CHANGES IN ANTIOXIDANT ACTIVITY AND CHEMICAL COMPOSITION OF APPLE FILLLINGS AFTER THERMAL PROCESSING AND 6-MONTH STORAGE

OČUVANJE AKTIVNOSTI ANTIOKSIDANATA VOĆNIH PUNILA NAKON 6 MESECI SKLADIŠTENJA

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ABSTRACT

The goal of the study was to determine the effect of thermal treatment and 6-months storage on antioxidant activity and chemical composition of heat-stable apple fillings. The aim was fully accomplished by analyzing apple fillings prepared in laboratory conditions under atmospheric pressure from aspetic apple puree (45 %) in a wide range of soluble solids and with different amounts of inulin and pectin according to the planned 2² factorial design with added central point. A correlation between antioxidant capacity evaluated by 2,2-diphenyl-1-picrylhydrazil radical (DPPH•) assay and other chemical parameters of apple fillings such as water activity, pH, total polyphenols, 5-hydroxymethylfurfural content and non-enzymatic browning index were reported in the study. Antioxidant activity and the total polyphenols were directly correlated with each other (p<0.05) and manifested a decrease with increasing the total soluble solids content of apple fillings.

Key words: antioxidant activity, apple filling, polyphenols, non-enzymatic browning.

REZIME

Cilj istraživanja bio je da se utvrdi uticaj termičkog tretmana i 6 meseci skladištenja na antioksidativne aktivnosti i hemijski sastav toplotno-stabilnih punjenja od jabuke. Cilj je u potpunosti ostvaren analizom punjenja od jabuke pripremljenog u laboratorijskim uslovima od jabukovog pirea (45%) pod atmosferskim pritiskom u širokom spektru rastvorljivih materija i sa različitim količinama inulina i pektina prema planiranom 2² faktorijalnom dizajnu sa dodatom centralnom tačkom. Korelacija između antioksidantnog kapaciteta procjenjenog pomoću 2,2 - difenil – 1 - pikrilhidrazil radikalno g (DPPH

INTRODUCTION

Apples present an abundant source of biologically active compounds, mainly polyphenols, which significantly contribute to the reduction of oxidative stress, by acting as strong antioxidants in the human diet. Raw apples have been demonstrated to suppress proliferation of cancer cell, reduce lipid oxidation, and decrease liver cholesterol and triglycerides (Eberhardt et al., 2000). Nevertheless, apples are not just consumed fresh; a big part of the global crop of produced apples (Oszmiański et al., 2008). The fruit -based raw material and ingredients used in the study were as follows: homogenized apple puree (14 °Bx, 6:41.13:631.563, 1821-4487 (2015) 19; 3; p 127-131 Original Scientific Paper
“Orhei-Vit”, Republic of Moldova), white sugar (moisture content 5.0 %, sucrose content 99.85 % d.m.), JV “Südzucker Moldova”, Republic of Moldova), long-chain inulin Orafti HP (moisture content 5.0 %, Beneo, Belgium), low-methoxyl pectin GRINDSTED SF 580 (moisture content 12 %, Danisco, Denmark) and citric acid (“EcoChimie” LTD, Chisinau, Republic of Moldova).

Preparation of apple fillings

Preparation of apple fillings was conducted in laboratory conditions under atmospheric pressure from apple puree, sugar, inulin, pectin and citric acid. The same amount of apple puree (45 % w/w) and citric acid (0.3 % w/w) was used for making the fillings. According to the planned experiment, reported in Table 1, in the filling formulations the content of stabilizing agents (pectin and inulin) and sugars were varied. The levels selected for each of the three independent variables from the experimental design were chosen on the base of preliminary experiments.

Table 1. Experimental design

<table>
<thead>
<tr>
<th>No</th>
<th>X₁ (inulin content) (%)</th>
<th>X₂ (pectin content) (%)</th>
<th>X₃ (total soluble solids (°Brix))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>4</td>
<td>+1</td>
</tr>
<tr>
<td>2</td>
<td>+1</td>
<td>8</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td>+1</td>
<td>8</td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>-1</td>
<td>4</td>
<td>-1</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>4</td>
<td>+1</td>
</tr>
<tr>
<td>7</td>
<td>+1</td>
<td>8</td>
<td>-1</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>+1</td>
<td>8</td>
<td>+1</td>
</tr>
<tr>
<td>10</td>
<td>-1</td>
<td>4</td>
<td>-1</td>
</tr>
</tbody>
</table>

Firstly, sugar added to apple puree, was stirred and boiled up to complete dissolution. Inulin and pectin were dissolved in hot distilled water under continuous mixing and then simultaneously introduced into the previously prepared apple filling mix. After homogenization under intense blending, the prepared solution of citric acid (50 % w/w) was added in amount of 0.6 %. The filling composition was boiled to reach the required °Brix according to the planned experiment (Table 1). The processing temperature was 89 °C for all fillings samples. Thermal treatment time was observed to rise from 10 to 20 minutes consequently, following the increase of the total soluble solids content of the fillings from 30 to 70 °Brix. The prepared fillings were put immediately into glass jars, sterilized and stored at room temperature (20 ± 2 °C) for 6 months before analysis. The further chemical assays were carried out randomly in three replicates.

Chemical analyses

Water activity (aₛ) value of the fillings was determined at 25 °C using a Acqualab (Decagon Devices Inc., Pullman, USA) and pH was measured by pH-meter (CRISON GLP 21), (AOAC 981.12-1982).

Estimation of the DPPH radical-scavenging activity of apple fillings was conducted by using a stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) according to Sánchez-Moreno et al. (2003) with some modifications. The results were expressed in μM trolox per 100 g dry weight.

HMF content was determined spectrophotometrically at 550 nm with a Shimadzu UV-VIS 1601 model double beam spectrophotometer, according to the procedure described by the ISO 7466 (1986) based on the colorimetric reaction between barbituric acid, p-toluidine and HMF, forming a red-colored complex.

The determination of the total polyphenol index (TPI₂₈₀) in apple fillings was conducted according to Somers et al. (1995) with some minor modifications.

The non-enzymatic browning index (NEBI) of apple fillings was determined according to the method described by Meydav et al. (1977) with some modifications measuring absorbance at 420 nm using UV-Vis spectrophotometer (Shimadzu UV-VIS 1601, Kyoto, Japan).

All experiments of the study described above were performed in triplicate.

Experimental design and statistical analysis

In order to analyze the common influence and interactions of the total soluble solids and amount of added stabilizers (inulin and pectin), a 2³ factorial design with two added central points was applied.

Statistical significance of the analyzed data was verified by using Student's t-test and the Analysis of Variance (ANOVA), followed by the Duncan test, to compare the means that showed significant variation (p<0.05). The regression models describing the common effect of the selected independent factors on analyzed response variables were derived using Statgraphics Centurion XVI software, version 16.1.15. The coefficients of determinations as well as R² values of all parameters displayed a good fit of the regression models derived in the study with the experimental data at the 95 % confidence level. In order to visualize the common effects of all factors having the main influence on the response variables, 3D surface plots were drawn by using MATHCAD v15.

RESULTS AND DISCUSSION

Table 2 shows the mean values and standard deviations of aₛ, pH, TPI, AC, 5-HMF content, and NEBI index of apple fillings prepared according to the planned experimental design.

Results of aₛ varied from 0.75±0.02 to 0.98±0.02 and decreased accordingly with increasing the total soluble solids of the apple fillings from 30 to 70 °Brix and the amount of added stabilizers (from 4 to 8 % for inulin and from 0.5 to 1.1 % for pectin), as expected. The 9th sample, prepared with the highest content of inulin, pectin and total soluble solids, had the lowest aₛ value.

The pH of the apple fillings ranged insignificantly; however there was observed an increase of pH values from 3.14±0.01 to 3.75±0.01 with growing the total soluble solids content. This phenomenon may be explained by the fact that all apple fillings were prepared with the same amount of citric acid and apple puree to exclude the “noise factor” in homogeneous formulations, while the amount of sugar added to the fillings varied as a function of the total soluble solids content according to the planned experiment.
Table 2. Average values and standard deviation (n=3) of chemical parameters of apple fillings

<table>
<thead>
<tr>
<th>No</th>
<th>a_w</th>
<th>pH</th>
<th>TPI_{280} (mg kg⁻¹ equiv. gallic acid)</th>
<th>AC (mg trolox eq g⁻¹ dry weight)</th>
<th>HMFₐ (mg kg⁻¹)</th>
<th>NEBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.98±0.02</td>
<td>3.15±0.01**</td>
<td>463.18±11.73**</td>
<td>1.26±0.01**</td>
<td>4.30±0.34</td>
<td>0.51±0.02**</td>
</tr>
<tr>
<td>2</td>
<td>0.95±0.03</td>
<td>3.20±0.02**</td>
<td>434.57±21.15</td>
<td>1.04±0.01**</td>
<td>3.87±0.27**</td>
<td>0.43±0.01**</td>
</tr>
<tr>
<td>3</td>
<td>0.97±0.02</td>
<td>3.14±0.01**</td>
<td>470.17±47.14**</td>
<td>1.28±0.01**</td>
<td>4.39±0.14</td>
<td>0.14±0.01**</td>
</tr>
<tr>
<td>4</td>
<td>0.93±0.01</td>
<td>3.65±0.02</td>
<td>418.52±17.51</td>
<td>0.86±0.01</td>
<td>4.41±0.15</td>
<td>1.05±0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.96±0.01</td>
<td>3.20±0.03**</td>
<td>441.12±32.57</td>
<td>1.04±0.01**</td>
<td>3.80±0.32**</td>
<td>0.13±0.01**</td>
</tr>
<tr>
<td>6</td>
<td>0.83±0.02</td>
<td>3.64±0.02</td>
<td>403.38±16.41</td>
<td>0.73±0.02**</td>
<td>3.61±0.46**</td>
<td>1.21±0.02**</td>
</tr>
<tr>
<td>7</td>
<td>0.81±0.03</td>
<td>3.65±0.01</td>
<td>398.87±64.25**</td>
<td>0.65±0.02**</td>
<td>5.31±0.22**</td>
<td>1.01±0.01**</td>
</tr>
<tr>
<td>8</td>
<td>0.93±0.01</td>
<td>3.65±0.02</td>
<td>420.23±14.22**</td>
<td>0.86±0.01</td>
<td>4.39±0.09</td>
<td>1.05±0.01</td>
</tr>
<tr>
<td>9</td>
<td>0.75±0.02**</td>
<td>3.75±0.01</td>
<td>402.24±27.12</td>
<td>0.64±0.02**</td>
<td>3.88±0.82**</td>
<td>1.35±0.03**</td>
</tr>
<tr>
<td>10</td>
<td>0.80±0.01**</td>
<td>3.55±0.03</td>
<td>377.11±41.27**</td>
<td>0.61±0.03**</td>
<td>4.39±0.46</td>
<td>1.42±0.02**</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± standard deviation (p<0.05): a_w – water activity, pH – pH value, TPI_{280} – the total polyphenol index, AC – antioxidant capacity of apple fillings, HMF – 5-hydroxymethylfurfural content in apple fillings.

** indicates data statistically different (p<0.05).

The TPI of initial apple puree was 798.18±11.09 mg/kg equivalent gallic acid. The amount of polyphenols was almost reduced twice in apple fillings after thermal processing and 6-months storage (Table 2). Thus, in samples nr. 1 and 3 it reduced by about 41 %, in samples nr. 2, 4, 5 and 8 by 45-48 % and, finally in samples nr. 6, 7, 9 and 10 by 49-53 %. Nevertheless, the values of the TPI varied from 377.11±41.27 to 463.18±11.73 mg/kg dry weight for all apple filling samples. In order to evaluate the individual influence of each independent factor on apple fillings TPI during thermal treatment and storage, the following regression equation in terms of actual values (Eq. 1) was derived from the experimental design (p<0.05):

\[
TPI_{280} = 457.318 + 1.316 \cdot I + 36.375 \cdot P - 1.422 \cdot TSS
\]

where \( I \) is inulin content (%), \( P \) is pectin content (%) and \( TSS \) is the total soluble solids content (%Bx).

F-test for analysis of variance indicated that the derived model was statistically significant and displayed a linear behavior with a slope close to 1 (0.9282 and \( R^2>0.95 \)).

The most positive impact on the TPI in apple fillings, after thermal processing and 6-months storage, is principally attributed to pectin. This may be due to a specific ability of certain carbohydrates, such as pectins, to behave as polyphenol protectors, while establishing cooperative hydrogen bonding between the oxygen atom of the carbohydrate and the phenolic hydroxyl group as well as by hydrophobic interactions (Vernhet et al., 1996). Based on the positive regression coefficient of inulin (+1.316) in Eq. 1, it is possible to make an assumption that this carbohydrate also possess polyphenol-protecting capacity, however much lower than one of pectin. Afterwards, the TSS content negatively influences the TPI of apple fillings after thermal processing and storage, in fact its regression coefficient has a negative value (-1.422) in the derived regression equation (Eq. 1). According to our hypothesis, as the TSS content increases, the breakdown of phenolic compounds, due to their oxidation into o-quinones and further polymerization into complex dark-colored pigments (Nicolas et al., 1995; Oszmiański et al., 2008), is intensified. The figure 1 (a, b) shows the overall influence of inulin, pectin and total soluble solids on the total polyphenol index of apple fillings. The highest level of total antioxidant activity (Table 2) was observed for the 3-rd apple filling sample, which also had the highest total polyphenol index. Generally, the results of antioxidant capacity were in reasonable agreement with the total polyphenol content for all apple filling samples and displayed a positive linear relationship (Fig. 2) with a slope of 0.99; \( R^2>0.99 \) and p<0.05.

The correlation between TPI and AC in apple-derived products has been reported in many studies (Loots, van der Westhuizen, & Jerling, 2006; Tsao et al., 2005).

However, according to our assumption, the antioxidant capacity of apple fillings was not only related to the total polyphenol content, but also to the 5-HMF formation, occurring during thermal processing.
The formation of HMF in apple fillings as a primary intermediate of the Maillard reaction is due to the chemical reaction between amino acids, derived from apple puree and sugars, with a reactive carbonyl group (Cohen et al., 1998); therefore, high-sugar apple fillings (70 °Bx) contained a higher amount of HMF than the other ones prepared with less amount of sugar (Table 2). Moreover, it can be mentioned that with the increase of total soluble solids content, the NEBI of apple fillings proportionally increased, ranging from 0.13±0.01 to 1.42±0.02 according to data from Table 2. As in the case of HMF, the results showed that apple fillings prepared with the highest total soluble solids content had higher NEBI values than other fillings. Moreover, with the decrease in moisture level in the product, the reaction rate may be significantly increased, leading to intensified formation of brown pigments (Porretta, 1992) evidenced by the increase of non-enzymatic browning index. Besides moisture level, this reaction is highly affected by pH, impurities (salts) and sucrose content in the product (Clarke et al., 1997). Thus, apple fillings with lower values of $a_w$ and higher values of pH and soluble solids content had higher values of non-enzymatic browning index (Table 2). Another explanation for formation of browning pigments may refer to a possible caramelization, which represents a group of reactions occurring when carbohydrates are exposed to high temperatures with no amino groups involved (Quintas et al., 2007) and including removal of water from the sugar. The formation of 5-HMF could be also a result of caramelization, through different chemical mechanisms (Antal et al., 1990). Besides, water activity of the product affects a lot the rate of non-enzymatic browning reactions (Acevedo, Schebor, & Buera, 2008).

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