

Ergosterol and Patulin Contents of Conventional and Homemade Red Peppers and Hot 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 over 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 annum*, 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 annum* 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 *Byssoschlamys* 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 pepper pastes were collected from 7

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different provinces (Adana, Hatay, Kahramanmaraş, Denizli, Bursa, Gaziantep and Manisa) of Turkey on September 2010 were used as material. In addition 6 different conventional and 6 different hot red pepper pastes which are produced in Turkish paste factory (Manisa, Gaziantep, Balıkesir, Manisa, Bursa and Denizli) were purchased from Turkish supermarkets on October 2010.

Standard solutions

All reagents used were analytical or HPLC grade, crystalline form of patulin (P1639, analytical reagent grade, Sigma-Aldrich Chemie GmbH Deisenhofen, Germany) and crystalline form ergosterol (E6510, analytical reagent grade, Sigma-Aldrich Chemie GmbH Deisenhofen, Germany) were used to prepare standard solutions. Also ultra pure distilled water was used for chromatographic analysis.

Methods

Determination of ergosterol: The HPLC method of Schwadorf and Müller [11] modified by Ghiretti et al. [12] was used for the determination of ergosterol in the samples. Ten grams of pepper paste samples were transferred to 500 ml volumetric flask. 50 ml of double distilled water was used in the transferring 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 followed 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 into a 10 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 isoamyl 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 282 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 (r^2) was found to be 99.98% for ergosterol. The detection limits (S/N=3) [13] ranged from 0.1 mg.L⁻¹ to 0.5 mg.L⁻¹.

Recovery of ergosterol: In the recovery experiment, samples, for which ergosterol concentrations were predetermined, were spiked with the different concentrations of ergosterol, using aliquots at 1, 2, 4, 8 and 20 mg.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 ergosterol in the three different pepper pastes for 5 different concentration added to samples ranged from 98.87%, 98.90%, 99.01% within average percent recovery of 98.93%.

Determination of patulin: The determination of patulin in the samples was carried out by using a Shimadzu Class-VP V5.01 high pressure liquid chromatography apparatus (Shimadzu Corp., Kyoto, Japan), as suggested by ISO (1993) for apple juice with a slight modification in extraction step. Subsample (25 g) was weighed into a stomacher bag added with 25 ml of ethyl acetate and homogenized in a

lab blender for 3 min. Then the contents of the bag were filtered through a filter paper into a separating funnel. Paste pieces on the filter paper were put into the same stomacher bag and re-extracted with a second portion of ethyl acetate. The contents of the bag were filtered through the same filter paper into the same separating funnel. This process was repeated one more time (a total of three extractions per sample). Ten ml of 3% sodium carbonate solution was added to the separating funnel and shaken for 1 min. Then, the phases were allowed to separate and to drained the lower aqueous layer into another separating funnel and immediately extracted with 25 ml of ethyl acetate by shaking for 1 min. Top layers (organic phases) were combined, transferred into a round-bottomed flask and acidified with glacial acetic acid. This extract was evaporated to dryness in a rotary evaporator. The residue in the flask was transferred into a 5 ml volume tube by portions of 1ml of ethyl acetate. Then the extract was evaporated just to dryness in a water bath at 40°C under a gentle stream of nitrogen. The residue was dissolved in 0.5 ml mobile phase. This final solution was kept at -18°C in deep freezer until the chromatographic measurements were made.

Apparatus for HPLC: The mobile phase employed was a 10% aqueous acetonitrile solution with a flow rate of 0.5 ml min⁻¹. For the analysis, a Nucleosil 100-7 C18 (250×4.6 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 (r^2) was found to be 99.98% for patulin. 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 (PL-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. Paste 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 (RFM340 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 tests. Significance was defined at $P \leq 0.05$. 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

Sample	Number of Samples	Brix (%)				pH				Acidity* (%)				Salt (%)				Ash (%)			
		Min	Max	Mean	SD**	Min	Max	Mean	SD**	Min	Max	Mean	SD**	Min	Max	Mean	SD**	Min	Max	Mean	SD**
HPP	13	14.95	45.00	34.14	9.310	3.66	4.55	4.11	0.315	0.56	3.56	1.24	0.857	5.26	15.78	9.54	3.431	5.56	18.34	12.33	4.235
HHPP	13	15.10	42.75	35.71	7.310	3.58	4.44	4.17	0.251	0.55	2.41	1.26	0.640	6.98	14.05	9.27	2.692	8.82	18.08	11.72	2.920
CPP	6	21.00	32.00	25.25	4.875	4.11	4.58	4.27	0.198	0.56	1.26	0.89	0.248	1.72	8.50	4.79	3.287	2.49	10.10	6.06	3.409
CHPP	6	20.00	36.00	28.33	7.118	4.09	4.64	4.40	0.187	0.52	0.99	0.74	0.166	1.79	7.98	5.68	2.733	3.61	10.00	7.99	2.540

*Citric acid value of titration acidity

** SD : Standard Deviation

HPP : Homemade Pepper paste.

HHPP : Homemade Hot Pepper Paste.

CPP: Conventional Pepper Paste.

CHPP: Conventional Hot Pepper paste

Table 1: Brix, pH, acidity, salt and ash content of homemade and conventional red pepper and hot red pepper pastes samples

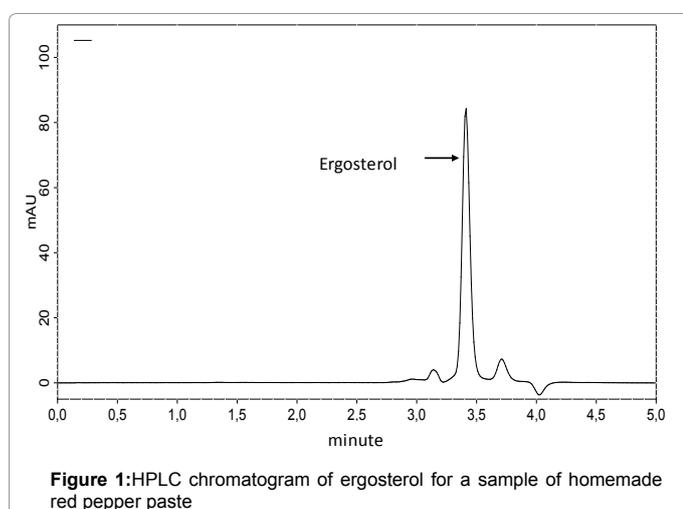


Figure 1: HPLC chromatogram of ergosterol for a sample of homemade red pepper paste

homemade red pepper 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 was detected to be at higher levels. High ash contents in the conventional red pepper paste samples were thought to arise from high salt addition to conventional red pepper paste. The standard deviation for the homemade red pepper paste has been determined higher than that for other types of pepper pastes. Moreover, the least standard deviation value has been obtained from the conventional red pepper paste averagely.

The separation of ergosterol and patulin of homemade red pepper paste samples by HPLC were illustrated in Figures 1 and 2, respectively. The analytical method proved reliable, with detection limits of 0.1 mg.kg⁻¹ and 0.1 µg.kg⁻¹ for ergosterol and patulin, respectively. 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].

Patulin and ergosterol contents of conventional red pepper paste samples with different characteristics collected from pepper paste processing plants is shown in Table 2. Ergosterol contents of pepper pastes have been measured as total ergosterol, which includes free and esterified ergosterol. Ergosterol contents of conventional pepper pastes ranged from 3.10 to 7.25 mg.kg⁻¹ and patulin contents of conventional pepper pastes ranged from 1.50 to 3.90 µg.kg⁻¹. Ergosterol, a suggested

measure of fungal growth in plant materials, has a proposed limiting value of 15 mg.kg⁻¹ for tomato products [12,19-21]. So, all these values were significantly lower than the limit. 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.

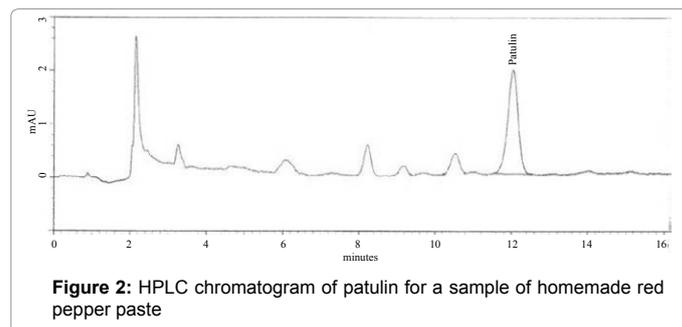


Figure 2: HPLC chromatogram of patulin for a sample of homemade red pepper paste

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

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

Table 2: Ergosterol and patulin contents of pepper pastes

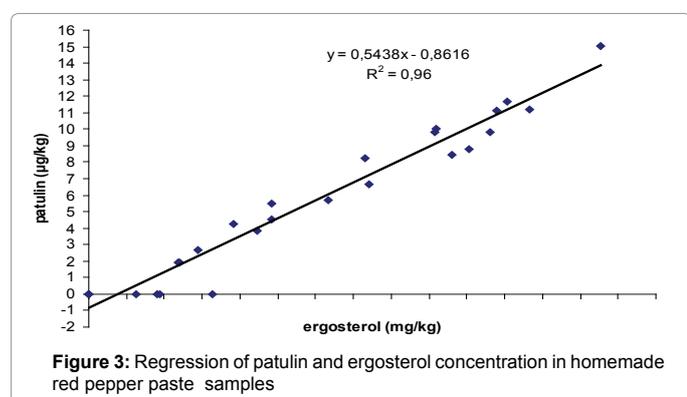


Figure 3: Regression of patulin and ergosterol concentration in homemade red pepper paste samples

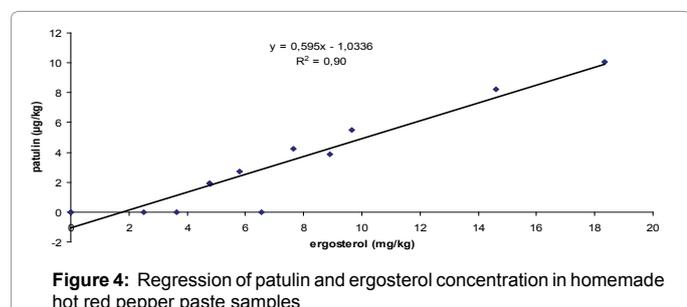


Figure 4: Regression of patulin and ergosterol concentration in homemade hot red pepper paste samples

Regressions showing ergosterol and patulin contents of homemade red pepper paste samples can be seen in Figure 3. There was significant relationship between ergosterol and patulin contents of homemade red pepper paste samples ($r^2=0.96$, $p<0.002$).

Ergosterol contents of homemade pepper pastes ranged from non-detectable level to 18.35 mg.kg⁻¹ and patulin contents of homemade pepper pastes ranged from non-detectable level to 10.05 µg.kg⁻¹ (Table 2)

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 was thought to arise from the substances that give hot red peppers their intensity when ingested or applied topically are capsaicin which may demonstrate inhibitory properties of mold growing. In addition none of the homemade red pepper paste samples of patulin content were fairly higher than maximum acceptable levels of 50 µg.kg⁻¹ for fruit (apple) juices.

Regressions showing ergosterol and patulin contents of homemade hot 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 ($r^2=0.90$, $p<0.002$).

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.

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.

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