard score calculation for oxalic and formic acid.

Normalized scores of the rest of organic acids contents are evaluated according to optimal values, using trapezoidal function, as follows:

$$\overline{x}_i = \begin{cases} \min_i x_i \le x_i < m, & \dfrac{x_i - \min_i x_i}{m - \min_i x_i} \\ m \le x_i < n, & 1 \\ n \le x_i < \max_i x_i, & 1 - \dfrac{x_i - n}{\max_i x_i - n} \end{cases}, \quad \forall i \tag{3}$$

where m and n are minimum and maximum of optimal range values. Optimal values for lactic acid were in the range of 5000-20000 mg/L, optimal acetic acid range was between 0-7000 mg/L, malic acid range was in the range of 50-100 mg/L, optimal citric acid content was between 500-1000 mg/L, an succinic acid content was in the range between 0 and 200 mg/L.

RESULTS AND DISCUSSION

Figure 1 shows oxalic acid content in the cabbage leaves during fermentation.

In the hybrid "Bravo" brine, from 55th day to 80th day , lactic acid content increased from 11.3 ·103 mg/L to 15.7 ·103 mg/L. , while in the brine of cabbage cultivar "Futoški", lactic acid content after 37, until 80 day didn't changed (12.7 ·103 mg/L. Values of lactic acid content in both cabbages are in line with some researchers' claims *(Johanningsmeier et al., 2005; Johanningsmeier et al., 2007)*, and commercial sauerkrauts values *(Trail et al., 1996)*, and slightly higher than certain claims *(Wolkers-Rooijackers et al., 2013)*. Higher values of acetic acid were found in hybrid "Bravo" brine and in the cabbage leaves in relation to cabbage cultivar "Futoški", but in both cabbages obtained values for acetic acid content are slightly lower than in statements of other authors *(Fleming, 1987; Johanningsmeier et al., 2007; Trail et al., 1996; Plevingdyhua et al., 2007)*. The recorded acetic acid content in "Bravo" cabbage brine was 1.0·103 mg/L, after 37th day of fermentation, while the concentration of 1.4·103 mg/L was noticed after 80th day. Acetic acid content reached 1.0·103 mg/L, after 37 days in "Futoški" cabbage brine, and its concentration remain unchanged till 80th day. All of the above indicates that fermentation is slower in hybrids and salt much more slowly diffuses into the cabbage tissue which results in slower growth of acids content. In the cabbage leaves, content of acetic acid was higher in hybrid cultivar, after 50 days of fermentation it was 1.8 ·103 mg/L, while in cabbage cultivar "Futoški" was about 1.2 ·103 mg/L.

"Bravo" hybrid cultivar has a higher content of oxalic acid and formic acid in relation to cabbage cultivar "Futoški". Malic, citric and succinic acid were equally attended in both varieties during the fermentation of cabbage in the quantities that are in line with other authors.*(Fleming, 1987; Trail et al., 1996)*. Propionic and butyric acid where not detected in the cabbage head and brine samples during fermentation of cultivar "Futoški" and hybrid "Bravo".

"Futoški" achieved the end of fermentation after 20 days for cabbage head, while"Bravo" reached the end of fermentation after 50 days for cabbage head.

The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models. ANOVA analysis revealed that the linear terms contributed substantially in all of the cases to generate a significant SOP model.

Linear term of temperature in SOP model, was the most influential for oxalic, lactic, acetic and malic content calculation in cabbage heads for "*Futoški*" cultivar, while quadratic term of time duration was most influential for citric, succinic and formic acids content. Obtained coefficients of determination for organic acids calculation ranged between 0.771 and 0.999 were considered as adequate for organic acids evaluation.

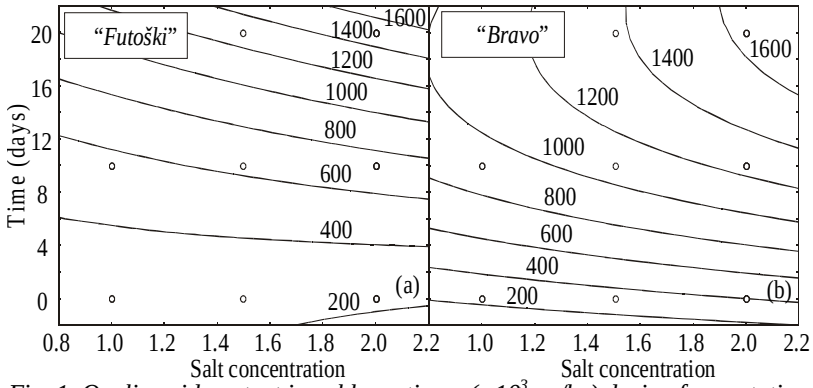

Fig. 1. Oxalic acid content in cabbage tissue (×10³mg/kg) during fermentation, for cultivar "Futoški" and hybrid "Bravo", at 20°C

During fermentation of "*Bravo*" hybrid cabbage heads, linear temperature term in SOP model was the most influential for oxalic, lactic, acetic, malic and citric acids content evaluation, while quadratic term in SOP model was most important in succinic and formic acids content calculation. Obtained coefficients of determination for organic acids calculation ranged between 0.717 and 0.993 were considered as adequate.

Quadratic term of fermentation time was the most important for organic acids calculation using SOP models, for both "*Futoški*" cultivar and "*Bravo*" hybrid. Obtained coefficients of determination for "*Futoški*" cultivar were in the range between 0.724 and 0.960, and between 0.867 and 0.966 for "*Bravo*" hybrid. These values can be considered as adequate for organic acids calculation.

Standard scores analysis showed that the optimum organic acids content have been experienced with "*Futoški*" cultivar. Optimal processing parameters for both cabbage heads were: 20 days of fermentation process, 2.0% salt content, 20°C temperature. The PCA allows a considerable reduction in a number of variables and the detection of relationship structure between measuring parameters. PCA gives complimentary information about analyzed cabbage cultivars and process parameters. All samples have been produced with different cabbage cultivars and various conditioning treatment and predicted by PCA score plots (Figure 2). The full auto scaled data matrix consisting of two different varieties of cabbage heads with different technological treatment are submitted to PCA. As can be seen, there is a neat separation of the different processing conditions, according to used assays for all samples. Quality results show that the first two principal components, accounting for 98.35 % of the total variability for "Futoški" cabbage and 98.83% for hybrid "Bravo" can be considered sufficient for data representation

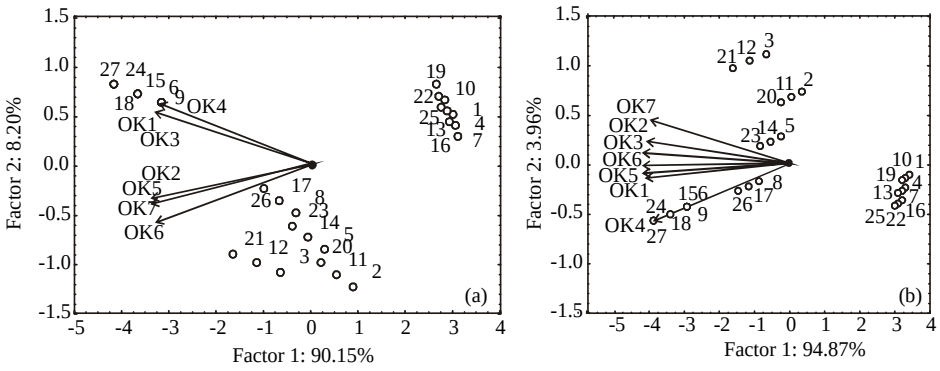

Fig. 2. Biplots of organic acids content in "Futoški" (a) and "Bravo" during fermentation process (b)

CONCLUSION

Organic acid analysis in fermentation of cultivar"Futoški" and hybrid "Bravo" shows that fermentation is slower in hybrids and salt much more slowly diffuses into the cabbage tissue which results in slower growth of acids content. More acetic acid and lactic acid were found in brine and the cabbage leaves in hybrid in relation to cultivar "Futoški".Cultivar "Futoški" achieved the end of fermentation after 20 days for cabbage head, while "Bravo" reached the end of fermentation after 50 days for cabbage head. Analysis and comparison of two cabbage varieties, shown that Serbian cultivar "Futoški" is more suitable material for fermentation of cabbage heads than hybrid "Bravo". Compacted hybrid cabbage heads possibly require higher concentration of salt for fermentation process.

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