# Assessment of the Thermal Stability of Patulin in Apple Puree and of Possibilities for Reduction of Patulin Contamination Level Through Apple Processing

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*Apples are an important raw material for the production of puree, nectar, jam, and a widely used ingredient in composition of pastry products. Patulin is a mycotoxin that appears in moldy apples, especially, attacked by Penicillium, Aspergillus and Byssochlamys. This study aimed to evaluate the thermal stability of patulin in apple puree and the possibilities to reduce the patulin contamination level through processing apples to apple puree and nectar. Within the experiments, samples of apple puree from Jonathan apple cultivar, spiked with patulin at four concentration levels (10 μg/kg, 20 μg/kg 40 μg/kg and 80 μg/kg) were heat treated at 100 °C for 10, 15, 20 and 25 min, respectively. It was found a reduction of the patulin level, due to the thermal degradation of this mycotoxin. Apples from Golden Delicious cultivar, attacked by Penicillium expansum and contaminated with native patulin were processed to puree and nectar. Through processing of apples contaminated with patulin it was registered an important decrease of it.*

*Keywords: apple, patulin, puree, nectar, processing*

Patulin is a mycotoxin with toxic potential which occurs in moldy apples, especially, attacked by *Penicillium, Aspergillus* and *Byssochlamys.* Among these molds, *Penicillium expansum,* shows the highest incidence of patulin contamination in apples and other fruits [1]. The most important factors which influence mycotoxins presence in raw materials are: insects attack, fruits and vegetables damage at harvesting, as well as temperature and humidity during storage [2]. The highest level of patulin contamination occurs on apples stored at temperatures between 4 and 25 °C [3, 4].

Apples are important raw materials in manufacturing products destined to infants' and children's nutrition. Apple products made from apples contaminated with patulin are an important source to introduce patulin in diet [5].

Patulin contamination in apples appears initially in the areas attacked by mold, then mycotoxin invades apples tissue to a depth of 1-2 cm. In general, the performed studies have concluded that, patulin contamination of processed apple products could be avoided by cutting and removing of apple healthy tissue at a distance of 2 cm from the area attacked by mold [6].

Influence of apple processing on patulin content of concentrated apple juice or apple puree was studied and presented by many researchers. Welke *et al.* (2009) [7] studied the influence of different technological operations in the manufacturing process of concentrated juice on patulin level. Within experiments, they used apple paste resulted from milling process, with a high level of patulin contamination. After pasteurization, enzymatic treatment, micro-filtration and evaporation, the average losses of patulin were 39.6, 28.3, 20.1 and 28.4%, respectively. By dilution of concentrated apple juice (69 °Brix) to a soluble dry matter of 12 °Brix, it was obtained an apple juice with a patulin content in the range 15-46 μg/L, so under maximum level allowed by Commission Regulation (EC) 1881/2006 (50 ìg/kg) [8].

Janotova *et al.* (2011) [9] studied the effect of processing of apple puree on patulin content. Apple samples taken into study were spiked with patulin at four levels of concentration (539 mg/kg, 140 mg/kg, 23 mg/kg and < 2 mg/kg). It was found that all technological operations of the manufacturing process of apple puree contribute to the reduction of patulin level. After apples washing, it was registered a differential reduction of patulin contamination level in the range -50%, depending on the initial patulin concentration and the degree of microbiological contamination (visible fungus growth on the surface versus fungus only inside the apple). Patulin concentration in washing water was in the range 14 - 50 mg/L. Pulping technological operation determined a reduction from 29 to 80% of the initial content. However, in the study conducted by Janotova et al. [9], apple puree samples with patulin concentration of 23 and 140 μg/kg, and thermal treated at 90°C, for 30 min, registered a decreasing of patulin concentration of 17.4 and 20.7%, respectively. For these puree samples, after the applied thermal treatment, the authors found a negative influence on color.

Kokkinidou *et al.* (2014) [10] studied kinetics of the thermal degradation of patulin in apple juice (model system consisting of 0.5% malic acid adjusted to *p*H 3.75) in the temperature range 25 °C-85 °C, with and without added ascorbic acid. Results showed that ascorbic acid increased the rate of patulin degradation ($p < 0.05$). The zero-order kinetics model was adequate for the degradation of patulin in the absence of ascorbic acid, and the non-linear Weibull model described the reaction of patulin-ascorbic acid for all the temperatures. This study confirmed that patulin stability decreased in the presence of ascorbic acid.

Li *et al.* (2014) [11] studied removal of patulin from aqueous solution using cross-linked chitosan beads. The results showed that maximum adsorption capacity of chitosan beads for patulin was 626.4 μg/g at *p*H 7.0, 40 °C for 24 h. Kinetics of the adsorbtion process of patulin on chitosan beads could be described by pseudo second-order model and Freundlich adsorption isotherm model, and

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chitosan beads were very good biosorbent for patulin removal from aqueous solution.

The purpose of this research was to study the thermal stability of patulin in apple puree and also to establish the possibilities for reduction of patulin contamination level through processing of contaminated apples to puree and nectar. The study undertaken aimed to establish to what extent apples contaminated with patulin can be used in the industrialization process, so as to obtain final products with patulin content below the maximum level allowed by the legislation into force.

## Experimental part

*Materials and methods*

Food matrices and samples

In order to study the influence of thermal treatment on patulin stability in apple puree, within the Pilot Experiments Plant for Fruits and Vegetables Processing of INCDBA- IBA Bucharest it was prepared apple puree from *Jonathan* apple cultivar (purchased from commerce), in which patulin was not detected after HPLC-DAD analysis. Technological flow for obtaining apple puree included the following operations: washing, cutting, blanching, pulping and deaeration. Apple puree was spiked with patulin in the following concentrations: 10, 20, 40 and 80 μg/kg (using a solution with patulin concentration of 10 mg/mL). For preventing enzymatic browning in apple puree citric acid (0.2 g/kg puree) was added. Homogenisation of apple puree with citric acid and patulin solution was made with a Braun vertical mixer. After homogenisation, apple puree spiked with patulin was manually dosed in glass recipients of 220 mL capacity, ermetically closed (Twist–off system) with help of a semi-automat system (Tecmon Italy), pasteurized at 100 °C in a cooking kettle (Tecmon Italy), for different time periods (V1 -10 min, V2 - 15 min, V3 - 20 min and V4 - 25 min) and cooled at 40 °C.

To study reduction of patulin contamination, by processing contaminated apples to puree and nectar, apples from *Golden Delicious* cultivar, for industrialization, attacked by *Penicillium expansum* were used. In order to expand the contamination, apples were maintained at room temperature for 7 days. Technological flow for manufacturing apple puree had the following operations: removing of moldy apple tissue, washing, cutting, blanching, pulping, deaeration and dosing. Then, it was pasteurized at 100 °C in a cooking kettle (Tecmon Italy), for different time periods (V1 – 10 min, V2 - 15 min, V3 - 20 min and V4 - 25 min) and cooling at 40°C. The areas attacked by mold were removed using a stainless steel knife. Washing operation was done with water, in an inox basine, using a plastic brush, then, apples were rinsed under running cold water. Cutting operation of apples in quarters was done manually using a stainless steel knife, and blanching operation was performed in cooking kettle with water at 100°C, for about 4 min. At the same time, unpasteurized apple puree was used to obtain apple nectar based on the following technological flow: preparation of sugar syrup with soluble dry matter of 16.7 °Brix, blending, deaeration, dosing in recipients of 330 mL capacity, closing, pasteurization for different time periods (V1 – 13 min, V2 - 16 min and V3 - 19 min) and cooling at 40 °C.

Samples of apple, apple puree and apple nectar were chemical-physical analysed using the following methods:

- SR ISO 2173:2008. Fruit and vegetable products. Determination of soluble dry matter by refractometry
- SR ISO 1842:2008. Fruit and vegetable products. Determination of *p*H.

Samples of apple puree and apple nectar, prepared from apples from *Golden Delicious* cultivar, were microbiological analysed using the following methods:

- SR ISO 21527-1:2009 Microbiology – General directives for enumeration of yeasts and moulds. Part 1. Colony-count technique in products with water activity higher than 0.95. Part 2. Colony-count technique in products with water activity less than or equal to 0.95.
- SR ISO 21528-2:2007 Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of *Enterobacteriaceae*. Part 2: Colony-count method.

*Determination of patulin*

Reagents and materials

Glacial acetic acid, ethyl acetate and methanol of HPLC grade were purchased from Sigma-Aldrich. Optigrade acetonitrile was purchased from LGC Standards and ultrapure water was obtained in house using ELGA water ultrapurification system. For calibration curves, a solid analytical patulin standard (5 mg, purity = 99.5%) was obtained from Sigma-Aldrich.

AFFINIMIP® SPE Patulin cartridges (6 mL-200 mg) and pectinase enzyme have been obtained from AFFINISEP – POLYINTELL.

Sample preparation

The steps of the method for determination of patulin in apple puree and nectar were: homogenization of sample, weighting, enzyme treatment, centrifugation, extraction and clean-up on AFFINIMIP® SPE Patulin cartridges, extract evaporation, residue redissolving and HPLC-DAD analysis. Sample preparation is based on Application note - AFFINIMIP® SPE *Patulin* 6 mg/200 mg - apple puree [12]. In a 50 mL centrifuge vial, 10 g of apple puree/apple nectar sample, 150 ìL of pectinase enzyme solution and 10 mL ultrapure water were mixed. Sample is maintained in a water bath at 40 °C for 2 hours, then is centrifuged at 6000 rpm, at 5 °C, for 25 min. The supernatant (5 mL) is purified on AFFINIMIP® SPE *Patulin* cartridge, which has been preconditioned with 2 mL acetonitrile and 1 mL of ultrapure water. In order to remove interferences, 4 mL of 0.1% acetic acid and 4 mL of ultrapure water were passed through the cartridge, then applied a vacuum for about 10s. Further, 500 μL ethyl ether were passed through the cartridge and finally patulin was eluted with 2 mL acetonitrile containing 0.1% acetic acid. The elution fraction was evaporated to near dryness under a nitrogen atmosphere at 40$^0$C, then re-dissolved in acetonitrile:ultrapure water (*p*H = 4) = 10:90.

The steps of the method for determination of patulin in apples were: sample preparation and homogenization, weighting, enzyme treatment, centrifugation, extraction and clean-up on AFFINIMIP® SPE Patulin cartridges, extract evaporation, residue redissolving and HPLC-DAD analysis. Sample preparation is based on Application Notebook for AFFINIMIP®SPE - Determination of patulin in whole apple [13]. After removal of apple core, apples were cut in pieces and homogenized into a blender with ultrapure water (apples:water = 2:1) for about 2 min. In a 50 mL centrifuge vial, 15 g of apple sample and 300 ìL of a pectinase enzyme solution were mixed. Sample was maintained in a water bath at 40°C for 2 h, then is centrifuged at 6000 rpm, at 5 °C, for 25 min. The supernatant (3 mL) was purified on AFFINIMIP® SPE *Patulin* cartridge, which was preconditioned with 2 mL acetonitrile and 1 mL ultrapure water. In order to remove interferences, 3 mL of 2% acetic acid were passed through the cartridge, then applied a

| Sample name | Recovery (%) | | |
|---|---|---|---|
| | c < 20 (µg/kg) | c = 20 - 50 (µg/kg) | c > 50 (µg/kg) |
| Apple | 92.57 | 83.10 | 76.85 |
| Apple puree | 91.36 | 81.60 | 75.17 |
| Apple nectar | 93.85 | 84.45 | 77.34 |

** c - patulin concentrations*

**Table 1**
RECOVERY IN THE CASE OF METHODS FOR DETERMINATION OF PATULIN IN APPLE, APPLE PUREE AND NECTAR

**Table 2**
REPEATABILITY OF THE METHODS FOR DETERMINATION OF PATULIN IN APPLE, APPLE PUREE AND NECTAR

| Sample name | Spiking level (µg/kg) | RSD(r) (%) |
|---|---|---|
| Apple | 10 (n = 6) | 7.85 |
| | 40 (n = 6) | 3.12 |
| Apple puree | 10 (n = 6) | 6.40 |
| | 40 (n = 6) | 2.39 |
| Apple nectar | 10 (n = 6) | 8.37 |
| | 40 (n = 6) | 3.85 |

vacuum for about 10s. Further, 250 µL ethyl ether were passed through the cartridge, a vacuum for about 10s was applied, and finally patulin was eluted with 2 mL acetonitrile containing 0.1% acetic acid. The elution fraction was evaporated to near dryness under a nitrogen atmosphere at 40 $^0$C, then re-dissolved in acetonitrile:ultrapure water (*p*H = 4) = 10:90.

*Parameters and conditions of HPLC-DAD method for determination of patulin in apple puree*

A *Surveyor Plus* (*Thermo Finnigan)* high performance liquid chromatograph (vacuum degasser, quaternary pump, autosampler with PELTIER sample temperature control, column compartment with PELTIER temperature control, Diode Array Detector, ChromQuest 4.2 software for data acquisition and data processing) was used. The separation was performed at 25°C, on a C18 (Hypersil GOLD 150 x 4 mm, 5µm) with a Hypersil Gold guard column (10 x 4 mm, 5 µm). The composition of mobile phase was water: acetonitrile (95:15, v/v). The injection volume was 25 µL, the flow rate of the mobile phase was 1.0 mL/min and the detection wavelength was 276 nm. Peak identification was based on retention time, spectral information and spiking technique. Peak quantification was based on the external standard method, using calibration curve [14].

*Validation studies*

Performance criteria of the method for determination of patulin in apple, apple puree and apple nectar were in accordance with the Regulation (EC) No 401/2006 [15]. Main performance parameters of the method were evaluated after analysis of apple, apple puree and apple nectar, spiked with patulin, at seven concentration levels (5, 10, 20, 40, 50, 60 and 80µg/kg). These samples were analyzed to determine patulin concentration (each concentration level was analyzed in 6 parallel prepared samples).

The obtained values for recovery for the three matrices taken into study are presented in table 1. Also, the repeatability RSD(r), and the limit of detection (LOD) and the limit of quantification (LOQ) are presented in table 2 and table 3, respectively.

According to the obtained results, recovery for the ranges of concentrations mentioned in table 1 and the repeatability RSD(r) mentioned in table 2 were under the provisions of the Regulation (EC) no. 401/2006 of 23 February 2006 [15].

**Table 3**
LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION OF THE METHODS FOR DETERMINATION OF PATULIN IN APPLE, APPLE PUREE AND NECTAR

| Sample name | Limit of detection (µg/kg) | Limit of quantification (µg/kg) |
|---|---|---|
| Apple | 1.31 | 4.51 |
| Apple puree | 1.36 | 4.55 |
| Apple nectar | 1.27 | 4.41 |

*Statistical analysis*

Data were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey test were applied to determine difference between means. Differences were considered to be significant at $p < 0.05$.

## Results and discussions

Apple puree used in experiments to study the influence of thermal treatment on patulin stability had a soluble dry matter of 9.3 °Brix and *p*H = 3.44. Thermal treatment (at 100 °C) applied to apple puree spiked with patulin, to assure preservation, determined a differential reduction of patulin content depending on two variable factors: patulin concentration and time period of thermal treatment. Thus, after 10 min of pasteurization, patulin concentration decreased in the range 19.68- 23.35% (minimum value was registered for the apple puree spiked with 10 µg/kg patulin, and the maximum one for the apple puree spiked with 80µg/kg patulin). However, the application of pasteurization for 20 min resulted in a reduction of patulin concentration with 28.78 and 31.03% to initial concentration, in case of apple puree spiked with 10 µg/kg patulin and spiked with 80 µg/kg patulin, respectively. Noteworthy is the fact that after pasteurization time period of 25 min, a reduction of patulin concentration of apple purees ranging from 32.58 to 34.68% was achieved. Changing of their color due to non-enzymatic browning was also observed. Results obtained are in accordance with those obtained by Janotová et al. (2011) [9]. They found a reduction of patulin content with 8.6 to 17.9%, and also a negative influence on color of apple puree due to high temperature, after maintaining of the apple puree contaminated with patulin (initial concentration of 23 and 140 µg/kg) at 90°C, for 30 min. Reduction of patulin concentration in processed apple products, thermal treated, is determined by degradation reaction of patulin with sulfites, thyols and other compounds in these food matrices [16].

For apple purees spiked with 10, 20, 40 and 80 µg/kg patulin, respectively, the pasteurization time period had a significant influence ($p < 0.05$) in patulin reduction. Thus, the higher the pasteurization period, the higher patulin reduction is (fig. 1). Apple purees spiked with 20 and 40 µg/kg patulin, respectively, and at a pasteurization time period of 15 min, did not significantly differ ($p > 0.05$) in patulin reduction. For pasteurization time period of 20 min, there were no significant differences ($p > 0.05$) in patulin reduction in apple purees spiked with 10 and 20 µg/kg patulin, respectively. The same trend was also observed for apple puree spiked with 40 and 80 µg/kg patulin,

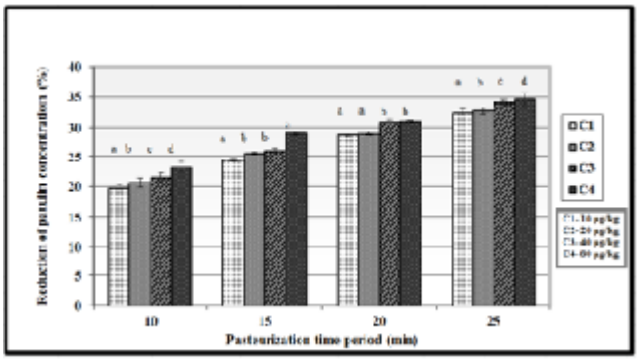


Fig. 1. Reduction of patulin content of apple purees spiked with patulin, through pasteurization at 100 °C. Results are expressed as mean ± standard deviation (n=6). Different letters mean significant differences ($p < 0.05$) among samples for the same pasteurization time period

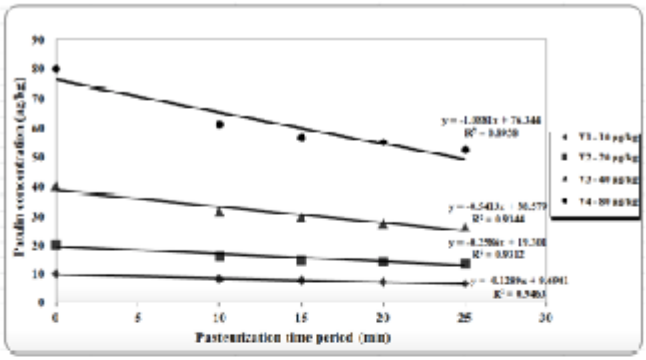


Fig. 2. Variation of patulin concentration depending on the time period of thermal treatment in case of apple purees spiked with patulin (10 - 80 µg/kg)

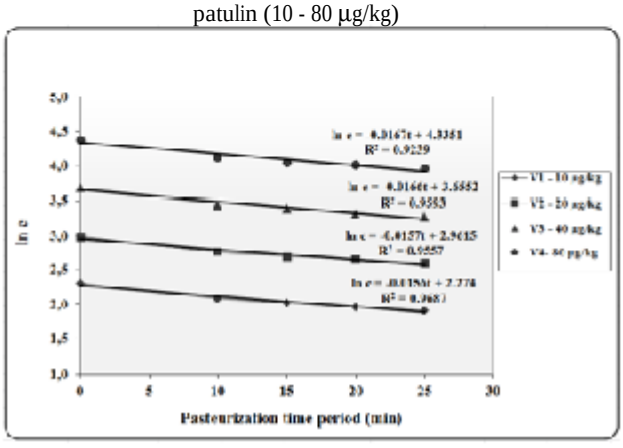


Fig. 3. Thermal degradation of patulin in case of apple purees spiked with patulin (10 - 80 µg/kg)

respectively. Comparing the apple puree spiked with 10 µg/kg patulin with that spiked with 80µg/kg patulin, there are significant differences ($p < 0.05$) in patulin reduction for all the pasteurization time period investigated (10, 15, 20 and 25 min).

Regarding the experiments performed for evaluation of thermal stability of patulin in apple puree, a linear dependence was found bettween the patulin concentration in apple puree and time period of the thermal treatment applied at 100°C; *the regression coefficient* $R^2$, ranging from 0.8958 to 0.9463 (fig. 2).

However, there is a linear dependence between the natural logarithm of patulin concentration of apple puree and time period of the thermal treatment applied at 100 °C; *the regression coefficient* $R^2$ ranging from 0.9239 to 0.9687 (fig. 3).

Based on the regression equations describing the linear dependence and taking into account the values of the *regression coefficient* $R^2$, resulted that thermal degradation of patulin at 100 °C, followed the kinetics of a first order chemical reaction (1):

$$\ln c = \ln c_0 - kt \quad (1)$$

where:

c - patulin concentration in apple puree, expressed as µg/kg

$c_0$ - initial patulin concentration, expressed as µg/kg

t - pasteurization time period at 100 °C, expressed as min

From the slope of the straight lines ln c = f(time), the rate constant (k) of the thermal degradation reaction of patulin from apple purees subjected to pasteurization at 100 °C was obtained. Thus, k was 0.01516 ± 0.0006 $min^{-1}$ (mean arithmetic rate constants obtained in case of apple purees with initial patulin concentrations of 10, 20, 40 and 80 µg/kg: 0.0167, 0.0166, 0.0157 and 0.0156, respectively). Kokkinidou *et al.* (2014) [10] studied kinetics of the thermal degradation reaction (in the temperature range 25 °C to 85 °C) of patulin in the presence and absence of ascorbic acid. According to the obtained results, thermal degradation of a patulin solution with initial concentration $C_0$ = 6.40 µM and *p*H = 3.75 (this *p*H is characteristic to apple juice, being reported by Mattick and Moyer (1983) [17]), followed a kinetics of zero order. In thermal degradation reaction of patulin, in the temperature range 25 to 85 °C, rate constant varied in the range: 0.0024 ± 0.0003 M . $h^{-1}$ - 0.0138 ± 0.0037 M . $h^{-1}$. In this study for the apple purees with no ascorbic acid into composition, the thermal degradation reaction of patulin followed a kinetics of first order. These results could be explained by the effect of the matrix.

Technological operations for apple processing have an important role in reduction of patulin contamination of the final products (juice, concentrated juice, puree etc.). In this study, apples from *Golden Delicious* cultivar, attacked by *Penicillium expansum,* were processed to puree and nectar. After technological operations of removal of moldy apple tissue, washing and cutting, patulin content of apples was 532.43 µg/kg. To this concentration, the influence of the technological operations of blanching and pulping, in reduction of patulin content will be reported. Within the performed experiments, technological operations of blanching and pulping, determined a reduction of patulin concentration with 57.17%. Patulin concentration of the achieved apple puree (unpasteurized) was 228.04 µg/kg. These results are according to those obtained by Janotová et al. (2011) [9], which reported a reduction of patulin content from 29 to 80% (to the initial concentration), after pulping operation. Pasteurization of apple puree at 100 °C, for 10 to 25 min, determined a reduction of patulin content with 18.55 to 33.19%. The most important reduction was registered after the first 10 min of pasteurization, namely 18.55% (fig. 4). After a thermal treatment of 25 min, a

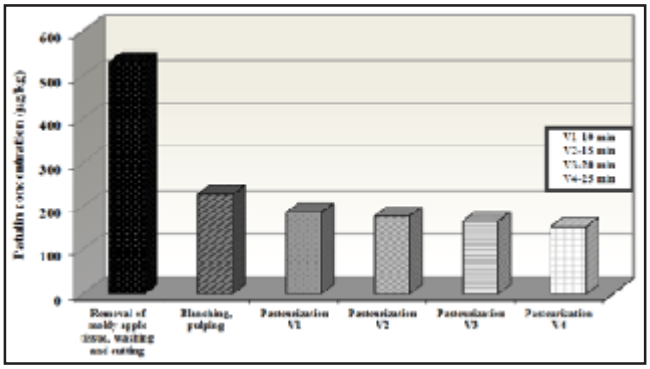


Fig. 4. Influence of processing on patulin content of apple puree

degradation of apple puree color due to the non-enzymatic browning was found. Increasing pasteurization time period in apple puree from 10 to 25 min produced a significant increase ($p < 0.05$) in native patulin reduction.

In the performed experiments, by processing of apples from Golden Delicious cultivar, attacked by Penicillium expansum, even on the technological flow are registered significant decreases of patulin content, finally it was obtained an apple puree contaminated with patulin (185.74 µg/kg in case of pasteurization for 10 min and 165.37 µg/kg after pasteurization for 20 min), exceeding the maximum level of the Regulation (EC) 1881/2006 (25µg/kg) [8].

Thermal degradation (at 100 °C), of patulin from apple puree contaminated with native patulin respected a kinetic of a first order chemical reaction. It was a linear dependence (y = -0.0155x + 5.4108), between the natural logarithm of patulin concentration of apple puree and time period of the applied thermal treatment. The *regression coefficient* was $R^2 = 0.9803$ (fig. 5). Rate constant of the reaction of thermal degradation of patulin from apple puree contaminated with native patulin was k = 0.0155 $min^{-1}$.

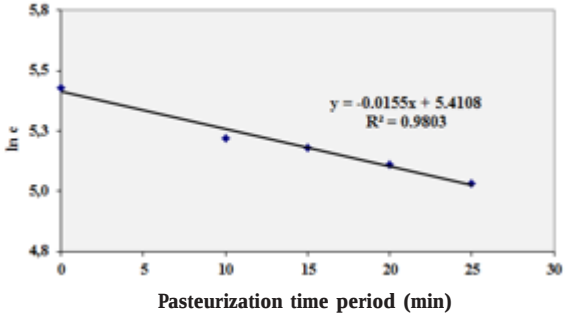


Fig. 5. Thermal degradation of patulin in case of a contaminated apple puree with native patulin 228.04 µg/kg

Apple puree is a valuable foodstuff for the nutrition of infants and children, but, in the same time, it is an important ingredient for nectars and jams producing. Therefore, patulin content of apple puree has to be carefully controled taking into account toxicity of this mycotoxin. In the performed study, the unpasteurized apple puree, contaminated with patulin (concentration of the native patulin of 228.04 µg/kg), was used to prepare apple nectar with a content of 25% pulp, soluble dry matter of 14.6 °Brix and *p*H = 3.27.

Technological operations of blending of sugar syrup and lemon juice with apple puree and deaeration of the obtained nectar determined a reduction of patulin content 77.77%. Deaeration of apple nectar was achieved by its boiling for about 2 min. Technological operation of pasteurization of apple nectar, at 100 °C, in the time period ranged from 13 to 19 min, determined a reduction with 28.11 to 35.17% of patulin level (fig. 6).

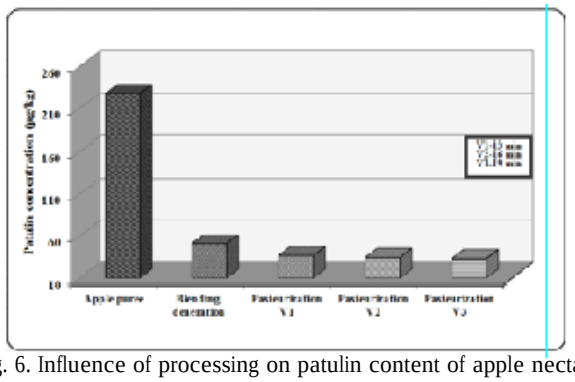

Fig. 6. Influence of processing on patulin content of apple nectar

Apple nectar pasteurized for 13 min had a patulin content of 36.45 µg/kg, being under the maximum level of the Regulation (EC) 1881/2006 (50 µg/kg) [8], and the apple nectar, pasteurized for 19 min had a patulin content of 32.87 µg/kg, but a significant non-enzymatic browning. Increasing pasteurization time period in apple nectar from 13 to 19 min produced a significant increase ($p < 0.05$) in native patulin reduction.

In the conducted study, the thermal degradation of patulin in apple nectar followed also a kinetics of first order, the rate constant being k = 0.0235 $min^{-1}$ (fig. 7).

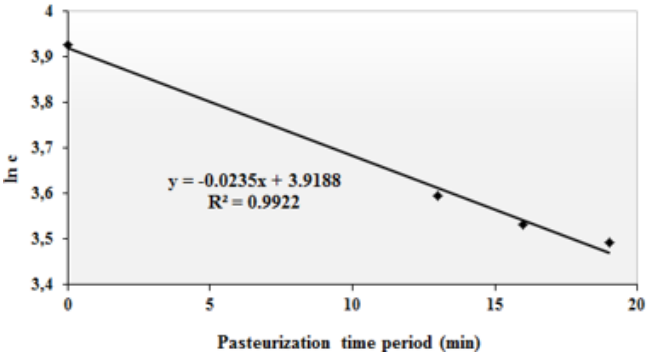


Fig. 7. Thermal degradation of patulin in case of apple nectar contaminated with native patulin 50.7 µg/kg

According to the microbiological analysis, apple puree and apple nectar obtained by processing of apples from *Golden Delicious* cultivar, attacked by *Penicillium expansum,* showed no contamination with molds and *Enterobacteriaceae* (Molds < 10 cfu/g, *Enterobacteriaceae* < 10 cfu/g).

## Conclusions

Experiments performed showed that it was possible a reduction of patulin contamination by processing the contaminated apples to apple puree and apple nectar, respectively. But, in order to obtain safe products for the consumers, it is very important the initial contamination level of apples. Patulin is a marker of apple and processed apple products quality, and concentration of this mycotoxin has to be carefully verified by rapid and sensitive methods.

In the performed study it was highlighted the fact that the thermal treatment at 100°C, applied to apple puree from *Jonathan* apple cultivar, spiked with patulin, in the concentrations range 10 - 80 µg/kg, for 10 - 25 min, determined a reduction of patulin concentration from 19.68 to 34.68%. Application of a thermal treatment at 100 °C, a time period higher than 20 min (for glass recipients of 220 mL capacity), it is not feasible at industrial level, because determines a degradation of color of apple puree, due to the non-enzymatic browning.

Through the processing of apples from *Golden Delicious* cultivar, attacked by *Penicillium expansum,* to puree, it was obtained a reduction of patulin concentration with 57.17%, following the application of technological operations of blanching and pulping. Also, it was highlighted the fact that pasteurization at 100 °C of the obtained apple puree (dosed into recipients with 220 mL capacity), for 10 to 25 min, determined a reduction of patulin level with 18.55 to 33.19%. Application of the thermal treatment at 100 °C for a time period higher than 20 min, determined a pronounced degradation of the color of apple puree.

By application of the specific technology for nectar production, using an apple puree from *Golden Delicious* apple cultivar, contaminated with native patulin, at a level of 228.04 µg/kg, an apple nectar (packed into glass

recipients of 330 mL capacity) with a patulin content lower than 50 μg/kg was obtained (maximum level allowed by the Regulation (EC) 1881/2006 [8]). Blending of sugar syrup and lemon juice with apple puree and deaeration by boiling for 2 min of nectar, determined a reduction of patulin content by 77.77%, and pasteurization at 100 °C, for 13 to 19 min, determined a reduction of patulin level with 28.11 to 35.17%. A time period for nectar pasteurization higher than 16 min, determined a pronounced non-enzymatic browning.

Thermal degradation at 100 °C, of patulin in apple puree and nectar taken into study followed kinetics of a first order chemical reaction.

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