Ergosterol and Patulin Contents of Conventional and Homemade Red Peppers Pastes

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Abstract

In this study, ergosterol and patulin contents of homemade and conventional pepper paste and hot paprika paste were determined and the relationships between these substances were investigated. Patulin and ergosterol were determined using high performance liquid chromatography (HPLC). A limiting value of 15 mg kg⁻¹ ergosterol has been proposed as an index of acceptable quality for tomato products. Ergosterol content of the 13 homemade pepper pastes were found to be lower than 15 mg kg⁻¹ while 8 of these over limits. In contrast, for the ergosterol content, only one of the homemade hot pepper pastes was above proposed limit while all of the conventional hot pepper pastes were lower than limits. In addition, patulin contents of all conventional and homemade pepper pastes were lower than the allowed level of 50 µg kg⁻¹. Patulin content was significantly correlated with the ergosterol content in conventional and homemade paprika paste as 0.90 and 0.96, respectively.

Keywords: Ergosterol; Patulin; HPLC; Pepper paste; Paprika paste

Introduction

Pepper, specifically Capsicum annuum, is a general name for plants coming from Capsicum species of Solanaceae family, whose products are used as vegetables and spices [1]. Red hot pepper is cultivated and consumed in a variety of ways in Turkey and Middle East Countries. Generally, red hot pepper is used as a spice, and in recent years it is also used in the production of hot pepper paste [2].

Pepper paste is a traditionally consumed in many countries. In its traditional use, mashed fresh fruits of Capsicum annuum are subjected to spontaneous fermentation and preserved by addition of high amounts of salt and boiling [1]. Recently, inadequate amounts of traditionally produced pepper paste and consumer demand towards more hygienic products makes the industrial production of pepper paste inevitable. Industrial production of red pepper paste is dependent on some major factors: initial micro flora, thermal processes, pH, total soluble solids, and addition of salt and chemical preservatives [3].

Fresh hot pepper has 77–313 mg 100 g⁻¹ of ascorbic acid, 2.7–5.9 g 100 g⁻¹ of reducing sugars [4], and hot pepper paste contains 115–493 mg 100 g⁻¹ of ascorbic acid and 5.43–35.97 g 100 g⁻¹ of reducing sugars [5,6].

Hot pepper paste is a food product known in Turkey, Spain, Mexico and Korea. Generally, hot pepper paste in Turkey is produced traditionally by sun drying. Recently, hot pepper paste has been manufactured in a plant similar to the manufacturing of tomato paste [3,5,7,8]. There are two main techniques applied for manufacturing of the hot pepper paste. These are concentration in an open pan and under vacuum. Production steps involve; separation of seeds and stem, washing, breaking, and concentration. In this technique hot pepper paste is manufactured without fermentation and the desired aroma does not form properly [3]. Fermentation is one of the main changes that take place during the first days of concentration of hot pepper using traditional technique. Fermentation of hot pepper paste occurs by means of natural contaminant bacteria (lactic acid bacteria) in the hot pepper [2,3].

The most important problem in dried tomatoes, dried pepper and paste industry is growth of mold. Ergosterol (3β-Hydroxy-5,7,22-ergostatriene) is an important constituent of fungal cell wall and measurement of it is a useful method to detect fungal activity, which correspond to mycotoxine production. Thus ergosterol, has been recently recognized as a potential objective parameter useful for the characterization of the quality of processing tomatoes. The poor precision of the “percentage of discarded fruits” and “HMC” methods have increased the importance of ergosterol for the microbiological quality evaluation of tomato and tomato products [9].

Patulin [4-hydroxy-4 H-furo (3,2 c)-pyran-2-(6H) one], a mycotoxin which is highly toxic effect for plant and animal cells and tissues produced by several species of Aspergillus, Penicillium and Byssochlamys fungi where it is produced as a secondary metabolite, is often detected in apples. Studies with fungi or animals have demonstrated that patulin is mutagenic, carcinogenic and teratogenic. Molds contaminated from natural resources such as soil, water and air to raw materials and processed foods creating economic damage in both quality problems and led to the loss of product, as well as cause health risks. Consequently, patulin is an important quality parameter in food industry for human health [10].

The purpose of this study was to determine the contents, if any, of ergosterol and patulin in samples of pepper pastes (homemade and conventional) showing different characteristics collected from different regions of Turkey and to analyze the relationships between them.

Materials and Methods

Materials

In this research, 13 different homemade red pepper pastes and 13 different homemade hot red peppers pastes were collected from 13 different homemade hot red peppers pastes were collected from 7

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which ergosterol concentrations were predetermined, were spiked with
modification in extraction step. Subsample (25 g) was weighed into a
Kyoto, Japan), as suggested by ISO (1993) for apple juice with a slight
high pressure liquid chromatography apparatus (Shimadzu Corp.,
pastes for 5 different concentration added to samples ranged from
level. The recovery rates of ergosterol in the three different pepper
determination of ergosterol in the samples. Ten grams of pepper paste
were transferred to 500 ml volumetric flask. 50 ml of double
distilled water was used in the transfering process. After that pepper
paste was saponified with 50 ml of methanol, 50 ml of ethanol and
10 g of potassium hydroxide. The mixture was boiled for 45 min and
the resultant reflux was filtered followed by separation in a separatory
funnel with water and n-hexane (water/hexane: 1:2) shaking 1 min. After
that, the lower layer was collected into the erlenmeyer and the upper one
was filtered over anhydrous Na2SO4. The lower layer was re-
transferred to the separatory funnel and shaken for 2 min after adding
50 ml of n-hexane following by filtration over Na2SO4. The combined
organic extracts were evaporated using a rotary evaporator (R-114,
Buchi Rotavapor, Switzerland) to approximately 1 ml and transferred
to the separatory funnel and shaken for 2 min after adding
50 ml of 3% sodium carbonate solution was added to the separating funnel. The phases were allowed to separate and then immersed in 1 ml test tube, and the content of the tubes was evaporated to
dryness at 40°C (heating block) under a gentle stream of nitrogen. The residue was dissolved in 5 ml n-hexane and 20 µl portion of the
solution was injected into the HPLC for the analysis.

Apparatus for HPLC: The mobile phase was n-hexane and isooamyl
alcohol (95/5, v/v) with a flow rate of 2 ml min⁻¹. For the analysis, a Nucleosil 100-7 C18 (250x4.6 ID mm) column, a photodiode array detector (Shimadzu, model SPD-M20 A) set at 272 nm, a LC-20AD Shimadzu HPLC pump, a column oven (Shimadzu, CTO-20A) set at 25°C and a Software program (Shimadzu) were used. The sample (20 µL) was injected with a syringe (Hamilton Co., Reno, NV, USA) into the HPLC. Coefficient of determination (r²) was found to be 99.98% for ergosterol. The detection limits (S/N=3) [13] ranged from 0.1 µg.L⁻¹ to 0.5 µg.L⁻¹.

Recovery of patulin: In the recovery experiment, samples, for
which patulin concentrations were predetermined, were spiked with
the different concentrations of patulin, using aliquots at 10, 25, 50, 100, 200, 400, 800 µg.L⁻¹, to determine the recovery of the extraction procedure in the initial step. Three determinations were carried out for each addition level. The recovery rates of patulin in the three different pepper pastes for 7 different concentration added to samples ranged from 97.7%, 98.6%, 98.9% within average percent recovery of 98.4%.

Further determinations: The pH of the samples was measured using a pH meter (PH-700PV Gondo-Taiwan) equipped with an electrode [14]. The pH meter was standardized by a two point method against buffer standards of pH 7.0 and pH 4.0. Paint samples from each treatment were ground in a blender (Waring, USA) and juice was
used to determine the soluble solids (brix) using a digital refractometer (BFM340 Bellingham Stanley, UK). The machine was standardized using purified water before readings were taken. Titratable acidity (TA) was determined as g citric acid/100 ml using the method of AOAC [15]. Ash amount was performed according to AOAC [16] method using burning in a furnace (Nuve MF 110, Turkey) at 650 ± 25°C.

Statistical analysis: Measurements were carried out in duplicates, and data were expressed as means ± standard deviation (SD). Data were evaluated using analysis of variance (ANOVA) described by Snedecor and Cochran [17]. Comparisons of means for treatments were also done by using Duncan's multiple range test. Significance was defined at P ≤ 0.05. Statistical analyses were performed using the SPSS statistics software package (version 16.0; IBM Corporation, Armonk, NY, USA).

Results and Discussion

Some physical and chemical properties of homemade and
conventional red pepper and hot red pepper pastes obtained from
different provinces of Turkey are presented in Table 1. Ash content of
Ergosterol and patulin were baseline separated from other matrix components with good resolution. Identification of the compounds was achieved by comparing their retention times and UV spectrums with the data obtained from the pure reference substances [18].

Samples of pepper pastes ranged from non-detectable level to 27.1 mg.kg⁻¹ and patulin contents of homemade pepper pastes ranged from non-detectable level to 15.05 µg.kg⁻¹.

Homemade red pepper paste was found higher compared with conventional red pepper paste. High ash contents in the homemade red pepper paste samples were thought to arise from high salt addition to homemade red pepper paste. Remaining parameters were significantly lower than the Turkish Food Codex limits. Ash content of some conventional red pepper paste had been detected as a 1.2 mg.kg⁻¹. Rest of these samples did not detect ergosterol content. In addition, these samples did not detect patulin content.

Ergosterol values of all these conventional hot red pepper pastes were significantly lower than the limits. Also patulin level of these samples detected lower than maximum acceptable level of 50 µg.kg⁻¹ for fruit (apple) juices (Table 2).

The results obtained for patulin and ergosterol from the conventional hot red pepper paste samples with different characteristics collected from pepper paste processing plants is shown in Table 2. Ergosterol content of hot red pepper paste samples from one out of six processing plants had been detected as a 1.2 mg.kg⁻¹. Rest of these samples did not detect ergosterol content. In addition, these samples did not detect patulin content.

Ergosterol values of all these conventional hot red pepper pastes were significantly lower than the limits. Also patulin level of these samples detected lower than maximum acceptable level of 50 µg.kg⁻¹ for fruit (apple) juices.

Patulin and ergosterol contents of homemade red pepper paste samples also were given in Table 2. Ergosterol contents of homemade pepper pastes ranged from non-detectable level to 27.1 mg.kg⁻¹ and patulin contents of homemade pepper pastes ranged from non-detectable level to 15.05 µg.kg⁻¹.

Homemade red pepper paste samples from eight out of thirteen samples had high ergosterol levels exceeding the allowed level of 15 mg.kg⁻¹ for tomatoes and tomato products. Homemade pepper paste is a sun dried (drying in shadow about 2 days) product. Therefore, the high ergosterol values in homemade pepper paste compared to conventional one may be attributable to the growing of mould in homemade pepper paste during the sun drying in shadow. It is well known that mould can propagate under sun drying conditions in shadow. In addition none of the homemade red pepper paste samples of patulin contents were fairly higher than maximum acceptable levels of 50 µg.kg⁻¹ for fruit (apple) juices.

Regressions showing ergosterol and patulin contents of homemade red pepper paste samples can be seen in Table 2. There was significant relationship between ergosterol and patulin contents of homemade red pepper paste samples (r²=0.96, p<0.002).

Ergosterol contents of homemade pepper pastes ranged from non-detecTable level to 18.35 mg.kg⁻¹ and patulin contents of homemade red pepper paste samples can be seen in Figure 3. There was significant relationship between ergosterol and patulin contents of homemade red pepper paste samples (r²=0.90, p<0.002).

Homemade hot red pepper paste samples from one out of thirteen samples had high ergosterol levels exceeding the allowed level of 15 mg.kg⁻¹ for tomatoes and tomato products. It is thought there is health risks for consumers. Probably high ergosterol and patulin contents of homemade red pepper paste is result of concentrated in uncontrolled production conditions at open pan.

There was significant relationship between patulin and ergosterol contents both conventional and homemade red pepper paste samples. So the relationship between the patulin and ergosterol concentrations of pepper paste was examined to determine whether ergosterol and patulin may also be an indication of the microbiological quality of pepper pastes. Nevertheless more advanced research is needed for using ergosterol and patulin content as a quality parameter in the pepper pastes and to determine the acceptable limits in pepper paste is bring about the next stage of this study.

Table 2: Ergosterol and patulin contents of pepper pastes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Samples</th>
<th>Ergosterol (mg.kg⁻¹)</th>
<th>Patulin (µg.kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min Max Mean SD*</td>
<td>Min Max Mean SD*</td>
<td></td>
</tr>
<tr>
<td>HPP</td>
<td>13 nd 27.10 16.442 7.645</td>
<td>nd 15.05 7.908 4.277</td>
<td></td>
</tr>
<tr>
<td>HHPP</td>
<td>13 nd 18.35 6.708 5.096</td>
<td>nd 10.05 2.958 3.201</td>
<td></td>
</tr>
<tr>
<td>CPP</td>
<td>6 3.10 7.25 5.083 1.548</td>
<td>1.50 3.90 2.725 0.864</td>
<td></td>
</tr>
<tr>
<td>CHPP</td>
<td>6 nd 1.20 0.200 0.447</td>
<td>nd nd - -</td>
<td></td>
</tr>
</tbody>
</table>

nd : Non detectable level ,  
SD : Standard Deviation

HPP : Homemade Pepper paste.  
HHPP : Homemade Hot Pepper Paste.  
CPP: Conventional Pepper Paste.  
CHPP: Conventional Hot Pepper paste

Figure 3: Regression of patulin and ergosterol concentration in homemade red pepper paste samples

Figure 4: Regression of patulin and ergosterol concentration in homemade hot red pepper paste samples

Regressions showing ergosterol and patulin contents of homemade red pepper paste samples can be seen in Figure 4. There was significant relationship between ergosterol and patulin contents of homemade hot red pepper paste samples (r²=0.90, p<0.002).

Conclusion

This is the only report known to the authors concerning the presence of ergosterol in homemade and conventional pepper paste. It is also the first time that pepper products were examined in Turkey for the presence of ergosterol. In addition, there are no published data available from other countries for comparison of results of ergosterol in Turkish pepper products. Higher levels of ergosterol were found in homemade pepper paste, and much lower amounts in conventional pepper paste.

As a result of the above mentioned analyses, conventional red pepper and hot red pepper paste has been found quite safe for the human consumption. In contrast many of homemade red pepper paste samples had high ergosterol levels exceeding the allowed level of 15 mg.kg⁻¹ for tomatoes and tomato products. It is thought there is health risks for consumers. Probably high ergosterol and patulin contents of homemade red pepper paste is result of concentrated in uncontrolled production conditions at open pan.

References


