VOLATILE COMPOUNDS OF FUNCTIONAL DAIRY PRODUCTS

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Volatile compounds, affecting flavour of traditional and probiotic fresh cheese, were determined. Functional dairy product-fresh cheese was produced from milk of 2.5% fat content and milk of 4.2% fat content, under the semi-industrial conditions. The traditional starter culture Flora Danica (FD) and a combination of probiotic starter ABT-1 and FD (ABT-1:FD=1:1) were applied as starters. The volatile fractions were isolated by employing the combined simultaneous distillation-extraction technique (SDE). The compounds were identified by gas chromatography – mass spectrometry (GC-MS) and quantified by using standard procedure. Following 19 compounds have been identified: 8 hydrocarbons (decane, undecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane and 2, 6, 10, 14-tetramethyl hexadecane); 6 ketones (2-heptanone, 2-nonanone, 2-undecanone, 2-pentadecanone, 2-heptadecanone and 2-tridecanone); 3 aldehydes (nonanal, tetradecanal and hexadecanal); 1 fatty acid (decanoic acid) and disulfide, bis (1-methylethyl). The highest levels were associated with hexadecanal, 2-pentadecanone, 2-tridecanone, and 2-undecanone in all examined samples, regardless to the starter culture and type of milk used.

KEY WORDS: Fresh cheese, volatile flavour components, probiotics.

INTRODUCTION

Functional dairy product is highly valuable products, particularly if it contains probiotics. However, main obstacle to its broader consumption could be lower sensory characteristics, compared to the traditional fresh cheese. Very important factor, which affects flavour, is great number of volatile compounds appearing as a result of an action of enzymes on the milk components: protein, fat, lactose, and citrate. Proteolytic enzymes from lactic acid bacteria cause the degradation of casein and peptides, leading to production of free amino acids that contribute directly to the basic taste of cheese and indirectly to cheese flavour, as the precursors for other catabolic reactions (1, 2, 3). These reactions and side-chain modification may yield keto-acids, ammonia, amines, aldehydes, acids and alcohols, which are essential contributors to cheese taste and aroma. For exam-
ple, bitterness is due to hydrophobic peptides, rancidity to fatty acids, and fruitiness to esters (3).

Volatile fatty acids in fresh cheese are the products of various metabolic pathways, mostly microbial. Their further degradation leads to a generation of very important group of compounds - aldehydes and ketones. Besides β-oxidation of fatty acids, they can be synthesized by direct oxidation of hydrocarbons (4). Panseri et al. (number ) investigating flavour of the Italian mountain cheese- Bitto have detected aldehyde hexadecanal, which is associated with the waxy, floral aroma; 2-pentadecanone, giving delicate musk aroma; 2-undecanone, giving citrus, rose and iris aroma and n-nonanal, giving floral, citrus and green aroma (5). Many authors have found 2-undecanone, 2-heptanone and 2-nonanone as flavour compounds in semi fat and fat cheeses, exposed to a certain period of ripening (6-9). Nogueira et al. (10) identified 2-undecanone, which gives fruity, oral notes to Minas cheese, while Beuvier and Buchin detected 2-heptanone in Emmental, Gruyere and Padano cheese (10, 11).

Important flavour compounds are esters, formed by condensation of an acid and an alcohol either spontaneously or mediated by microbial esterases. Sulphur compounds are particularly present in mould- or smear-surface cheese, and provide typical cabbage or garlic flavours (11-14). Cheeses can contain also a great number of hydrocarbons, which belong to a family of secondary products of lipid antioxidants (15). They do not have a major contribution to aroma in cheese, but may serve as precursors for the formation of other aromatic compounds (16).

Starter culture is an important factor which affects flavour of the final product. When starters are new systems, as probiotics, very little knowledge about their aroma impact exists. Recently, an investigation was performed by Kourkoutas et al. (17). The investigators immobilized *Lb. casei* cells on fruit (apple and pear) peaces and used them as adjunct culture in probiotic cheese making. Sensory evaluation revealed the fruity taste of the obtained probiotic cheese. It was found that the commercial Feta cheese has a more sour taste, whereas no significant differences concerning two cheeses flavour were reported. A significant content of n-hexadecanoic acid was found in both products. Other investigation dealt with the use of freeze-dried kefir coculture as a starter in the production of Feta-type cheese (18). The main active microbial associations were members of the genera *Pseudomonas* and *Lactococcus*. The effect of the starter culture on the production of aroma-related compounds was studied. Among 18 carbonyl compounds, 2-undecanone and hexadecanone were identified. Broadbent et al. (19) made the reduced-fat Cheddar cheeses with *Lc. lactis* starter only, starter plus *Lb. casei* ATCC 334, and starter plus *Lb. casei* ATCC 334 transformed with pTRKH2: *dhic* (19). They found that the culture system used significantly affected the concentrations of various ketones, aldehydes, alcohols and esters, after 3 months of ripening. Among other volatile compounds, hexadecanal ranging 0.22-0.52 µg/g as well as 2-undecanone varying 0.01-0.03 µg/g were quantified.

Very limited data on fresh cheese are available when probiotics as the starters are applied. The aim of this study was to investigate the volatile compounds in functional dairy products-probiotic fresh cheese produced with combination of probiotics and traditional cultures from partially skimmed and full-fat milk.
MATERIAL AND EXPERIMENTAL

Starter Culture

Traditional culture Flora Danica - FD (Lc. lactis subsp. lactis, Lc. lactis subsp. cremoris, Ln. mesenteroides subsp. cremoris, Lc. lactis subsp. lactis biovar diacetylactis) was applied for production of control samples, while the 1:1 combination of FD and probiotic starter culture ABT-1 (Lb. acidophilus-5, Bifidobacterium-12, Str. thermophilus) was used for production of probiotic cheese. Both starters are commercially available (Chr. Hansen, Denmark).

Cheese manufacture

Two series of functional dairy products - fresh cheese samples with different fat content were manufactured under semi-industrial conditions. Milk was pasteurized at 71°C, during 15s, and cooled to 28°C.

Starter culture (0.01%) and 0.005% enzyme for coagulation were added into milk at 28°C. Coagulation lasted 18 hours (pH= 4.6). After that, coagulum was cut, pasteurized by gently stirring at 60°C (5 min) and quickly cooled and drained. Cheese samples were homogenized by mixing and packed in cups of 1.8 dl volume.

Series I was produced from milk of 2.5% fat; it consisted of the traditional fresh cheese - I.FD and probiotic fresh cheese - I.ABT-1: FD=1:1. Series II was produced from milk of 4.2% fat; it consisted of the traditional fresh cheese - II.FD and probiotic fresh cheese - II.ABT-1: FD=1:1.

Chemical analyses

Milk composition and quality of fresh cheese samples were analysed by standard methods. Dry matter content was measured in milk (20) and cheese by drying at 105°C (21). Fat content in milk and cheese (F) were analysed according to Gerber (22) and Van Gulik (23), respectively. Total nitrogen content (TN) was determined according to Kjeldahl method (24), while total proteins (TP) were calculated by multiplying TN by 6.38%. Ash content was determined by ignition at 550°C (AOAC, 2000), while pH value was measured by pH-meter Iskra, MA 5713, Kranj, Slovenia.

All results are based on 3 to 5 measurements of each parameter.

Analyses of volatile flavour compounds

The volatile flavour components were isolated from fresh cheese samples employing the combined simultaneous distillation-extraction procedure widely used in cheese analysis (25).

10 g of cheese sample was poured into the distillation vessel with 40 mL distilled water. Distilled water (1.5 mL) and 1 mL n-pentane were mixed in the extractor. Extraction was performed for 2 hours. The obtained extract was evaporated and reconstituted to 100 µL with ethyl-acetate. The concentrated extracts were analysed by Agilent Technologies.
G1777A Gas Chromatograph, equipped with flame ionization detector and capillary column DB-5 30m x 0.25mm. The oven temperature was programmed: 50°C, 1 min; from 50 to 100°C at 5°C/min, from 100 to 200°C at 9°C/min, for 2.9 min. The carrier gas was nitrogen (2mL/min). The injected volume was 3μL. The injector and detector temperature was set at 250°C. The volatile components were identified by gas chromatography - mass spectrometry (GC-MS).

RESULTS AND DISCUSSION

Chemical composition of functional dairy products-fresh cheese samples is presented in Figure 1.

![Figure 1. Composition of fresh cheese samples](image)

This analysis shows that some differences between cheeses produced by traditional starter culture application: I.FD and II.FD and starter combination: I. ABT-1:FD=1:1 and II. ABT-1:FD=1:1 exist. Probiotic cheese I.ABT-1:FD=1:1 produced in series I, from milk with 2.5% fat, contains less dry matter than the traditional cheese. On the contrary, probiotic cheese produced from milk with 4.2% fat in series II, has greater content of dry matter. In cheeses manufactured from partially skimmed milk content of proteins is greater than content of fat, while in the cheeses produced from full-fat milk content of fat overcomes the content of proteins. Nutritive value of obtained cheeses is high and all cheeses can be suggested as valuable food under the condition that their sensory characteristics are acceptable.

Due to analyses of aromatic fractions of the manufactured traditional and probiotic cheeses, the SDE-procedure was applied.

Figure 2 and 3 shows the obtained capillary gas chromatogram of the SDE volatile fraction of cheeses produced from partially skimmed milk in series I and from full-fat milk in series II, respectively.
Figure 2. Chromatogram of the SDE fraction of cheese from milk of 2.5% fat: a) traditional fresh cheese; b) fresh cheese produced by application of starter culture combination ABT-1:FD=1:1

Figure 3. Chromatogram of the SDE fraction of cheese from milk of 4.2% fat: a) traditional fresh cheese; b) fresh cheese produced by application of starter culture combination ABT-1:FD=1:1

In the traditional fresh I.FD cheese manufactured from skimmed milk, 77 compounds were identified by GC-MS; 79 compounds were identified in the probiotic I.ABT-1 : FD = 1 : 1 cheese, produced from the same milk. Quantitively, 18 compounds were determined by internal standard procedure in both cases (Table 1).

In the traditional fresh II.FD cheese produced from full-fat milk, 71 compounds were detected by GC-MS; 58 compounds were detected in the probiotic II.ABT-1:FD=1:1 cheese, manufactured from the same milk. Quantitively, 19 compounds were determined by internal standard procedure (Table 1) in traditional cheese and 15 compounds of the probiotic cheese.

The obtained values of the quantified compounds are shown as the relative concentrations (%). Following 19 compounds have been identified: 8 hydrocarbons (decane, n-undecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane and 2,6,10,14-tetramethyl hexadecane); 6 ketones (2-heptanone, 2-nonanone, 2-undecanone, 2-pentadecanone, 2-heptadecanone and 2-tridecanone); 3 aldehydes (nonanal, tetradecanal and hexadecanal); 1 fatty acid (decanoic acid) and disulfide, bis (1-methylethyl).
The main compounds are hexadecanal, 2-pentadecanone, 2-tridecanone and 2-undecanone, present in all examined samples (Table 1).

**Table 1.** The quantified volatile components detected in SDE fraction of functional dairy samples

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>SERIES I</th>
<th></th>
<th></th>
<th>SERIES II</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.FD</td>
<td>LABT-1:FD=1:1</td>
<td>II.FD</td>
<td>ILFD</td>
<td>II.ABT-1:FD=1:1</td>
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<tr>
<td></td>
<td>RTa (min)</td>
<td>RCb (%)</td>
<td>RTa (min)</td>
<td>RCb (%)</td>
<td>RTa (min)</td>
<td>RCb (%)</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>5.61</td>
<td>0.12</td>
<td>5.61</td>
<td>0.17</td>
<td>5.62</td>
<td>0.21</td>
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<tr>
<td>Decane</td>
<td>8.50</td>
<td>0.03</td>
<td>8.50</td>
<td>0.06</td>
<td>8.51</td>
<td>0.07</td>
</tr>
<tr>
<td>Disulfide, bis (1-methylethyl)</td>
<td>9.08</td>
<td>0.04</td>
<td>9.08</td>
<td>0.06</td>
<td>9.08</td>
<td>0.08</td>
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<td>2-Nonanone</td>
<td>11.1</td>
<td>0.52</td>
<td>11.1</td>
<td>0.43</td>
<td>11.1</td>
<td>0.47</td>
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<td>Undecane</td>
<td>11.38</td>
<td>0.05</td>
<td>11.37</td>
<td>0.07</td>
<td>11.38</td>
<td>0.08</td>
</tr>
<tr>
<td>Nonanal</td>
<td>11.51</td>
<td>0.03</td>
<td>11.5</td>
<td>0.04</td>
<td>11.51</td>
<td>0.11</td>
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<td>2-Undecanone</td>
<td>15.66</td>
<td>0.81</td>
<td>15.65</td>
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<td>0.4</td>
<td>15.8</td>
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<td>15.8</td>
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<td>Decanoic acid</td>
<td>16.88</td>
<td>0.21</td>
<td>16.86</td>
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<tr>
<td>Tetradecane</td>
<td>-</td>
<td>-</td>
<td>17.49</td>
<td>0.08</td>
<td>17.49</td>
<td>0.09</td>
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<tr>
<td>2-Tridecanone</td>
<td>18.98</td>
<td>1.48</td>
<td>18.95</td>
<td>1.35</td>
<td>18.94</td>
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<tr>
<td>Pentadecane</td>
<td>19.02</td>
<td>0.05</td>
<td>19.02</td>
<td>0.06</td>
<td>19.02</td>
<td>0.08</td>
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<tr>
<td>Hexadecane</td>
<td>20.41</td>
<td>0.27</td>
<td>20.42</td>
<td>0.1</td>
<td>20.41</td>
<td>0.09</td>
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<tr>
<td>Tetradecanal</td>
<td>20.6</td>
<td>0.61</td>
<td>20.6</td>
<td>0.4</td>
<td>20.6</td>
<td>0.29</td>
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<tr>
<td>2-Pentadecane-none</td>
<td>21.7</td>
<td>1.59</td>
<td>21.7</td>
<td>1.36</td>
<td>21.7</td>
<td>1.44</td>
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<tr>
<td>Octadecane</td>
<td>23.02</td>
<td>0.59</td>
<td>23.03</td>
<td>0.7</td>
<td>28.02</td>
<td>0.4</td>
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<tr>
<td>2, 6, 10, 14-Tetra-methyl hexadecane</td>
<td>23.12</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>23.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Hexadecanal</td>
<td>23.29</td>
<td>7.41</td>
<td>23.28</td>
<td>2.9</td>
<td>23.55</td>
<td>3.7</td>
</tr>
<tr>
<td>2-Heptadecane-none</td>
<td>24.66</td>
<td>0.34</td>
<td>24.66</td>
<td>0.22</td>
<td>24.66</td>
<td>0.36</td>
</tr>
</tbody>
</table>

\[^a{\text{RT}} – \text{Retention time}; \quad ^b{\text{RC}} – \text{Relative concentration}\]

The highest level of hexadecanal (7.4%) was found in the traditional I.FD cheese. Also, very high level (3.7%) was detected in the traditional II.FD cheese. The probiotic cheeses contain lower levels of hexadecanal; probiotic I.ABT-1:FD=1:1 cheese 2.9% and probiotic II.ABT-1:FD=1:1 cheese 2.1%. Second important volatile compound is 2-pentadecanone. Its level is the highest (2.2%) in the probiotic II.ABT-1: FD=1:1 cheese, which is followed by the traditional I.FD cheese (1.5%) and the other traditional II.FD cheese (1.44%). The lowest level was found in the probiotic I.ABT-1: FD=1:1 cheese (1.36%). Very similar results were obtained in the case of the third compound present in all samples, 2-tridecanone. The fourth volatile compound, present in all samples, was 2-undecanone. It reached 0.02% in the probiotic II.ABT-1:FD=1:1 cheese, 0.81% in the traditional I.FD cheese, 0.75% in the probiotic I.ABT-1:FD=1:1 cheese and 0.63% in the traditional II.FD cheese.

Based on the analysis of values presented in Table 1, it can be concluded that probiotic cheeses in both series contained less quantity of the main volatile compound (hexadecanal) than the traditional cheeses. Some authors (5) associated the presence of hexadecanal with the waxy, floral aroma of cheese. The remaining three important compounds (2-pentadecanone, 2-tridecanone and 2-undecanone) were present in probiotic cheeses pro-
duced in series I and series II even at a higher level than in the traditional cheeses, especially when full-fat milk was used (Table 1). Presence of 2-pentadecanone can be related to the delicate musk aroma of cheese (5). Many authors have found 2-undecanone as a compound giving citrus, rose and iris aroma flavour to semi fat and fat cheeses (5-10). Unfortunately, there are not literature date about aroma of fresh cheeses particularly cheeses produced with probiotics application.

Volatile compounds, present in smaller quantities in investigated cheeses, are: tetradecanal, octadecanal, 2-nonanone, tridecane, 2-heptadecanone, hexadecane and decanoic fatty acid. These compounds were detected in all samples, except 2-heptadecanone in probiotic cheese II.ABT-1:FD=1:1. Their quantity varied from 0.7% to 0.1%. They were identified by other authors, but in semi-hard and hard cheeses. So, 2-heptanone and 2-nonanone were identified by Moio et al. (1998), Fernandez-Garcia et al. (2002) and Lanciotti et al. (2006) (6,8,9). Panseri et al. (5) associated presence of n-nonanal with floral, citrus and green flavour of Italian Bitto cheese (5).

Finally, a great number of n-alkanes, such as decane, undecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane etc., were present in all fresh cheese samples. These compounds also were found as volatile flavour components of buffalo Mozzarella cheese in a study of Moio et al. (26). Although the hydrocarbons do not have a major contribution to aroma in cheese, they may serve as precursors for the formation of other aromatic compounds (15).

CONCLUSION

On the basis of the comparative study carried out it can be concluded that there is an acceptable level of similarity between aroma of traditional fresh cheese and probiotic fresh cheese. Consequently, the functional dairy product-probiotic cheeses manufactured from milk of 2.5% fat content and full-fat milk (4.2% fat) by applying the combination of the traditional and probiotic starter culture in ratio 1:1, possess good characteristics, regarding to their taste and flavour.

Acknowledgements

The financial support from the Ministry of Education and Science (Project No. 46009) of Serbia is gratefully acknowledged. The authors thank also MTC, Sombor, Serbia for supply of probiotic starters Chr. Hansen, Denmark and AD Imlek, Division Novi Sad Dairy, Serbia for cooperation in experiments in their plant.

REFERENCES


ИСПАРЉИВЕ КОМПОНЕНТЕ АРОМЕ ФУНКЦИОНАЛНОГ МЛЕЧНОГ ПРОИЗВОДА

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Испарљиве компоненте аrome су одређене у традиционалном и пробиотском свежем сиру-функционалном млечном производу. Свежи сир произведен је из млека са 2,5% и 4,2% млечне масти уз коришћење традиционалне стартер културе Flora Danica (FD) и комбинације пробиотске стартер културе АВТ-1 и традиционалне културе FD у односу 1:1. Фракције испарљивих компонената аrome сира изоловане су применом симултанске дестилације и екстракције (СДЕ). Компоненте аrome сира идентификоване су применом гасне хроматографије и масене спектрометрије (GC-MS) и квантификоване коришћењем стандардне процедуре. Идентификовано је 19 компонената: 8 угљоводоника (декан, н-ундекан, тридекан, тетрадекан, пентадекан, хексадекан, октадекан и 2,6,10,14-тетраметил хексадекан); 6 кетона (2-хептанон, 2-нонанон, 2-ундеканон, 2-пентадеканон, 2-хептадеканон и 2-тридеканон); 3 алдехида (нонанал, тетрадеканал и хексадеканал); 1 масна киселина (деценска киселина) и дисулфид, бис (1-метилетил). У свим узорцима сира најзаступљенији су хексадеканал, 2-пентадеканон, 2-тридеканон и 2-ундеканон.

Кључне речи: Свеж сир, испарљиве компоненте аrome, пробиотици

Received: 26 June 2012
Accepted: 10 September 2012