FATTY ACID PROFILE CHANGES IN RICOTTA-FILLED PASTRY DURING STORAGE INVESTIGATED BY A GC/MS-ANOVA

Article Highlights
• Ricotta-filled pastry was packed in a special multilayered packaging material
• Fatty acids profile of Ricotta cheese filled pastry was examined by GC/MS
• Significant changes of most fatty acids were not observed after product storing
• Significant change was observed only for cis,cis-9,12-octadecadienoic (linoleic) acid
• It is appropriate to pack and storage Ricotta-filled pastry for the period of 4 weeks

Abstract
Fatty acid composition of Ricotta cheese filled bakery product was examined using a GC-MS method immediately after production and packaging in the case of a control sample, and after production, packaging under air atmosphere in a seven-layer packaging material consisting of PE/Ad/PA/Ad/PE/Ad/PET, and storing during a four weeks period at room temperature, in the case of the experimental samples. The statistical significance of the fatty acid profile change was examined using ANOVA method. The results of this research showed that there are no significant changes of fatty acids composition and content after defined storing period, with the exception of diunsaturated cis,cis-9,12-octadecadienoic (linoleic) acid, whose average content was reduced by 83.705%. However, a small amount of linoleic acid was converted to cis,trans-9,11-octadecadienoic (conjugated linoleic) acid. Therefore, it could be considered as appropriate to pack and storage Ricotta-filled pastry for the period of four weeks, considering the insignificant changes of fatty acid composition and content.

Keywords: fatty acid, GC-MS, packaging material, Ricotta-filled pastry, storage.
bakery products with cheese filling are subjected to
heat treatment, the result could be a reduction of a
total number of microorganisms and the change in
nutritional and sensory characteristics of the product.

The packaging material and the packaging pro-
cess might play an important role in preserving the
initial product characteristics. Packaging materials
with good barrier properties allow the stable storing
conditions and preserve food products from external
conditions, to a certain extent [5]. Some synthetic-
based multilayer packaging materials possess good
barrier properties towards the oxygen, moisture and
aromatic compounds, and therefore could affect the
preservation of freshness [6,7]. Polyamide(PA)-based
packaging materials possess good barrier properties
towards gasses and aromatic compounds. Thus,
using polyamide with polyethylene (PE) films,
enhanced moisture barrier properties might be
obtained [8]. However, it is necessary to determine
whether the packaging in the air atmosphere affects
potential change of fatty acids in ricotta-filled lamin-
ated pastry. Alam & Goyal pointed out that oxygen
remained inside the packaging could affect the
cheese quality [9]. Considering that, it is assumed
that pastry containing dairy products could undergo a
certain change of fatty acids during the storing pro-
cess. A significant fatty acid change might cause a
certain modification of the nutritional and sensorial
properties of a food product.

Besides microbiological defects and starch retro-
gradation, as the main causes of the pastry prod-
products quality deterioration, it is also necessary to fol-
low the changes of fatty acids contents, especially in
food with high fat concentrations. During the storing
period, the pastry products with increased fat content
are subjected to oxidation, which lowers nutritional
and sensorial values of the products [10,11]. Oxid-
ation and lipolyses are certainly some of undesirable
appearances that might occur during cheese product
storage, whereby the negative effect is the change of
nutritional and sensorial properties of the product [12].

Autooxidation - the reaction of double bonds in
unsaturated fatty acids with the atmospheric oxygen -
could have a detrimental effect on organoleptic and
toxicological changes of the product [11], and could
cause the food spoilage, but may also potentially
threaten the health of consumers [13].

Lately, the impact of various fatty acids on
human health has been increasingly studied. The
cause and effect relationships, between daily intake
of fatty acids and cardiovascular diseases, degener-
ative and inflammatory arthritis, cancer and osteo-
poroses, were determined. Moreover, some fatty
acids are recognized as a trigger of the serum low
density cholesterol (LDL) increase. Changes in fatty
acids composition and content, during the storing
period, are particularly interesting, due to the potential
transformation of double bonds from cis to trans form,
whose daily intake, according to the references,
should be limited to the minimal amount [14]. Milk is a
good source of fat in human nutrition, and especially
saturated fatty acids, because they are present in the
amount of 70% [15]. However, the concentration of
trans fatty acids (TFA) ranges between 3 and 6%. An
excessive intake of TFA could have a negative impact
on human health, but the moderate intake of these fats,
especially milk originated, is considered safe [16].

Several different analytical methods were recog-
nized for determination of lipid oxidation. However,
the standard method for all types of food products has
not been established. Gas chromatography coupled
with mass spectrometry proved to be a good choice to
determine the lipid and carbohydrate content of ama-
ranth flour [17]. Lipid oxidation can also be assessed
by quantitatively measuring the loss of initial sub-
strates. In foods containing fats or oils, unsaturated
fatty acids are the main reactants whose composition
changes significantly during oxidation. Changes in
fatty acid composition provide an indirect measure of
the extent of lipid oxidation.

The aim of this work was to determine whether a
significant change of fatty acid composition and con-
tent would occur in ricotta-filled pastry, packed under
air atmosphere in specially selected packaging mat-
erial, after four weeks of storage at 4 °C (±2 °C).

EXPERIMENTAL

Ricotta-filled pastry production

Two identical dough batches were prepared in
Ekomi pite d.o.o. factory, in Bačka Palanka, Serbia. Every batch consisted of wheat flour (255 g), water
(90 g) and salt (5 g), which is a measure for one
pastry product. The mixtures were kneaded in the
mixer (Kemper SP 30, Germany) for 8 min in the first
gear and 4 min in the second gear. The laminating of
the dough was conducted applying a special machine
(Mateks Makina, Turkey) in order to reach 0.4 mm
dough thickness, followed by the addition of Ricotta
cheese (100 g) and the final shaping into the pipe
form. The pastry was then baked for 40 min at 220 °C
and cooled down for 20 min at 20 °C. Cold pastry
was packed into the seven-layer packaging material, con-
sisted of PE/Ad/PA/Ad/PE/Ad/PET in the air atmo-
sphere by DZQ vacuum packager (DZQ-600/2SB,
China). After the packaging process, a control sample
was taken from the paste created from one batch, while the experimental sample was taken from the paste created from the other batch. The control sample was analyzed immediately after the packaging process (A). The pastry samples were taken both from the edges (A1), and from the middle part of the pastry, in which cheese dominates (A2). Experimental sample was stored at 4 °C (±2 °C), and the analysis was performed after four weeks (B). Likewise, the edges of the pastry were labeled as B1, and the middle part as B2.

Preparation of samples

Five grams of the edge samples (A1, B1) and five grams of the middle parts (A2, B2) were weighed and homogenized in a blender. A sample portion (1.61 g each) was collected from every sample and transferred to centrifugation test tubes. The 3 ml of methylene-chloride was added for extraction of liposoluble substances, and the prepared mixture was firstly homogenized for 1 min in the Vortex mixer. Furthermore, the samples were centrifuged for 10 min at 2000 rpm. One ml of the lipid supernatant from every sample was transferred into vials, and 50 μl of the derivatization reagent (0.2 M TMSH, trimethylsulphonium-hydroxide in methanol, Macherey-Nagel) was added, by which trans-esterification reaction of fatty acids, from triacylglycerol into corresponding evaporative fatty acid methyl esters, was conducted [18-22].

GC-MS Analysis

Analysis of lipid (fatty acid) profile from the edge and the middle parts of the investigated samples was conducted by the application of gas chromatography (GC, Agilent Technologies 7890) coupled with the mass-spectrometric detection device (MS, Agilent Technologies MSD 5975). The standard conditions were applied for the analysis of fatty acid methyl esters by GC-MS system. An electron ionization method with the energy of 70 eV was applied. A DB-5 MS column (60 m×0.25 mm×25 μm) was used and the following temperature program was applied: 50-130 °C, 30 °C·min⁻¹ and 130-280 °C, 15 °C·min⁻¹, whereas at the end of every analysis the temperature was held for 8 min at 280 °C. The temperature of the injector was 250 °C, and the helium flow, as a gas carrier, was 1.1563 ml·min⁻¹. One μl of a solution from every analyzed sample was injected with the split-ratio of 1:50 [23,24].

Data processing

The obtained chromatograms were processed using a MSD ChemStation Data Analysis (Agilent Technologies) program, and the fatty acid peaks, in the form of the corresponding methyl esters, were identified by the examination and comparison of their characteristic fragmentations with the Wiley 275 mass spectra library, using the AMDIS, with a probability-based matching (a match quality of 95% minimum was used as a criterion). Surface areas of detected fatty acid methyl esters were integrated from total ion current (TIC) chromatograms, both automatically and manually in control and experimental pastry samples. Mean values were determined and afterwards compared using ANOVA statistical method.

RESULTS AND DISCUSSION

Figure 1 shows total ion current chromatograms (TIC) of the edge samples of ricotta-filled pastry (A1 and B1, Figure 1A) and the middle parts of the same pastry (A2 and B2, Figure 1B), whereas the pastry samples A1 and A2 were analyzed immediately after the packaging process and samples B1 and B2 after packaging and storing for the defined period of four weeks. By looking at the presented chromatograms a high similarity of eluting peaks could be observed.

Table 1 lists the detected fatty acids, their retention times, relative content with regard to the most abundant hexadecanoic (palmitic) acid (100%), and standard deviations.

It might be concluded that the presence of fatty acids, which originate from Ricotta cheese applied for the pastry production, has been typical for the milk fat, whose composition and content could be influenced by various factors [25]. Numerical values of the peak surface areas were obtained by a more rapid and convenient automatic integration of the detected fatty acid methyl esters. The relative contents of every fatty acid detected compared to the most abundant palmitic acid (abundance 100%) were subjected to statistical calculations. By the application of the analysis of variance (ANOVA), the relations between the variance of intergroup variability were tested for every detected fatty acid in analyzed edge and middle part pastry samples.

The results did not show statistically significant differences in the contents of any detected fatty acid between the samples of the pastry product analyzed after the packaging (A1 and A2) and the pastry product subjected to storing (B1 and B2). Obtained F value did not exceed an F-critical value in any case. Likewise, p value was higher than 0.05 in every case.

An exception was the diunsaturated cis,cis-9,12-octadecadienoic acid (linoleic acid), whose F value (42.8850) exceeded F-critical (9.5520). Moreover, p value (0.0062) was lower than 0.05. That implies the
Figure 1. Total ion current fatty acids chromatogram of the edges (A1 and B1) and the middle part (A2 and B2) of Ricotta-filled pastry after packaging (A) and after packaging and storing (B).

Table 1. Retention times and relative content of fatty acids detected in Ricotta-filled pastry after packaging (A) and after packaging and storing (B).

<table>
<thead>
<tr>
<th>No.</th>
<th>Rt / min</th>
<th>Fatty acid</th>
<th>Index</th>
<th>A1, %</th>
<th>A2, %</th>
<th>SD / %</th>
<th>B1, %</th>
<th>B2, %</th>
<th>SD / %</th>
</tr>
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<td>1</td>
<td>6.88</td>
<td>Octanoic (caprylic acid)</td>
<td>C8:0</td>
<td>2.57</td>
<td>2.87</td>
<td>0.12</td>
<td>2.17</td>
<td>2.34</td>
<td>0.21</td>
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<td>2</td>
<td>8.39</td>
<td>cis-4-decenoic acid</td>
<td>C10:1ω6</td>
<td>0.60</td>
<td>0.64</td>
<td>0.15</td>
<td>0.22</td>
<td>0.43</td>
<td>0.03</td>
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<td>3</td>
<td>8.45</td>
<td>Decanoic acid</td>
<td>C10:0</td>
<td>7.06</td>
<td>7.90</td>
<td>0.17</td>
<td>6.19</td>
<td>6.43</td>
<td>0.59</td>
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<tr>
<td>4</td>
<td>10.05</td>
<td>Dodecanoic (lauric acid)</td>
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<td>9.42</td>
<td>10.37</td>
<td>0.11</td>
<td>8.31</td>
<td>8.46</td>
<td>0.67</td>
</tr>
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<td>0.03</td>
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<td>C14:0</td>
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<td>cis-9-Tetradecenoic acid (myristoleic acid)</td>
<td>C14:1ω5</td>
<td>2.86</td>
<td>3.09</td>
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<td>3.10</td>
<td>2.50</td>
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<td>39.14</td>
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<td>34.36</td>
<td>33.44</td>
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<td>3.76</td>
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<td>11</td>
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<td>C16:1ω7</td>
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<td>C16:0</td>
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<td>0</td>
<td>100.00</td>
<td>100.00</td>
<td>0</td>
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<td>13</td>
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<td>C17:0</td>
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<td>1.41</td>
<td>0.08</td>
<td>1.70</td>
<td>1.58</td>
<td>0.14</td>
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<td>14</td>
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<td>cis-10-Heptadecenoic acid</td>
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<td>1.07</td>
<td>0.07</td>
<td>0.91</td>
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<td>Heptadecanoic acid</td>
<td>C17:0</td>
<td>2.26</td>
<td>2.49</td>
<td>0.22</td>
<td>2.31</td>
<td>2.00</td>
<td>0.16</td>
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<tr>
<td>16</td>
<td>14.22</td>
<td>cis,cis-9,12-Octadecadienoic acid (linoleic acid)</td>
<td>C18:2ω6</td>
<td>46.63</td>
<td>44.61</td>
<td>1.05</td>
<td>9.68</td>
<td>8.19</td>
<td>1.43</td>
</tr>
<tr>
<td>17</td>
<td>14.26</td>
<td>cis-9-Octadecenoic acid (oleic acid)</td>
<td>C18:1ω9</td>
<td>93.18</td>
<td>93.36</td>
<td>3.85</td>
<td>87.75</td>
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<td>11.89</td>
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<td>48.82</td>
<td>0.12</td>
<td>48.08</td>
<td>47.91</td>
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Table 1. Continued

<table>
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<tr>
<th>No.</th>
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<th>Fatty acid</th>
<th>Index</th>
<th>A1, %</th>
<th>A2, %</th>
<th>SD / %</th>
<th>B1, %</th>
<th>B2, %</th>
<th>SD/ %</th>
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</thead>
<tbody>
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<td>21</td>
<td>14.58</td>
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<td>C18:2ω7</td>
<td>2.17</td>
<td>2.35</td>
<td>0.15</td>
<td>2.67</td>
<td>2.46</td>
<td>0.13</td>
</tr>
<tr>
<td>22</td>
<td>15.03</td>
<td>cis-10-Nonadecenoic acid</td>
<td>C19:1ω9</td>
<td>0.29</td>
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<td>23</td>
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<td>Nonadecanoic acid</td>
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<td>0.25</td>
<td>0.01</td>
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<tr>
<td>24</td>
<td>15.87</td>
<td>cis-11-Eicosenoic acid (gondoic acid)</td>
<td>C20:1ω9</td>
<td>0.96</td>
<td>0.94</td>
<td>0.15</td>
<td>0.83</td>
<td>0.62</td>
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<tr>
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<td>16.09</td>
<td>Eicosanoic acid (arachidic acid)</td>
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<td>0.87</td>
<td>0.05</td>
<td>0.60</td>
<td>0.53</td>
<td>0.04</td>
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</table>

significant statistical change in this fatty acid content after the defined storage period.

Due to the aforementioned, it was decided to select each fatty acid containing 18 carbon atoms in the molecule, and eluting time between 14.16 and 14.50 min, as the area of interest (Figure 2). The aim was to perform another method of chromatogram integration - a manual integration, to verify the results obtained using an automatic integration, and make a statistical comparison between contents of the fatty acids that elute between mentioned retention times, obtained from the samples A and B. Indicated fatty acids include: hexadecanoic (palmitic acid), cis,cis-9,12-octadecadienoic (linoleic acid), cis-9-octadecenoic (oleic acid), trans-9-octadecenoic (elaidic acid), trans-15-octadecenoic, octadecanoic (stearic acid) and cis,trans-9,11-octadecadienoic acid (conjugated linoleic acid).

Figure 2 presents a zoomed part of a TIC chromatogram with the focus on the retention time interval of the area of interest, for the samples of the edge (A1 and B1, Fig. 2.A) and the middle (A2 and B2, Fig. 2.B) of Ricotta-filled pastry samples analyzed immediately after production and packaging (A1 and A2) and the pastry samples packed and stored for the defined time period (B1 and B2).

![Fig. 2. Enlarged total ion current chromatogram of fatty acids with focus on the area of interest from the edges (A1 and B1) and the middle part (A2 and B2) of Ricotta-filled pastry after packaging (A) and after packaging and storing (B).](image-url)
The difference in linoleic acid (cis,cis-9,12-octadecadienoic) content between the samples of edge (A1 and B1) and the middle parts (A2 and B2, Figure 2) of the bakery product analyzed immediately after the packaging (A1 and A2) and the same product analyzed after packaging and storing in a defined time period (B1 and B2), could be obviously observed in Fig. 2.

By repeated statistical data analysis of the area of interest, using the ANOVA test, it was concluded that only diunsaturated cis,9,12-octadecadienoic (linoleic) acid suffered statistically significant losses during the storing period, owing to oxidation and degradation ability, due to the presence of two double bonds in the molecule. The content of linoleic acid was decreased by 85.55% in the samples of the edges and by 81.85% in the samples of the middle parts of the pastry (mean value 83.7%). Considering the moderate increase of conjugated linoleic (cis,trans-9,11-octadecadienoic) acid content in the middle parts of the pastry (mean value 83.7%), the increase of the fatty acid content in the trans form was detected. However, conjugated linoleic acid was frequently labeled as beneficial for human health, because it reportedly shows anticarcinogenic, antiatherogenic and antidiabetic effects [16,27]. On the other hand, EFSA reported (2010) that there are no convincing evidences about the beneficial effect of conjugated linoleic acid on human health. In accordance to above mentioned report, there are no recommended daily intakes of conjugated linoleic acid.

CONCLUSION

The storing of Ricotta-filled pastry product packed in a specially selected multilayered packaging material during the defined time period could be significant as appropriate, considering the insignificant changes of fatty acids composition and content. GC-MS analysis proved that the choice of packaging material, storing period and packaging conditions were adequate for the fatty acid preservation in Ricotta-filled pastry. However, it is important to emphasize that fatty acid analysis, performed without microbiological and sensory analysis, shouldn’t have an effect on decision of storing period and the best before date of the product. The composition of initially detected fatty acids remained unchanged after specified time period of storage, except in the case of diunsaturated cis,cis-9,12-octadecadienoic (linoleic) acid. A slight increase of cis,trans-9,11-octadecadienoic acid (conjugated linoleic acid) occurred, due to the oxidation process, but it didn’t essentially affect the nutritional value of the bakery product, due to its low concentration. Additionally, a certain amount of linoleic acid was probably transformed into the products of lower molecular mass. The general good manufacturer practice requires the maintenance of microbiological and sensory properties of the starting food product, without any reference to the potential nutritional changes, such as fatty acid profile changes. Therefore, the necessity of fatty acid analysis due to a food product sustainability measurement, should be considered. GC-MS fatty acid analysis perform along with the microbiological and sensory analysis, should answer the questions whether Ricotta-filled pastry product could be stored for a selected period of time.

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NAUČNI RAD

PROMENE U PROFILU MASNIH KISELINA U PECIVU PUNJENOM RICOTTA SIROM U TOKU SKLADIŠTENJA ISPITANE PRIMENOM GC/MS-ANOVA

Sastav masnih kiselina pekarskog proizvoda sa Ricotta sirom ispitan je metodom GC-MS, odmah nakon proizvodnje i pakovanja u slučaju kontrolnog uzorka, a u slučaju eksperimentalnih uzoraka nakon proizvodnje, pakovanja u atmosferi vazduha u sedmostrukom ambalažnom materijalu koji se sastoji od PE / Ad / PA / Ad / PE / Ad / PET i skladištenja u periodu od četiri nedelje na sobnoj temperaturi. Statistički značajna promena u profilu masnih kiselina ispitana je pomoću ANOVA. Rezultati ovog istraživanja pokazali su da nema značajnih promena u sastavu i sadržaju masnih kiselina i nakon definisanog perioda čuvanja, sa izuzetkom dinezasićene cis,cis-9,12-oktadekadienske (linolne) kiseline, čiji je prosečni sadržaj smanjen za 83,705%. Međutim, mala količina linolne kiseline pretvorena je u cis,trans-9,11-oktadekadiensku (konjugovanu linolnu) kiselinu. Prema tome, pakovanje i skladištenje peciva napunjeno Ricotta sirom u trajanju od četiri nedelje može se smatrati odgovarajućim, s obzirom na beznačajne promene sastava i sadržaja masnih kiselina.

Ključne reči: masne kiseline, GC-MS, ambalažni materijal, punjeno pecivo Ricotta sirom, skladištenje.