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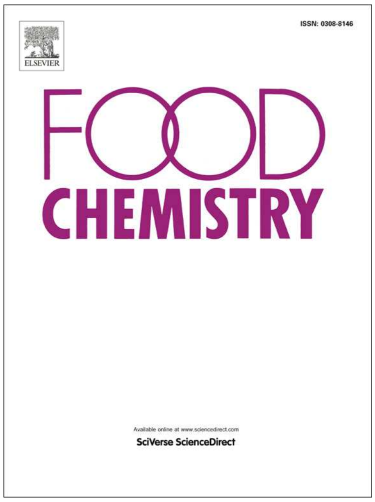


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# The optimization of traditional fermentation process of white cabbage (in relation to biogenic amines and polyamines content and microbiological profile)

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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, and Verpoorte (2009); (Martinez-Villaluenga, Peñas, Frias, Ciska, Honke, Piskula, 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, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997). Spontaneously fermented cabbage as source of autochthonous functional starter cultures (Beganović, Kos, Leboš Pavunc, Uroić, Jokić, & Šušković, 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, Niebuhr, Dickson, Mendonca, Koziczkowski, & Ellingson, 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 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 optimize 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 characterized 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 carbonydrates and more acid was produced (Tamang & Kailasapathy, 2010).

Response Surface Methodology (RSM) is used as an effective tool for optimizing 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 folowing intervals, 16-18, 18-20 and 20-22$^{o}$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 50$^{th}$ 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 homogenized with 20 ml of 0.1 M HCl. Further extraction and derivatization were done according to (Peñas, Frias, Sidro, & Vidal-Valverde, 2010). HPLC analysis was performed according (Tasić, Ikonić, Mandić, Jokanović, Tomović, Savatić, 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 $kg^{-1}$ for PUT and SPD, 0.17 mg $kg^{-1}$ for CAD and TY, 0.25 mg $kg^{-1}$ for TR, PHE and HI and 0.50 mg $kg^{-1}$ 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:

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

where: $\beta_{k0}^l$, $\beta_{ki}^l$, $\beta_{kii}^l$, $\beta_{kij}^l$ 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 "*Futoški*" ($l$=1) or "*Bravo*" hybrid ($l$=2) cabbage heads; $X_1$ - time; $X_2$ - temperature and $X_3$ - salt concentration.

In this article, ANOVA was conducted by StatSoft Statistica, ver. 10 to show the significant effects of independent variables to the responses, and to show which of responses were significantly affected by the varying treatment combinations.



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In order to enable more comprehensive comparison between investigated samples, standard score (SS) was introduced. Principal component analysis (PCA) was applied to classify and discriminate analysed samples. Fuzzy synthetic optimization method (FSE) was implemented using the results of models proposed, to represent quantity of observed biogenic amines and polyamines, as well as microbiological profile, according to Eqn. 1. FSE is commonly used technique to solve problems with constraints involving non-linear functions (Gordana B. Koprivica, Lato L. Pezo, Biljana L. Ćurčić, Ljubinko B. Lević, & Danijela Z. Šuput, 2013). These methods aim to solve a sequence of simple problems whose solutions converge to the solution of the original problem. Fuzzy composite operator O=M (•,⊕), used in this study (where O represents the optimization function), was chosen according to study (Wang, LU, Jiang, LI, & Tian, 2009) .

Standard scores were calculated for each assay, and were used for complex comparison of observed samples, regarding the content of biogenic amines and polyamines and microbiological profile. The ranking procedure between different samples was performed, based upon the ratio of raw data and extreme values for each applied assay (Brlek, Pezo, Voća, Krička, Vukmirović, Čolović, et al., 2013; Prior, Wu, & Schaich, 2005) according to these equations:

$\overline{x}_i = 1 - \dfrac{\max_i x_i - x_i}{\max_i x_i - \min_i x_i}, \quad \forall i$ in case of "the higher, the better" criteria (used for LAB), or

$\overline{x}_i = \dfrac{\max_i x_i - x_i}{\max_i x_i - \min_i x_i}, \quad \forall i$, in case of "the lower, the better" criteria (used for other assays),

where $x_i$ represents the raw data. The higher content of biogenic amines and polyamines was considered as negative for final product properties, as well as the increased number of microorganisms, except lactic acid bacteria content. An optimization procedure was performed according to FSE algorithm, using MicroSoft Excel 2007 to determine the workable optimum conditions for traditionally fermented white cabbage production.

## 3. Results and discussion

Experimental results are presented in Table 1 and Table 2, with calculated SS values used for optimization.

### *3.1. Biogenic amines and polyamines*

TR, PHE and SPM were not detected in any of cabbage samples. According to experimental results (Table 1), higher biogenic amines and polyamines content were observed at higher temperatures regimes and at higher salt concentrations. PUT ranged between 25.56 and 48.08 mg/kg, for "*Futoški*" cabbage head, while "*Bravo*" hybrid head reached lower values, between 3.58 and 22.79 mg/kg, which is lesser than some researches claims (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Peñas, Frias, Sidro, & Vidal-Valverde, 2010). CAD in both cabbage varieties is similar (between 3.58 and 22.35 mg/kg, for "*Futoški*", while "*Bravo*" hybrid reached values between 4.96 and 19.83 mg/kg). HI and TY for "*Bravo*" hybrid head was much lesser in comparison to "*Futoški*", while SPD content reached a bit higher value for "*Bravo*" hybrid head. HI production is more typically associated with Gram negative bacteria such as the *Enth.* However, these bacteria only occur in significant numbers during the early stages of white cabbage fermentation (Holzapfel, Schillinger, Buckenhüskes, & Farnworth, 2003) Smaller amounts of HI, TY and PUT in "*Bravo*" could be explained by slower fermentation in compacted hybrid heads as compared to cultivar "*Futtock*"(B. R. Cvetković, Pestorić, Gubić, Novaković, Mastilović, Kevrešan, et al., 2012).

Quantities obtained for all detected amines are lower than some researchers claims (Kalač, Špička, Křížek, Steidlová, & Pelikánová, 1999; Moret, Smela, Populin, & Conte, 2005), and higher than other authors statements (Kosson & Elkner, 2010). Standard scores calculation showed that lower biogenic amines and polyamines content were obtained for gentler temperature regime (18$^{o}$C), with lesser salt concentration (1%) which is in accordance with (Kalač, Špička, Křížek, & Pelikánová, 2000; Peñas, Frias, Sidro, & Vidal-Valverde, 2010). These results may be explained by stimulation of microbial decarboxylase enzymes at higher osmotic pressure and higher salt content (Peñas, Frias, Sidro, & Vidal-Valverde, 2010).

### *3.2. Microbiological profile*

The initial microbial profile of raw "*Futoški*" for LAB, TN, YM and *Enth* (logCFU/g) was 3.18±0.17, 4.83±0.25, 3.48±0.26, 4.33±0.12 , and 3.33±0.12, 4.43±0.4, 3.57±0.22, 4.78±0.24 for hybrid "*Bravo*", respectively. These results were in the range of typical natural microflora for fresh cabbage (Fleming, McFeeters, & Humphries, 1988). Table 2 shows descriptive statistics results for microbiological profile of "*Futoški*" and "*Bravo*" hybrid cabbage heads during fermentation. Higher values of LAB, TN, YM and *Enth.* were observed at higher temperature content. LAB and TN of microorganisms grew with salt concentration increase, while yeasts and moulds and *Enth.* were lesser at higher salt concentration which is in accordance with other authors (Viander, Mäki, & Palva, 2003; Wiander & Palva, 2008). As seen from Table 2, microbiological profile seems to be similar for "*Futoški*" and "*Bravo*" hybrid cabbage heads. According to standard score calculation, better results were obtained with higher salt concentration.

*3.3. Standard score analysis*

Standard score analysis revealed that optimal process parameters regarding the presence of biogenic amines and polyamines, for "*Futoški*" cabbage heads should be: relatively high salt concentration (2%), at low temperature regime (18$^{o}$C). A bit different process parameters were obtained for optimal fermentation process for "*Bravo*" hybrid: low salt concentration (1%), at mild temperature regime (20$^{o}$C).

*3.4 Principal Component Analysis*

PCA allows a considerable reduction in a number of variables and the detection of structure in the relationship between assays (different biogenic amines and polyamines), different cabbage cultivars and process parameters that give complimentary information. All samples were produced with two cabbage cultivars and various conditioning treatment and predicted by PCA score plot (Figure 1). "*Futoški*" samples were marked as "F", "*Bravo*" hybrid were marked as "B" and numerical signs behind these marks represent the time of fermentation. As it can be seen, there is a neat separation of the two varieties of cabbage, according to used assays. Quality results show that the first two principal components, accounting for 88.04 % of the total variability can be considered sufficient for data representation and the first two principal components. CAD (26.5%), HI (23.2%) and TY (20.3%) content showed more affective for first principle component calculation, while PUT (28.5%), CAD (20.4%) and SPD (46.3%) content were more influential for second factor coordinate calculation. PCA graphic showed good discrimination characteristics between cultivars, six oval areas can be drawn on the graphic, which were found different mostly due to duration of cultivar type and fermentation. Higher biogenic amines and polyamines content were gained with "*Futoški*" cabbage heads samples, increasing with fermentation time. PUT and HI were more affected by temperature, while SPD was more influenced by salt content.

*3.5. Response Surface Methodology*

ANOVA exhibits the significant independent variables as well as interactions of these variables.

The analysis revealed that the linear terms of SOP model were found significant in all model calculation. ANOVA test shows the significant effects of the independent variables to the responses and which of responses were significantly affected by the varying treatment combinations, Table 3. Linear term of fermentation duration was the most important variable for PUT, CAD, HI, TY and SPD in SOP model calculation (statistically significant at $p<0.01$ level, 95% confidence limit). Linear term of salt concentration, as well as quadratic term of fermentation time was also found to be very influential for biogenic amines and polyamines contents calculation. The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models.

A three-dimensional response surface plot was plotted for experiment data visualization and for the purpose of observation the fitting of regression models to experimental data (Fig. 2.). Obtained regression models for HI and SPD, for "*Futoški*" cabbage was as follows:

$$HI=4.96-0.39\cdot T+0.33\cdot t-0.01\cdot t^2-0.04\cdot C\cdot t+0.02\cdot T\cdot t$$

$$SPD=0.99+0.56\cdot C+0.01\cdot T+0.07\cdot t+0.01\cdot C\cdot t$$

while for "*Bravo*" hybrid was:

$$HI=-15.25-3.66\cdot C+0.98\cdot T+1.19\cdot t-0.01\cdot t^2+0.22\cdot C\cdot t-0.06\cdot T\cdot t$$

$$SPD=-8.83+0.90\cdot C+0.62\cdot T-0.13\cdot t-0.01\cdot C\cdot t+0.01\cdot T\cdot t .$$

The analysis revealed that the linear terms of SOP model were found significant in all model calculation. ANOVA test shows the significant effects of the independent variables to the responses and which of responses were significantly affected by the varying treatment combinations, Table 4. Linear term of fermentation duration was the most important variable for LAB, TN, YM content in SOP model calculation (statistically significant at $p<0.01$ level, 95% confidence limit). Linear term of salt concentration, as well as quadratic term of fermentation time was also found to be very influential for biogenic amines and polyamines contents calculation. The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models.

A three-dimensional response surface plot was plotted for experiment data visualization and for the purpose of observation the fitting of regression models to experimental data (Fig. 3.). Obtained regression models for LAB and TN, for "*Futoški*" cabbage was as follows:

$$LAB=4.03+0.05\cdot t-0.02\cdot t^2+0.05\cdot T-0.02\cdot C-0.02\cdot t\cdot T+0.04\cdot t\cdot C+0.01\cdot T\cdot C$$

$$TN=2.49+0.86\cdot t-0.03\cdot t^2+0.06\cdot T+1.43\cdot C-0.22\cdot t\cdot C$$

while for "*Bravo*" hybrid was:

$$LAB=5.56-0.17\cdot t-0.01\cdot t^2+0.01\cdot T-0.43\cdot C-0.01\cdot C^2-0.01\cdot t\cdot T+0.10\cdot t\cdot C+0.01\cdot T\cdot C$$

$$TN=8.25+0.14\cdot t-0.03\cdot t^2+0.08\cdot T-3.29\cdot C+0.14\cdot C^2-0.01\cdot t\cdot T+0.36\cdot t\cdot T+0.03\cdot T\cdot C$$

A significant lack of fit generally shows that the model failed to represent the data in the experimental domain at which points were not included in the regression. All SOP models had insignificant lack of fit tests, which means that all the models represented the data satisfactorily. A high coefficient of determination, $r^2$, is indicative that the variation was accounted and that the data fitted satisfactorily to the proposed model (SOP in this case). The $r^2$ values for observed responses were found very satisfactory and showed the good fitting of the model to experimental results.

## 4. Conclusions

Analysis of biogenic amines and polyamines content and microbial profile of fermented whole cabbage, cultivar "*Futoški*"and hybrid “*Bravo*” showed that biogenic amines and polyamines content of fermented whole cabbage heads are not higher than the stated values for shredded cabbage.  Standard scores calculation showed that lower biogenic amines and polyamines content were obtained for gentler temperature regime, with lesser salt concentration.  PUT and HI were more affected by temperature, while SPD was more influenced by salt content. Fermentation duration was the most important variable for PUT, CAD, HI, TY and SPD content, also for LAB, YM and TN. Standard score analysis showed the optimal process parameters regarding the presence of biogenic amines and polyamines and microbiological profile data. Higher salt concentration is desirable for better microbiological profile for both "*Futoški*"and hybrid “*Bravo*”. The most appropriate process parameters, regarding low biogenic amines and polyamines content, for "*Futoški*" cabbage where: high salt concentration (2%), low temperature (18°C). The most acceptable process parameters obtained for "*Bravo*" cabbage were as follows: low salt concentration (1%), temperature (20°C).

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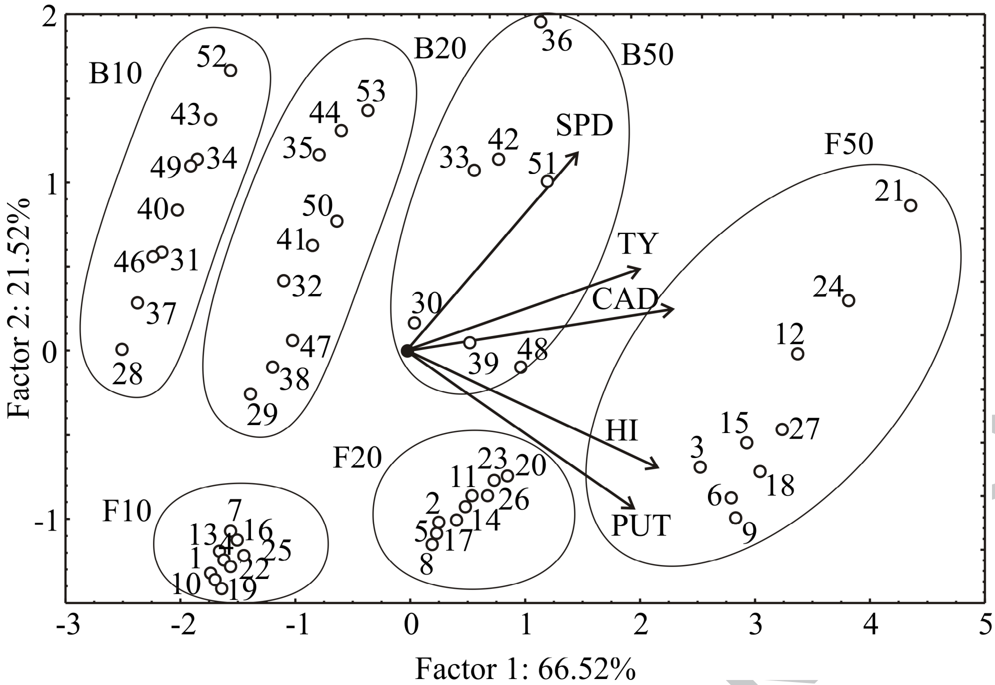
Figure legends:

Figure 1. Biplot for biogenic amines content in fermented cabbage heads (“F“ – *Futoški*, „B“ – *Bravo*)

Figure 2. Histamine (HI) and spermidine (SPD) content in "*Futoški*" (a and b) and "*Bravo*" (c and d) cabbage heads during fermentation

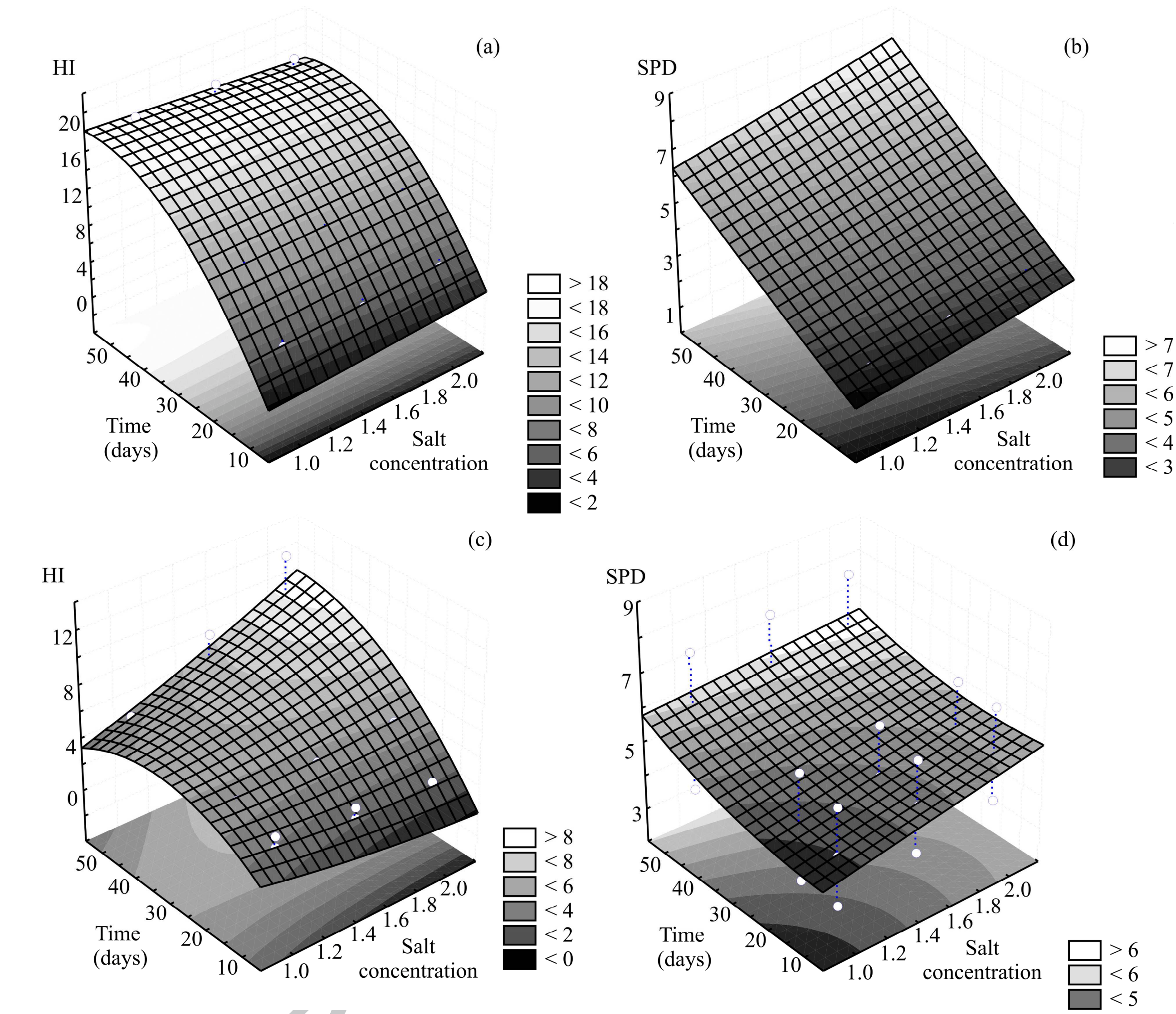
Figure 3. Lactic acid bacteria (LAB) and total number of microorganisms (TN) in "*Futoški*" (a,b) and “*Bravo*” (c,d) cabbage heads during fermentation



Factor 2: 21.52%
Factor 1: 66.52%
B10
B20
B50
F10
F20
F50
SPD
TY
CAD
HI
PUT


374

375



(a)
HI
20
16
12
8
4
0
50
40
30
20
10
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 18
< 18
< 16
< 14
< 12
< 10
< 8
< 6
< 4
< 2
(b)
SPD
9
7
5
3
1
50
40
30
20
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 7
< 7
< 6
< 5
< 4
< 3
(c)
HI
12
8
4
0
50
40
30
20
10
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 8
< 8
< 6
< 4
< 2
< 0
(d)
SPD
9
7
5
3
50
40
30
20
10
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 6
< 6
< 5

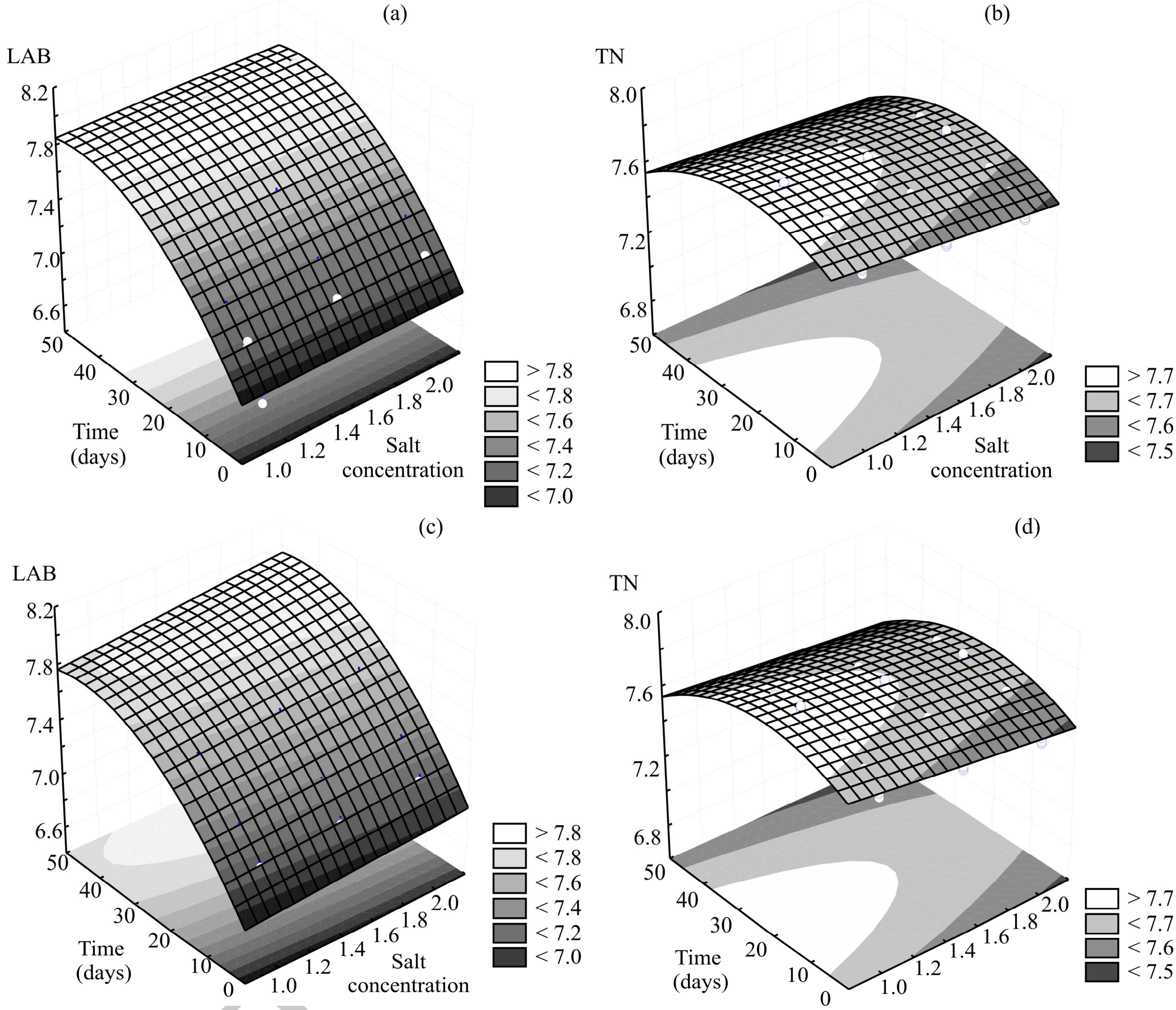

(a)
LAB
8.2
7.8
7.4
7.0
6.6
50
40
30
20
10
0
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 7.8
< 7.8
< 7.6
< 7.4
< 7.2
< 7.0
(b)
TN
8.0
7.6
7.2
6.8
50
40
30
20
10
0
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 7.7
< 7.7
< 7.6
< 7.5
(c)
LAB
8.2
7.8
7.4
7.0
6.6
50
40
30
20
10
0
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
Salt
concentration
> 7.8
< 7.8
< 7.6
< 7.4
< 7.2
< 7.0
(d)
TN
8.0
7.6
7.2
6.8
50
40
30
20
10
0
Time
(days)
1.0
1.2
1.4
1.6
1.8
2.0
> 7.7
< 7.7
< 7.6
< 7.5

Table 1. Experimental results of biogenic amines and polyamines content (mg kg-1) in cabbage heads, during fermentation

| | | | "*Futoški*" cultivar | | | | | | "*Bravo*" hybrid | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | T | t | PUT | CAD | HI | TY | SPD | SS | PUT | CAD | HI | TY | SPD | SS |
| 1 | 18 | 10 | 26.09 | 5.18 | 5.49 | 2.25 | 2.50 | 0.94 | 5.02 | 5.46 | 2.29 | 2.49 | 3.05 | 0.95 |
| 1 | 18 | 20 | 30.61 | 13.40 | 10.53 | 4.59 | 3.29 | 0.67 | 13.46 | 10.81 | 4.67 | 3.28 | 3.06 | 0.72 |
| 1 | 18 | 50 | 46.58 | 18.93 | 16.46 | 5.41 | 5.98 | 0.26 | 19.32 | 16.40 | 5.52 | 5.93 | 3.57 | 0.43 |
| 1 | 20 | 10 | 25.56 | 4.38 | 5.25 | 3.17 | 2.46 | 0.95 | 4.26 | 5.28 | 3.17 | 2.45 | 4.51 | 0.89 |
| 1 | 20 | 20 | 31.33 | 13.19 | 10.82 | 4.28 | 3.37 | 0.67 | 13.35 | 10.80 | 4.31 | 3.39 | 4.51 | 0.66 |
| 1 | 20 | 50 | 48.02 | 20.32 | 18.26 | 5.01 | 6.06 | 0.21 | 20.03 | 17.82 | 4.94 | 5.89 | 5.62 | 0.33 |
| 1 | 22 | 10 | 26.31 | 3.58 | 4.88 | 3.89 | 2.56 | 0.94 | 3.58 | 4.96 | 3.96 | 2.51 | 5.89 | 0.83 |
| 1 | 22 | 20 | 31.87 | 12.96 | 10.94 | 4.01 | 3.38 | 0.67 | 12.57 | 11.19 | 4.00 | 3.31 | 6.15 | 0.60 |
| 1 | 22 | 50 | 47.48 | 21.01 | 19.44 | 4.57 | 6.03 | 0.20 | 21.04 | 19.83 | 4.47 | 5.97 | 7.54 | 0.22 |
| 1.5 | 18 | 10 | 28.15 | 5.61 | 5.78 | 1.51 | 2.84 | 0.91 | 5.71 | 5.78 | 1.54 | 2.78 | 3.50 | 0.92 |
| 1.5 | 18 | 20 | 32.35 | 14.08 | 10.65 | 4.94 | 3.71 | 0.63 | 13.98 | 10.76 | 4.88 | 3.70 | 3.38 | 0.68 |
| 1.5 | 18 | 50 | 46.86 | 19.41 | 16.03 | 8.91 | 6.39 | 0.19 | 19.94 | 15.69 | 9.12 | 6.50 | 3.87 | 0.33 |
| 1.5 | 20 | 10 | 27.67 | 4.85 | 5.57 | 2.39 | 2.81 | 0.92 | 4.74 | 5.61 | 2.38 | 2.87 | 4.86 | 0.86 |
| 1.5 | 20 | 20 | 33.29 | 13.43 | 10.78 | 4.68 | 3.77 | 0.62 | 13.60 | 11.05 | 4.72 | 3.70 | 5.01 | 0.61 |
| 1.5 | 20 | 50 | 47.96 | 21.11 | 17.44 | 5.30 | 6.52 | 0.19 | 20.84 | 17.58 | 5.39 | 6.54 | 5.71 | 0.29 |
| 1.5 | 22 | 10 | 28.32 | 3.98 | 5.23 | 3.18 | 2.89 | 0.91 | 4.00 | 5.39 | 3.22 | 2.82 | 6.20 | 0.80 |
| 1.5 | 22 | 20 | 33.12 | 13.22 | 11.06 | 4.36 | 3.71 | 0.63 | 13.11 | 10.92 | 4.38 | 3.76 | 6.48 | 0.56 |
| 1.5 | 22 | 50 | 47.31 | 21.55 | 19.44 | 4.92 | 6.51 | 0.17 | 22.26 | 19.56 | 4.98 | 6.63 | 7.71 | 0.17 |
| 2 | 18 | 10 | 30.19 | 6.14 | 6.15 | 0.78 | 3.15 | 0.89 | 6.02 | 6.10 | 0.77 | 3.08 | 3.93 | 0.90 |
| 2 | 18 | 20 | 34.34 | 14.61 | 11.06 | 5.32 | 4.08 | 0.57 | 14.12 | 10.80 | 5.24 | 3.99 | 3.74 | 0.64 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 18 | 50 | 46.58 | 20.04 | 15.57 | 12.76 | 7.11 | 0.09 | 19.92 | 15.56 | 12.60 | 7.04 | 3.97 | 0.25 |
| 2 | 20 | 10 | 29.62 | 5.40 | 5.89 | 1.65 | 3.14 | 0.89 | 5.30 | 5.97 | 1.62 | 3.13 | 5.26 | 0.83 |
| 2 | 20 | 20 | 33.83 | 14.06 | 11.12 | 4.98 | 4.13 | 0.59 | 14.13 | 11.13 | 5.06 | 4.15 | 5.30 | 0.57 |
| 2 | 20 | 50 | 47.52 | 21.52 | 16.76 | 8.95 | 7.19 | 0.11 | 21.11 | 16.87 | 9.03 | 7.02 | 5.98 | 0.20 |
| 2 | 22 | 10 | 30.79 | 4.54 | 5.55 | 2.46 | 3.15 | 0.88 | 4.51 | 5.73 | 2.47 | 3.23 | 6.66 | 0.77 |
| 2 | 22 | 20 | 34.67 | 13.84 | 11.06 | 4.82 | 4.04 | 0.59 | 13.66 | 11.41 | 4.73 | 4.10 | 6.75 | 0.52 |
| 2 | 22 | 50 | 47.82 | 22.35 | 18.96 | 5.19 | 6.93 | 0.15 | 22.79 | 18.95 | 5.20 | 6.95 | 7.96 | 0.14 |
| Polarity | | | - | - | - | - | - | | - | - | - | - | - | |

Polarity: '+' = the higher the better criteria, '–' = the lower the better criteria, C-salt concentration (%), T-temperature ($^{o}$C), t-time (days)

Table 2. Descriptive statistics of microbiological profile

| Cultivar | "*Futoški*" cultivar | | | | "*Bravo*" hybrid | | | |
|---|---|---|---|---|---|---|---|---|
| Microorganism | LAB | TN | YM | *Enth.* | LB | TN | YM | *Enth.* |
| Average | 7.39 | 7.53 | 2.54 | 4.29 | 7.18 | 7.72 | 2.70 | 4.24 |
| St. Dev. | 0.64 | 0.94 | 0.73 | 1.36 | 0.78 | 0.93 | 0.93 | 1.34 |
| Minimum | 5.60 | 4.98 | 1.79 | 3.01 | 5.65 | 5.02 | 1.85 | 3.00 |
| Maximum | 7.99 | 8.34 | 3.65 | 5.55 | 8.01 | 8.32 | 5.07 | 5.48 |
| Variance | 0.41 | 0.88 | 0.53 | 1.85 | 0.61 | 0.86 | 0.87 | 1.79 |

*LAB-lactic acid bacteria, TN-total number of microorganisms, YM-yeasts and moulds, *Enth.*-*Entherobacteriaceae*. Results are expressed in log cfu $ml^{-1}$

Tabela 3 ANOVA table of biogenic amines and polyaminescontent during fermentation (sum of squares)

| | | *"Futoški"* cultivar | | | | | *"Bravo"* hybrid | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | PUT | CAD | HI | TY | SPD | PUT | CAD | HI | TY | SPD |
| C | 1 | 17.987$^{*}$ | 5.088$^{*}$ | 0.073 | 9.909$^{*}$ | 3.078$^{*}$ | 4.410$^{*}$ | 0.076$^{**}$ | 9.490$^{*}$ | 3.242$^{*}$ | 1.540$^{*}$ |
| $C^2$ | 1 | 0.013 | 0.017 | 0.001 | 0.258 | 0.001 | 0.088 | 0.003 | 0.149 | 0.009$^{**}$ | 0.000 |
| T | 1 | 2.101$^{*}$ | 0.330$^{*}$ | 7.392$^{*}$ | 9.367$^{*}$ | 0.001 | 0.468$^{*}$ | 9.943$^{*}$ | 9.682$^{*}$ | 0.011$^{**}$ | 48.631$^{*}$ |
| $T^2$ | 1 | 0.000 | 0.082 | 0.005 | 0.171 | 0.008 | 0.002 | 0.019 | 0.145 | 0.001 | 0.000 |
| t | 1 | 1670.799$^{*}$ | 1129.531$^{*}$ | 654.691$^{*}$ | 87.758$^{*}$ | 61.358$^{*}$ | 1153.799$^{*}$ | 647.742$^{*}$ | 88.059$^{*}$ | 60.925$^{*}$ | 3.609$^{*}$ |
| $t^2$ | 1 | 0.029 | 129.329$^{*}$ | 30.413$^{*}$ | 7.917$^{*}$ | 0.007 | 125.307$^{*}$ | 31.902$^{*}$ | 7.727$^{*}$ | 0.005 | 0.157$^{*}$ |
| C × T | 1 | 0.004 | 0.001 | 0.000 | 3.642$^{*}$ | 0.015$^{*}$ | 0.193$^{*}$ | 0.008 | 3.160$^{*}$ | 0.000 | 0.003 |
| C × t | 1 | 14.543$^{*}$ | 0.053 | 2.161$^{*}$ | 21.767$^{*}$ | 0.137$^{*}$ | 0.034 | 1.958$^{*}$ | 22.189$^{*}$ | 0.142$^{*}$ | 0.134$^{*}$ |
| T × t | 1 | 0.153 | 11.718$^{*}$ | 12.215$^{*}$ | 25.096$^{*}$ | 0.001 | 12.361$^{*}$ | 13.285$^{*}$ | 25.826$^{*}$ | 0.002 | 1.133$^{*}$ |
| Error | 17 | 3.318 | 0.467 | 0.460 | 10.959 | 0.053 | 0.601 | 0.397 | 10.148 | 0.048 | 0.037 |
| $r^2$ | | 0.997 | 0.999 | 0.999 | 0.895 | 0.999 | 0.999 | 0.999 | 0.903 | 0.999 | 0.999 |

$^{*}$Significant at $p<0.05$ level, $^{**}$Significant at $p<0.10$ level, 95% confidence limit, unmarked terms are not significant, *dF* – degrees of freedom, C-salt concentration (%), T-temperature (°C), t-time (days)

Tabela 4 ANOVA table of microbiological profile during fermentation (sum of squares)

| | | "*Futoški*" cultivar | | | | "*Bravo*" hybrid | | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | LAB | TN | YM | *Enth.* | LAB | TN | YM | *Enth.* |
| t | 1 | 12.823* | 19.705* | 60.785* | 127.867* | 6.707* | 6.946* | 57.554* | 139.228* |
| $t^2$ | 1 | 1.549* | 4.420* | 10.307* | 27.032* | 0.344* | 2.162* | 11.052* | 33.895* |
| T | 1 | 2.297* | 0.743* | 0.935* | 0.235 | 1.240* | 5.416* | 0.531* | 1.351 |
| $T^2$ | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.010 | 0.000 | 0.000 |
| C | 1 | 1.263* | 0.582* | 0.102 | 7.973* | 0.757* | 0.247 | 0.201 | 7.645* |
| $C^2$ | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.010 | 0.000 | 0.000 |
| t × T | 1 | 0.223** | 0.017 | 0.238** | 0.162 | 0.167* | 0.145 | 0.239 | 1.461 |
| t × C | 1 | 0.096 | 2.872* | 0.383* | 1.839** | 0.503* | 7.050* | 0.319** | 1.525 |
| T× C | 1 | 0.003 | 0.000 | 0.000 | 0.043 | 0.002 | 0.016 | 0.001 | 0.095 |
| Error | 26 | 0.840 | 0.740 | 1.998 | 15.699 | 0.537 | 5.269 | 2.296 | 15.965 |
| $r^2$ | | 0.941 | 0.966 | 0.964 | 0.883 | 0.923 | 0.736 | 0.957 | 0.899 |

*Significant at $p<0.05$ level, **Significant at $p<0.10$ level, 95% confidence limit, unmarked terms are not significant, *dF* – degrees of freedom, C-salt concentration (%), T-temperature (°C), t-time (days) traditional whole cabbage heads fermentation comparison between „*Futoški*“ and „*Bravo*“

analysis of biogenic amines and polyamines content and microbiological profile

optimum processing conditions determination by Response Surface Method