Improving the Processed Cheese Quality by the Addition of Natural Spice Extracts

K. Krumov, G. Ivanov, A. Slavchev and N. Nenov

1Department of Technology of Milk and Dairy Products, 
2Department of Food Preservation and Refrigerated Technology, 
3Department of Organic Chemistry and Microbiology, Technological Faculty, 
University of Food Technologies, Plovdiv, Bulgaria
4Extractum Ltd., Plovdiv, Bulgaria

Abstract: The aim of the present study was to establish the possibilities for improving the processed cheese quality by the addition of natural spice extracts. Four batches of processed cheese were produced by using of Piper nigrum L., Satureja hortensis L. and their natural extracts. Control and test samples were stored at 5±1°C for 10 days. Each batch of processed cheese was analyzed for dry matter, pH, titratable acidity, fat and salt content. Microbiological analysis of spices, spice extracts and processed cheese samples was performed. Sensory evaluation of all batches was also performed. A similarity in the composition of control and test samples was established. Total aerobic colony count of test samples was approximately 10 times lower than in the controls. Processed cheeses produced by using of spice extract had higher sensory evaluation coefficients than the controls. It was concluded that the replacement of Piper nigrum L. and Satureja hortensis L. spices with their extracts improved significantly the processed cheese microbiological and sensory quality.

Key words: Microbial contamination, processed cheese, sensory quality, shelf-life, spice extracts

INTRODUCTION

Processed cheese is a food product made by heating a mixture of cheese, water, emulsifying salts and further optional ingredients such as butter or spices. Production of processed cheese and factors influencing its characteristics have been described in many publications (Caric and Kalab, 1997; Guinee, 2004). According to Chambre and Daurelles (1997), processed cheese products usually retain their good quality for up to 6-12 months at room temperature. Processed cheese is not a preserved food, but a ‘semi-preserved food’ with a limited shelf-life (Berger et al., 1989).

Most of the problems of the shelf-life and storage of processed cheese were associated with problems caused by microbial contamination. Microbiological hazards during processed cheese production can be eliminated by UHT processing: even temperature-resistant spores such as Clostridium butyricum, Clostridium tyrobutyricum, Clostridium sporogenes can be destroyed (Schar and Bosset, 2002). Post-sterilization infection could be prevented by hot filling (85-95±1°C) into the packing (Sturm, 1998).

The spices added are the main source of contamination during manufacture of processed cheese. In the available literature there were not reports for application of spice extracts for decreasing microbial contamination of processed cheese. In some cases milk ingredients used for processed cheese mixture formulation could also be a source of microbial contamination (Bhowmick et al., 2006; Kumbhar et al., 2009). Usually these microorganisms did not survive melting process.

The aim of the present study was to establish the possibilities for improving the processed cheese quality by the addition of natural spice extracts.

MATERIALS AND METHODS

Processed cheese making: The experiments were performed during 2010 in the laboratories of University of Food Technology, Plovdiv, Bulgaria. Processed cheese samples were manufactured from blends of Kashkaval cheese, curd, butter, water and emulsifying salt (sodium citrates, sodium orthophosphates or sodium polyphosphates). The mixture was heated in a batch cooker with constant agitation, until a homogeneous mass is obtained. The melting temperature of processed cheese was 85±1°C and the total melting time was 15 min from the beginning of heating to the beginning of discharge. The spices and spice extracts were added to the mixture after 5 and 10 min of heating, respectively. Portions of 100 g melted cheese were poured into PET cups with sealable lids. The filled cups were divided into four parts...
according to the spices or spice extracts added: processed cheese with *Satureja hortensis* L. - control sample 1 (C1), processed cheese with *Piper nigrum* L. - control sample 2 (C2), processed cheese with *Satureja hortensis* L. extract - test sample 1 (T1) and processed cheese with *Piper nigrum* L. extract - test sample 2 (T2). Thereafter, these samples were cooled down to the temperature of 10±1°C and were stored at 5±1°C for 10 days.

**Spices and spice extracts:** For flavoring of processed cheese the following spices and extracts were used:

- Ground black pepper fruits (*Piper nigrum* L.), country of origin Vietnam, crop 2009, bulk density 600 g/L
- Dried savory herb (*Satureja hortensis* L.), country of origin Bulgaria, crop 2009

Standardized extracts *Botex*™ Black pepper and *Botex*™ Savory produced by Extractum Ltd., Bulgaria. The extracts were derived on semi-industrial scale extraction unit using sub-critical liquefied gas 1,1,1,2-tetrafluoroethane (CAS-No. 811-97-2, Solkane 134a) as food grade solvent according to EU flavoring regulations. The extraction raw materials were the same as used for cheese flavoring.

The standardized extract *Botex*™ Black pepper has the following technical data: INCI-Name (CTFA) *Piper Nigrum* Extract, CAS-No. 84929-41-9, EINECS-No. 284-524-7, appearance - brown clear oily liquid with yellow crystals, pungent taste and strong characteristic smell of raw material, volatile oil content - min.50% (v/w), piperin content - min. 40% (v/w), yield 25-32 Kg raw material per kg extract, solvent residue - less than 0.1 g solvent/kg extract. The extraction parameters: extraction time 60 min, temperature 20-23°C.

The standardised extract *Botex*™ Savory has the following technical data: INCI-Name (CTFA) *Satureia Hortensis* Extract, CAS-No. 84775-98-4, EINECS-No. 283-922-8, appearance - dark brown viscous liquid with strong characteristic smell of raw material, volatile oil content - min. 60% (v/w), yield 400-500 Kg raw material per kg extract, solvent residue - less than 0.1 g solvent/kg extract. The extraction parameters: extraction time 90 min, temperature 20-23°C.

**Chemical analysis:** Each batch of processed cheese was analyzed for dry matter, pH, titratable acidity, fat and salt content. Dry matter content was determined by drying at 102±2°C to a constant weight according to ISO 5534:2004; pH was measured by pH-meter MS 2011 (Microsyzt, Plovdiv, Bulgaria), with glass electrode (pH electrode Sensorety, Garden Grove, CA, USA) at 20±2°C; titratable acidity was expressed as % lactic acid and was determined by titration of water solution of 10 g processed cheese with 0.1 N NaOH using phenolphthalein as indicator; fat content was determined by the acidobutyric method of van Gulik according to ISO 3433:2008; salt content was determined according the method described by Reddy and Marth (1993). The chemical analysis of processed cheese samples were performed after 10 days of storage in a refrigerator at 6±2°C. Chemical analyses were conducted in triplicate.


Processed cheese samples were analyzed for aerobic colony count according to ISO 6610:2002, yeasts and moulds according to ISO 6611:2002, coagulase-positive staphylococci according to ISO 6888-1:2005+A1:2005 and anaerobic sulfite-reducing bacteria according to ISO 15213:2003 at the first day of the cold storage.

**Sensory analysis:** Processed cheese samples were coded and served in randomized order. Samples were presented to the sensory panel at room temperature (20±2°C). A panel of 30 customers evaluated the following sensory properties: color, aroma, flavor and texture by using of five points hedonic scale (Table 1). Sensory evaluation was performed by calculating a sensory quality coefficient for each cheese sample (Table 2).

**Statistical analysis:** Statistical analyses were carried out on the averages of the triplicate results. Data were
analyzed by the analysis of variance (one-way ANOVA) method with a significant level of p≤0.05 (Draper and Smith, 1998). The Duncan’s multiple comparison test (SPSS) with a significant difference set at p≤0.05 was used to compare sample means. Significant differences between means less than 0.05 were considered statistically significant (Kenward, 1987). All statistical procedures were computed using the Microsoft Excel and Sigma Plot 2001 software.

RESULTS AND DISCUSSION

Chemical analysis: The results obtained from the chemical analysis showed similarities in the composition of control and experimental batches of processed cheese (Table 3). The dry matter, fat and salt contents of control and test processed cheese samples did not differ significantly (p<0.05). According to Schar and Bosset (2002) the main factors influencing the changes in processed cheese during storage are product composition, processing, packaging and storage conditions (time and temperature). The similarity in chemical composition of all studied samples found in present study could be explained with the uniformity of the factors mentioned above. Minor differences were observed in pH values and lactic acid content of control and test samples (Table 3). The pH values of samples C1 and C2 were lower than those of samples T1 and T2 with 0.19 and 0.11, respectively. In correspondence with these results the lactic acid content of control samples C1 and C2 was higher with 0.35 and 0.25 %, respectively in comparison with the test samples T1 and T2. These tendencies could be explained with the higher microbial contamination of control samples (Table 5) contributing for more intensive microbial growth and activity during storage period. The results obtained (Table 3) for pH values and lactic acid content of control samples did not differ significantly (p<0.05). Similar tendency was found for the test samples.

Microbiological analysis: Results from microbiological analysis of natural spices and spice extracts (Table 4) used for processed cheeses production showed similarity of Escherichia coli and Salmonella counts in all studied samples. Small differences were found in moulds, yeasts, coliforms, coagulase-positive staphylococci and anaerobic sulfite-reducing bacteria counts of all studied samples. It was found that ground black paper samples had higher contamination with yeasts, coagulase-positive staphylococci and anaerobic sulfite-reducing bacteria in comparison with the dried savory samples. Results obtained (Table 4) showed higher contamination with moulds and coliforms of dried savory samples in comparison with their natural extracts. Most significant difference between spices and their natural extracts was established for aerobic colony count. Aerobic colony counts of ground black paper and dry savory were with 4 and 31 g, respectively higher in comparison with their extracts. All these results allow a conclusion to be made, that the microbiological contamination of spice extracts is significantly lower than that of the corresponding natural spices. As it is evident from the results for aerobic colony count, the spices could be an important source of processed cheese microbial contamination. In contrast to them the spice extracts had significantly lower microbial contamination.

Evaluation of the effect of replacement of spices with natural spice extracts on processed cheese microbiological

### Table 3. Physicochemical analysis of processed cheese samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Lactic acid (%)</th>
<th>Dry matter (%)</th>
<th>Fat (%)</th>
<th>NaCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>5.54±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.5±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.2±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td>5.73±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.8±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.4±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2</td>
<td>5.63±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.18±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.2±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.5±0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.4±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>5.74±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.93±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45.6±0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.5±0.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.1±0.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>a, b, c</sup>: means within same column bearing a common superscript did not differ significantly (p<0.05)

### Table 4: Microbiological quality of spices and spice extracts used for processed cheese making

<table>
<thead>
<tr>
<th>Microorganisms groups</th>
<th>Sample</th>
<th>Ground black pepper fruits (&lt;i&gt;Piper nigrum&lt;/i&gt; L.)</th>
<th>Standardized extract Botfex&lt;sup&gt;TM&lt;/sup&gt; Black pepper</th>
<th>Dried savory herb (&lt;i&gt;Satureja hortensis&lt;/i&gt; L.)</th>
<th>Standardized extract Botfex&lt;sup&gt;TM&lt;/sup&gt; Savory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU/g</td>
<td>CFU/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>CFU/g</td>
<td>CFU/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Aerobic colony count</td>
<td>1.7x10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2.5x10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>3.4x10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Moulds</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2x10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Yeasts</td>
<td>2x10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Coliforms</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>4x10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Coagulase-positive staphylococci</td>
<td>1.3x10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Anaerobic sulfite-reducing bacteria</td>
<td>8x10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

ND: not detectable
quality was performed by determination of microbial contamination of control and test samples (Table 5). Results obtained showed, that there were not significant (p<0.05) differences in coagulase-positive staphylococci and anaerobic sulfite-reducing bacteria counts of the studied samples. That fact could be explained with the effect of melting temperature (85±1°C) on the microorganisms presented in processed cheese mixture. Evidently, the microorganisms groups mentioned above did not survived during processed cheese production. Different tendency was established for aerobic colony count of studied samples. Results obtained (Table 5) showed, that total microbial contamination of test samples was approximately 10 times lower than in the controls. It could be explained with the presence of thermo-durable microorganism, which spores could survive during processed cheese manufacture. Higher aerobic colony count of control samples in comparison with test processed cheeses could be attributed to some spices of genus Bacillus which are common contaminants of spices. It could be assumed, that the lower total microbial contamination of test samples (Table 4) in comparison with the controls to the great extend is due to these heat-resistible microorganisms. Besides, some molds also could survive melting procedure. Regardless of the similarity in yeast and molds count at the first day of the cold storage (Table 5) an intensive molds growth on the control samples surface was observed at the 10-th day of experiment. Probably, some heat resistant mould spores have germinated during this period. Similar results were found by Nour El-Diam and El-Zubeir (2006) who compared the microbiological quality of processed and non processed Sudanese white cheese. The results obtained (Table 4 and 5), showed that replacement of Piper nigrum L. and Satureja hortensis L. sp. with their extracts improved significantly the processed cheese microbiological quality.

Sensory analysis: Results obtained from sensory evaluation of studied samples showed, that replacement of Piper nigrum L. and Satureja hortensis L. sp. with their extracts had significant effect on the processed cheese sensory quality (Fig. 1). TSQC of test samples T1 and T2 was higher than the control samples C1 and C2 with 0.41 and 0.43, respectively, which was statistically significant (p<0.05). The main difference between control and test samples was found in sensory evaluated color. The color SQC of T1 and T2 samples was higher than the control samples C1 and C2 with 0.6 and 0.4, respectively. There are several factors influencing color changes during storage of processed cheese: nonenzymatic browning, oxidation, enzymatic activity as well as interactions with packaging material. According to Kristensen et al. (2001) the light exposure had little if any influence on processed cheese browning. The authors reported for absence of browning of processed cheese during storage at 5°C. In the present study were not found significant differences in chemical composition of all tested samples (Table 3). Therefore, it could be assumed that microbial activity was responsible for the negative color changes during storage of control samples. This statement is confirmed by the intensive molds growth, observed on the surface of control samples at the end of the experiment (10th day of cold storage). Evidently, reduced microbial contamination of test samples (Table 5) contributed to better preservation of processed cheese sensory quality during cold storage. It was found that test samples had higher flavor and taste SEQ than the controls, while the body SEQ of all studied samples did not differ significantly (p<0.05). According to Piska and Stetina (2004), cheese ripening and rate of cooling of the processed cheese mixture influenced significantly rheological properties of processed cheese. In the present study these factors, as well as the cheese composition were identical. This could be an explanation for the similarity of body SEQ of control and test samples.

**CONCLUSION**

It could be concluded that replacement of Piper nigrum L. and Satureja hortensis L. sp. with their extracts

---

### Table 5: Microbiological quality of processed cheese samples

<table>
<thead>
<tr>
<th>Microorganisms groups</th>
<th>Sample</th>
<th>C1</th>
<th>T1</th>
<th>C2</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic colony count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moulds, Yeasts</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Coagulase-positive staphylococci</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Anaerobic sulfite-reducing bacteria</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SQC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Fig. 1: Sensory evaluation of processed cheese samples produced with Piper nigrum L. and Satureja hortensis L. sp. and spice extracts
improved significantly processed cheese quality. A decreased microbiological contamination and better sensory properties of test processed cheese samples were established.

ACKNOWLEDGMENT

The authors express their gratitude to the company Extractum Ltd. Plovdiv, Bulgaria for the technological and financial support of the present research.

REFERENCES


