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Research Article

Patulin and phenolic content in commercial fruity baby foods on the Turkish market

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Abstract: This study aimed to determine baby foods' safety, antioxidant content, and color. In this study, the mycotoxin patulin, which is toxic to humans, and phenolics (chlorogenic acid, p-coumaric acid, epicatechin), which are beneficial to human health and color, were analyzed in baby foods containing fruit puree. The patulin content in 12 baby foods purchased from the market in Türkiye was below 8.45 µg/kg. Patulin found in baby foods comes from spoiled fruit. By performing this study, the quality of fruits used in baby foods was evaluated. Fruits also have chlorogenic acid, p-coumaric acid, and epicatechin phenolics. These phenolics in baby foods were 9.316-598.428 µg/kg, 0.953-14.166 µg/kg, and 0.471-20.35 µg/kg, respectively. L*, a*, and b* color values of 12 baby foods were found between 12.77-23.00, 1.44-11.19, and 10.50-11.01, respectively.

1. INTRODUCTION

Patulin (see Figure 1) is a mycotoxin produced by *Penicillium* and *Aspergillus* molds, often found in moldy fruits and their products. The highest allowable amount of patulin in baby food is 10 µg/kg, according to the Turkish Food Codex (2011). In an experiment on mice, patulin's lethal dose (LD50) value was 5 mg/kg. The level of toxic effects was determined as 43 µg/kg according to the Provisional Maximum Tolerable Daily Intake Value and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The highest level of patulin that can be taken daily is 0.4 mg/(kg body weight x day). Determining patulin levels in foods is very important for consumers' health. The Codex Alimentarius and the Food and Drug Administration (FDA) have set the highest permissible level of 50 µg/kg for apple juices and other apple-containing food products. Patulin content in baby foods and apple puree is subject to regulations set by the authorities. In the European Union (EU, 2006), this upper limit was determined to be about 10 µg/kg for baby foods and apple puree. Research conducted in European Union countries, such as Cuba, South Africa, Iran, and Türkiye, found that the amount of patulin in apple juices differed. In most studies, patulin levels were below the Codex Alimentarius limits in Iran and Türkiye. In Australia, patulin levels exceeded the European Union limit (Li *et al.*, 2007).

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Patulin is known as polyketide lactone (4-hydroxy-4H-furo (3,2-c) pyran-2 (6H)-1) (Baert *et al*., 2007). It is colorless and crystalline, and its melting point is 110ºC. It is stable with a temperature of 105-125ºC and pH 3.5-5.5. The degree of disintegration increases with increasing levels of pH. At pH six and 100ºC, only 50% of patulin is broken. Therefore, it is not enough to complete the pasteurization for the inactivation of patulin (Gonzales-Osnaya *et al*., 2007). In one study, patulin in 120 homogenized baby foods was examined. Baby foods were purchased from the supermarket in Italy between 2008 and 2009. Sixty of these samples were apple flavored (40 from traditional agriculture, 20 from organic agriculture), and 60 were selected as mixed fruit flavors (40 from traditional agriculture and 20 from organic agriculture). Patulin levels in all of the samples were below the limit. In 22 samples, the amount of patulin was close to the limit (9µg/kg) (Bonerba *et al*., 2010). Funes & Resnik (2009) determined that there was patulin contamination in apples and products purchased from supermarkets in Argentina. In this study, 21.6% of 51 products were found contaminated (17-221 µg/kg, average 61.7 µg/kg), and the highest patulin level was determined as 123 µg/kg in 50% contaminated apple puree. The level of patulin in apple puree should be reduced. Zaied *et al*. (2013) examined the patulin content of 85 apple products sold in supermarkets in Tunisia in 2013. This study compared the patulin contamination in apple products and determined the factors accelerating mycotoxin formation. The contamination level was defined as an average of 20 µg/L in the range of 0-167µg/L in all samples. 28% of baby foods exceeded the allowable limit for patulin. Barreira *et al*. (2010) examined the level of patulin in 144 apple foods (76 of them homogenized apple puree) sold in markets in Portugal. Patulin was detected in 33 samples (23%) in the 1.2-42µg/kg range. Patulin was determined in five homogenized apple purees (7%). Janotova *et al*. (2011) reported that all stages of apple puree production led to patulin reduction, particularly pulping, which reduced patulin levels by 29-80% of its original content. Marín *et al*. (2011) reported that patulin content in fruit products purchased from a market in Spain was below EC limits.

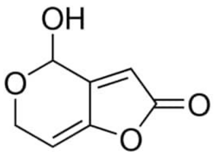

Figure 1. Formula of patulin.

Phenolic substances contain one or more hydroxyl groups in their aromatic ring (Shadidi & Naczk, 1995). In this respect, it is known that the simple phenolic substance is benzene, which contains one hydroxyl group and other phenolic substances derived from it (Cemeroğlu & Acar, 1986; Saygı, 2017; Saygi, 2021a; Saygi, 2021b). Phenolic substances are divided into two groups: simple phenolic substances and polyphenols. Phenolic substances commonly found in fruits and vegetables are examined in hydroxybenzoic acids, hydroxy-cinnamic acids, and flavonoids. Flavonoids are divided into five subgroups. These are catechins, anthocyanidins, flavanols, flavonoids, and proanthocyanins (Cemeroğlu & Acar, 1986). Phenolic acids vary according to the position and number of the hydroxyl group in the ring chain. These compounds have a strong antioxidant effect against free radicals that cause cancer and cardiovascular diseases (Yu *et al*., 2002). Phenolics effectively protect against oxidative stress in the human body (Papas, 1998). It is known that fruits are richer than vegetables in terms of phenolic substances. In addition, phenolic substances are present in almost every fruit and vegetable (Shadidi & Naczk, 1995). The main phenolic compounds in apple juice are procyanidins, epicatechin, chlorogenic acid, p-coumaric acid, phloretin 2-xylo glucoside, and phlorizin (Suárez *et al*., 1998).

Santos *et al*. (2014) examined the change in the phenolic profiles of baby food prepared from green vegetables during storage. Phenolic content was determined at the beginning and end of storage for ten days. It was found that storage affects the phenolic content of baby foods at different rates. Reyes *et al*. (2007) found a prolonged change in the total phenolic content of fresh fruits during storage. As a result of this change, the amount of ascorbic acid and phenolic compound decreased.

This study aimed to ensure an overview of patulin contamination, determine the antioxidant value, and provide data on the color values of fruity baby foods in Türkiye (produced in Türkiye, Hungary, and Poland). The assessment of antioxidant potential is of crucial importance in foods. The study about patulin content, phenolics, and color value in baby foods containing fruit puree purchased from a local market in Türkiye has not been performed so far. No study has examined patulin and phenolics in baby foods produced in Türkiye, Hungary, and Poland. Additionally, no publications perform all three analyses on baby foods.

2. MATERIAL and METHODS

2.1. Materials

Twelve baby fruit puree from four companies were purchased from a market in Türkiye. These fruit purees are given in Table 1.

Table 1. Fruity baby food samples.

Company	Samples
A	Carrot Apple Puree, Apple Peach Puree, and Apple Pear Puree
B	Mixed Fruit Oats, Organic Mixed Fruit, and Organic Apple Peach
C	Organic Peach Puree, Organic Apple Puree, and Organic Apple and Banana Puree
D	Organic Peach Apple Puree, Organic Cereal Mixed Fruit Puree, and Organic Fruit Cocktail

2.2. Patulin Analysis

The sample was homogenized. It was filtered through a coarse filter pan, and 10 mL of the filtrate was taken and placed in the separating funnel. Then, 20 mL of ethyl acetate was added and shaken for 1 minute. After performing phase separation, the ethyl acetate phase (clear phase on top) was transferred to another container. The yellowish phase at the bottom was treated twice with ethyl acetate, and then, the eluates were combined. The remaining aqueous phase was discarded. Agitation was carried out three times with 20 mL of ethyl acetate. 4 mL of 1.5% sodium carbonate was added over the combined ethyl acetate phases and shaken within 1 minute, and the underlying phase was taken in a beaker. 15 g of sodium sulfate was weighed in the upper phase and collected in an evaporator flask. The phase into the beaker was brought into the separatory funnel and shaken by adding 10 mL of ethyl acetate. The ethyl acetate phase was filtered through 15 g of sodium sulfate-weighed filter paper. This process was done once again. All ethyl acetate phases were collected in an evaporator flask. Ethyl acetate, the evaporator extraction solution, was blown off. 2 mL of mobile phase was added to the residue in the evaporator flask, mixed well, and put into the vial. Samples were analyzed by HPLC (Shimadzu-SIL-20AHT, Japan) at 276 nm with a flow rate of 0.7 mL/min according to the method of AOAC 2000.02 with slight modifications. The separation column was octadecylsilane (ODS), (3.5µ, 250x4.6mm). Analysis was done in duplicate.

2.3. Phenolic Analysis

The homogenized sample (1g) was extracted in 1000 mL of 80% methanol for 20 min in an ultrasonic bath and filtered. The residue was washed with 50 mL of 100% methanol. Extraction and washing processes were repeated. The solution mixture was evaporated at 40ºC. 1 mL of methanol was added and injected into HPLC (Shimadzu Prominence Brand, Japan). Phenolic compounds of samples were analyzed according to the method reported by Caponio *et al*. (1999) with some modifications (A: 3% Formic acid B: Methanol). Analysis was done in duplicate.

2.4. Color Analysis

The samples ' color values (L*, a*, b*) were determined using an X-rite instrument (USA). Analysis was done in duplicate.

2.5. Statistical Analysis

Data related to patulin, phenolic acids, and color values was statistically analyzed according to the Duncan test. The difference between groups was significant at $p < 0.05$. All data were analyzed using IBM Statistics SPSS 24.

3. FINDINGS

3.1. Patulin

Patulin level in all samples was found below 8.45µg/kg, below the upper limit of patulin in baby foods (10µg/kg). Ritieni (2003) reported that two samples of ten apple-based baby foods purchased from a supermarket in Italy were contaminated with 17.7 and 13.1 µg/L patulin.

3.2. Phenolic Compounds

The quantity of phenolic compounds in 12 baby foods is given in Table 2. LOD, wavelength, and retention times of phenolic standards are shown in Table 3. A chromatogram of baby foods is given in Figure 2. The highest chlorogenic acid was 598.428 µg/kg belonging to the organic apple puree of Company C, while the lowest chlorogenic acid was 9.316 µg/kg belonging to mixed fruit and oat of Company B. Chlorogenic acid in carrot and apple puree of Company A was statistically similar to that of apple and peach puree of Company C, apple and pear puree of Company A and organic cereal mixed fruit puree of Company D. The highest *p*-coumaric acid was 14.166 µg/kg belonging to organic peach and apple puree of Company D, while the lowest *p*-coumaric acid was 0.953 µg/kg belonging to apple and pear puree of Company A. *p*-coumaric acid in the organic peach puree of Company C was statistically similar to that of the organic apple puree of Company C. *p*-coumaric acid in apple and peach puree of Company A was statistically similar to that of mixed fruit oat of Company B, organic apple peach puree of Company B and organic cereal mixed fruit puree of Company D. *p*-coumaric acid in organic apple and banana puree of Company C was statistically similar to that of organic fruit cocktail of Company D. *p*-coumaric acid in carrot and apple puree of Company A was statistically similar to that of apple and pear puree of Company A. The highest epicatechin was 20.35 µg/kg belonging to the organic apple and banana puree of Company C, while the lowest epicatechin was 0.471 µg/kg belonging to mixed fruit oat of Company B. Epicatechin of the organic apple and peach of Company B was statistically similar to that of organic apple puree of Company C, organic peach apple puree of Company D and organic fruit cocktail of Company D. Epicatechin in an organic peach puree of Company C was statistically similar to that of organic cereal mixed fruit puree of Company D. Epicatechin in apple and peach puree of Company A was statistically similar to that of apple and pear puree of Company A, mixed fruit oat of Company B and organic mixed fruit puree of Company B. Similar results were reported by Casado *et al*. (2019). Casado *et al*. (2019) investigated two different extraction techniques for four baby foods purchased from a local pharmacy in Portugal. Casado *et al*. (2019) reported that chlorogenic acid in four baby foods containing bananas, apples, multi fruits with cereals, or chicken, beef, and vegetables was not detected, 815 µg/kg, 569 µg/kg, and 194 µg/kg, respectively. Epicatechin of those baby foods was 153µg/kg, 469µg/kg, 293µg/kg, and not detected, respectively. *P*-coumaric acid of those baby foods was 67µg/kg, 32.6µg/kg, 64µg/kg, and 65µg/kg, respectively. Osminaski *et al*. (2008) reported that the chlorogenic acid of apple purees was 63-200µg/kg, while epicatechin was 28.8-143µg/kg. Talcott & Howard (1999) reported that strained carrots had chlorogenic acid between 2.04 and 55.25µg/kg.

3.3. Color Values

L*, a*, and b* color values of baby foods are given in Table 4. L* color values of 12 baby foods ranged between 12.77 and 23.00. a* color values of 12 baby foods ranged between 1.44 and 11.19. b* color values of 12 baby foods ranged between 10.50 and 18.01. The highest L* color value was 23.00 belonging to the organic apple and banana puree of Company C, while the lowest L* color value was 12.77 belonging to the carrot and apple puree of Company A. The highest a* color value was 11.19 belonging to carrot and apple puree of Company A, while the lowest a* color value was 1.44 belonging to organic apple puree of Company C. The highest b* color value was 18.01 belonging to the carrot and apple puree of Company A, while the lowest b* color value was 10.50 belonging to the organic apple puree of Company C. Similar results were reported by Oszmiański *et al*. (2008). Oszmiański *et al*. (2008) reported that apple purees' L*, a*, and b* color values ranged between 47.99-60.98, 2.74-10.33, and 11.17-20.74, respectively.

Table 2. Phenolic content of fruity baby foods (μg/kg).

Fruity baby foods	Chlorogenic acid (μg/kg)	*p*-coumaric acid (μg/kg)	Epicatechin (μg/kg)
Company A Carrot and Apple Puree	66.316^{f}	1.282^{e}	3.219^{d}
Company A Apple and Peach Puree	71.041^{f}	6.662^{c}	1.349^{e}
Company A Apple and Pear Puree	79.867^{f}	0.953^{e}	0.638^{e}
Company B Mixed Fruit Oats	9.316^{h}	4.911^{c}	0.471^{e}
Company B Organic Mixed Fruit	40.388^{g}	7.027^{c}	1.304^{e}
Company B Organic Apple and Peach	449.842^{b}	6.115^{c}	9.301^{b}
Company C Organic Peach Puree	96.337^{e}	11.984^{b}	5.395^{c}
Company C Organic Apple Puree	598.428^{a}	10.459^{b}	9.230^{b}
Company C Organic Apple and Banana Puree	119.331^{d}	2.955^{d}	20.354^{a}
Company D Organic Peach and Apple Puree	286.252^{c}	14.166^{a}	9.054b
Company D Organic Cereal Mixed Fruit Puree	76.208^{f}	6.736^{c}	6.279^{c}
Company D Organic Fruit Cocktail	87.362^{e}	2.083^{d}	9.437^{b}

Values within columns with similar letters are not significantly different (Duncan's test).

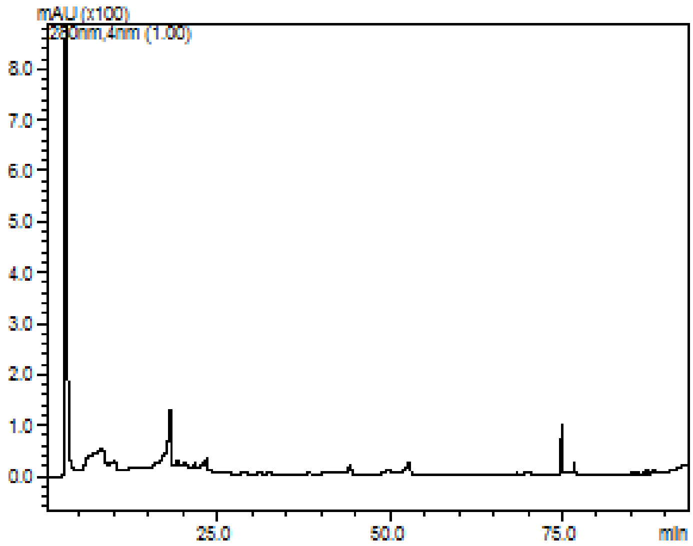

Figure 2. Chromatogram of phenolics in fruity baby food (Company A apple pear puree).

Table 3. LOD, wavelength, and retention times of phenolic compounds.

Standards	LOD (µg/g)	Wavelength (nm)	Retention times (min)
Chlorogenic acid	0.01	320	18.2
p-coumaric acid	0.01	320	26.1
Epicatechin	0.43	260	21.3

LOD: Limit of detection

Table 4. Color values (L*, a*, b*) of fruity baby foods.

Fruity baby foods	L*	a*	b*
Company A Carrot and Apple Puree	12.77^{e}	11.19^{a}	18.01^{a}
Company A Apple and Peach Puree	15.67^{d}	5.50^{c}	14.31^{b}
Company A Apple and Pear Puree	14.91^{d}	7.16^{b}	17.46^{a}
Company B Mixed Fruit Oats	17.35^{c}	2.14^{e}	11.13^{d}
Company B Organic Mixed Fruit	20.13^{b}	3.28^{d}	16.97^{a}
Company B Organic Apple and Peach	20.89^{b}	3.84^{d}	10.87^{d}
Company C Organic Peach Puree	16.42^{c}	3.38^{d}	12.24^{c}
Company C Organic Apple Puree	14.60^{d}	1.44^{f}	10.50^{d}
Company C Organic Apple and Banana Puree	23.00^{a}	2.87^{e}	12.72^{c}
Company D Organic Peach and Apple Puree	16.07^{d}	5.00^{c}	14.56^{b}
Company D Organic Cereal Mixed Fruit Puree	16.54^{d}	3.67^{d}	14.06^{b}
Company D Organic Fruit Cocktail	15.21^{d}	4.16^{d}	13.78^{b}

Values within columns with similar letters are not significantly different (Duncan's test).

4. DISCUSSION and CONCLUSION

The presence of patulin in apple juice indicates the microbiological quality of the raw material (Acar *et al*.,1998). Patulin in baby foods comes from fruit, which is spoilage. The daily intake of patulin is essential for babies and children who are sensitive to its toxic effects. According to the Turkish Food Codex, the maximum limit for patulin in baby foods is 10 µg/kg. It was concluded that the raw material of baby foods produced in Türkiye, Hungary, and Poland was of high quality. The patulin content did not exceed 8.45 µg/kg. Similar results were reported by Bonerba *et al.* (2010). Bonerba *et al*. (2010) reported that the patulin content in 120 baby foods purchased at a market in Italy was less than 10 µg/kg. The low level of patulin in Italian apple products is a parameter used to evaluate fruit quality, and the process is of a high standard (Ritieni, 2003). Patulin, an undesirable natural contaminant of fruits, can indicate fruit quality and processes (Burda, 1992; Gökmen & Acar, 1998).

The main phenolic compounds in apples were chlorogenic acid, *p*-coumaric acid, phloretin-2'-O-glucoside, phloretin-2'-O-xyloglucoside, (+)-catechin, (-)-epicatechin, and procyanidins (Wojdyło et al., 2007). Therefore, epicatechin, chlorogenic acid, and *p*-coumaric acid were found to have high ratios in organic apple-based fruit puree analyzed in this study. Apple contains considerable amounts of polyphenols, contributing to total antioxidant activity (Khanizadeh *et al*., 2007), and may reduce the risk of cardiovascular disease, coronary, and mortality risk (Knekt *et al*., 2000).

Determining the level of patulin in baby foods gives information about the quality of fruits used in baby food production. Children are exposed to patulin toxicity more than adults. Therefore, the amount of patulin in baby foods was investigated. The level of patulin was found below the upper limit (10 µg/kg) in all baby foods belonging to four companies. Phenolic contents were observed at a high rate in baby foods belonging to four companies. The highest amount of chlorogenic acid, p-coumaric acid, and epicatechin was determined in the organic apple puree of Company C, the organic peach and apple puree of Company D, and the organic apple and banana puree of Company C, respectively. The brightness values of the baby foods belonging to the four companies were close. There is no study about the patulin content, phenolic compounds, and color of baby foods purchased in Türkiye. The results obtained from this study will make a significant contribution to the creation of a detailed database.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Seda Yalçın: Design Analysis, Interpretation, and Writing. **Sevgül Coşkun:** Conception, Materials, and Literature Review

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