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EFFECT OF PEELING AND SANITIZING ON PATULIN LEVEL IN RECONSTITUTED ANNA APPLE JUICE

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ABSTRACT: The mycotoxin patulin is a common contaminant of apple products that poses a health risk to consumer. Control of patulin in Anna apples grown in Egypt was investigated using peeling step and washing treatment with some sanitizers. Results obtained from Anna apple juice were compared with reconstituted apple juices prepared from imported concentrates.

Peeling of apples before processing decreased the content of titratable acidity and vitamin C, while pH and sugar/acid ratio was increased. Quality parameters of reconstituted Anna apple juice was comparable to that prepared from one imported concentrate, but differed from the other one. Patulin level in Anna apple juice prepared from fresh fruits were 12.96 ppm while that prepared from spoiled apples contained 16.28 ppm. Imported apple juice concentrates resulted in reconstituted juices containing higher patulin level than that permitted by the international standard (50 ppb). Peeling of Anna apples led to complete removal of patulin from the resultant juice. Washing of apples with sodium hypochlorite solution and with H_2O_2 solution resulted in lowering of the patulin level by 97.5 and 98%, respectively. Complete elimination of patulin from apple juice was achieved when the fruits were washed with citric acid solution (pH 2.3).

The possibility of the detoxification of patulin by citric acid is discussed. It is recommended that for processing of Anna apples into apple juice the fruits must be subjected to washing with citric acid solution (pH 2.3). The peeling step, which alters quality parameters of the resultant juice, is not required.

Key words: Apple juice, apple concentrate, patulin, sanitizers.

INTRODUCTION

Patulin, a mycotoxin produced primarily by *Penicilium expansum*, is considered as a common contaminant and food safety hazard in apple fruits and apple juice establishments (Siliha and Askar, 1999). Since patulin is only partially destroyed during pasteurization, its presence in the juice is a good indicator of the quality of the fruits used for processing (Sylos and Rodriguez, 1999).

Animal tests revealed that patulin has a wide spectrum of activity and can act as carcinogenic, mutagen and teratogenic agent (Acar, 1993 and Mc Callum *et al.*, 2002). Moreover, patulin induces intestinal injuries including epithelial cell degeneration, inflammation, ulceration and hemorrhages (Mahford *et al.*, 2002). The immunosuppressive effects of patulin have been demonstrated in various live-stock species (e.g. turkey, chicken, and pigs) and also laboratory animals (Sharma, 1993).

Patulin is an unsaturated water soluble lactone, 4-hydroxy-4H-furo-(3,2-c)-pyran-2(6H)-one, has a molecular weight of 154. It may be formed on some vegetables and animal food stuffs (especially cheese) but it can not be formed, nor remain stable in some vegetables such as cabbage, radish, celery, onion and some juices such as orange juice (Askar and Siliha, 1999). In rotten apples the level of patulin may reach as high as 1120ppb. In a long term survey of the concentration of patulin in apple juice concentrates produced in Turkey. Gokmen and Acar (2000) found that the average patulin levels were 63, 43, 19 and 31 ppb in 1996, 1997, 1998 and 1999, respectively, while the percentage of concentrates exceeding the maximum permitted concentration of 50 ppb were 52, 34, 8 and 8%. Samples of apple juice prepared in laboratory from apples showing light, moderate and severe fungal spoilage contained 27 ppb, 75 ppb and 2.6 g/ L, respectively (Steiner *et al.*, 1999). The analysis of 111 processed fruit juice samples and 38 samples of whole fruit indicated that only 1 out of 30 samples of apple juice was positive for patulin (17 ppb) but not detected in other fruit juice samples (Sylos and Rodriguez, 1999). It was identified in 6 apple fruit samples (150 – 267 ppb), 4 pear samples (134 – 245

ppb) and 4 peach samples (92 – 174 ppb). Martins *et al.,* (2000) analyzed 351 samples of seven different varieties of apples with small rotten areas (Casanova, Golden Delicious, Red Delicious, Reineta, Richared, Rome Beauty and Starking) for the presence of patulin and citrinin using rapid multidetection thin layer chromatography method. Their data showed that the percentage of contamination with patulin only was higher (68.6%) than that with citrinin only (3.9%). Percentage of samples contaminated with both mycotoxins was 19.6%. The highest mean patulin content was 80.50 ppm for Richared variety, while the lowest mean contamination of patulin was found in Rome Beauty, Red Delicious, and Reineta ranging from 3.06 to 5.37 ppm. The patulin concentration in juice samples produced with Starking apples that were sound, 30, 60 and 100% decayed ranged between 0.0 - 15.9 ppb, 47.1 – 560.3 ppb, 156.4 – 2257.5 ppb and 54.9–2508 ppb, respectively (Kadakal and Nas, 2002).

The elimination of both patulin and the mould producing it from apples distained for processing has been the subject for several researches. Controlling the storage conditions of apple fruits before processing is the first effective measure for reducing patulin level (Siliha and Askar, 1999). The limited availability of suitable storage facilities may result in fruit being subjected to storage in the open (deck storage) for extended periods of time prior to processing (Sydenham *et al.,* 1997). Storage of apples under 3% CO_2 and 2% O_2 at 25°C totally inhibited patulin production (Paster, *et al.,* 1995). The implementation of good manufacture practices during processing of apple juice is a powerful tool for reducing patulin contamination. Mean patulin level in non-processed fruit ranged between 90 – 2445 ppb, which was dropped to values ranging between 75 and 695 ppb following water wash step (Sydenham *et al.,* 1997). Subsequent removal of rotten and damaged fruits decreased patulin further to 55 and 450 ppb. Acar *et al.,* 1998) studied the effect of different treatments (including washing, handling, clarification, filtration and concentration) on patulin content of apple juice and found that washing and handling were the most critical steps in removing patulin, since up to 54% of patulin could be removed using

high pressure water spray. Results obtained by Jackson *et al.* (2002) indicated that the avoidance of using ground-harvested apples and the careful culling of apples before pressing are good methods for reducing patulin levels in apple cider. However, it should be stressed that in rotten apples not only was the amount of patulin very high in the rotten areas (Beretta *et al.*, 2000), but the mycotoxin could be spread to areas unaffected by fungus.

The effect of some chemicals and sanitizers, frequently used in fruit processing, on patulin level in apple juice have been investigated. Compared to sorbic acid, formic acid and benzoic acid Podgorska, (1992) found that sulfur dioxide had the most inhibiting effect on mycelium growth and patulin production. On the other hand, Steiner *et al.* (1999) showed that decomposition of patulin by ascorbic acid was much quicker than decomposition by SO_2, as the maximum decomposition by ascorbic acid was 97.7%, while maximum decomposition by SO2 was 34.7%. Addition of thiaminehydrochloride (1g/kg), pyrodoxine hydrochloride (625 or 875 mg/kg) and calcium-d-pantothenate (1 or 2.5 g/kg) into apple juice concentrate and storage at 4°C for 6 months yielded 55.5 to 67.7% of patulin reduction (Yazici and Velioglu, 2002). Burrowes *et al.* (2002) examined the effect of Pleurocidin, a natural antimicrobial peptide isolated from edible winter flounders, on patulin producing *Penicillium expansum* isolated from rotten apples and found that the minimal inhibiting concentration was 20.6 μM. Electrolyzed-oxidizing water possessed significant activity against *Penicillium expansum* and could be useful sanitizing agent in operations such as apple processing where the organism is of concern (Okull, *et al.*, 2003). Most recently Moake *et al.* (2005) presented a comprehensive review of patulin control methods in foods.

The aim of the present work was to examine the effect of peeling and washing with some sanitizing substances on quality parameters and patulin level of juice of Egyptian Anna apples as compared with juice prepared from imported apple concentrates.

MATERIALS AND METHODS

Materials

Anna apple fruits (cultivated in Egypt) were obtained from a local market in Zagazig city. The fruits

were packed in carton boxes in 2 layers and stored at 0 ±1°C and 90-95% relative humidity for 3 months in a post harvest refrigerator in the Department of Food Science, Faculty of Agriculture, Zagazig University. Pectinex ultra spl, a commercial pectolytic preparation, was kindly provided by Novo Nordisk Ferment AG (Dittingen, Switzerland). Sodium hypochlorite, hydrogen peroxide and citric acid were food grade chemicals. Two imported apple juice concentrates were kindly provided by 2 juice factories. To maintain confidentiality the two imported apple juice concentrates were coded A and B.

Methods

Preparation of Anna Apple Juice Concentrate

Sanitizing of apple fruits was carried out by washing fruits in 200 ppm sodium hypochlorite (Chlorine), 5% H_2O_2 or citric acid solution of pH 2.3. Peeling of apple fruits was carried out by hand, taking care not to contaminate the pulp. Fresh, peeled or sanitized apples were mashed in electric meat grinder. Apple mash was treated with 200 ppm pectinx ultra spl and the enzymatic reaction was performed at 30°C for 2 hrs. The mash was raped in 3 layers of cheese cloth, pressed using manual fruit press and the free running juice was heated for 15 min at 85°C to terminate the enzymatic reaction and to aid in juice clarification. The juice was left undisturbed in refrigerator overnight and the clear juice was decanted. The clear apple juice was concentrated in a vacuum rotary evaporator (Büchi Rotavapor R-124), equipped with vacuum pump, at 40°C and 28-30 Hg to final 70°Brix total soluble solids. Reconstituted apple juices from Anna apple juice concentrates and from imported concentrates were prepared by dilution with distilled water to 13°Brix total soluble solids.

Analytical Methods

Total soluble solids, titratable acidity, pH, vitamin C and browning index (at 420 nm) were determined according to AOAC (1996).

Determination and Extraction of Patulin

Extraction of patulin was performed according to Burda (1992). One gram of juice was extracted with two 5ml portions of ethyl acetate. The combined ethyl

acctatc cxtract was dried using sodium sulfate and evaporated to near dryness under a gentle stream of nitrogen. The residue was dissolved in 1ml of 2% acetic acid. Patulin level in the extract was measured by HPLC technique using Waters HPLC System (USA) equipped with Nucleosil – C18 column 250x4mm (Machercy-Nagel, Germany) and UV detector at 276nm. The mobile phase was acetonitrile/water (15:85) at flow rate 0.5ml/min. An external standard of patulin was used to calculate the levels of patulin. The results were integrated using Mellinium 2010 software program.

Determination of Sugars

Sucrose, glucose and fructose concentrations were determined by HPLC technique using Aminex Carbohydrate HPX-87C column and refractive index detector (HP 1047A). Deionized water was used as mobile phase at flow rate 0.8ml/min. Juice samples were filtered prior injection through 0.4μm disposable filter.

RESULTS AND DISCUSSION

Quality Parameters of Reconstituted Juices

Table 1 shows some quality parameters of the reconstituted apple juices prepared from peeled and from sanitized apples. All reconstituted juices were made up to total soluble solids (TSS) content of 13°Brix. Titratable acidity ranged between 0.32 and 0.63% (w/v) as malic acid. The highest acidity was observed in apple juice prepared from citric acid-washed apples, while the lowest value was found in juice from peeled fruits. This indicates that Anna apple peels are characterized by high acidity compared to apple pulp. Reconstituted juice prepared after washing apples with sodium hypochlorite solution and 5% H_2O_2 had similar acidity levels. Sugar/acid ratio, which plays an important role in the sensory properties and palatability of fruit juices, was within the standard values (20 to 25) necessary for industrial processing in case of juices prepared from fresh fruits and citric acid-washed fruits. On the other hand, removal of the peels before processing resulted in apple juice with too high sugar/acid ratio (40.63%). Vitamin C content of Anna reconstituted juices ranged from a low of 0.49 mg/100 ml to a high of 0.98 mg/100 ml. Considerable concentration of vitamin C was found to be present in the peel and

its removal resulted in reduction of vitamin C by 40% compared to unpeeled fresh fruit. These results show that Anna apples are poor in vitamin C content when compared to other apple varieties (Frei, *et al.*, 1993).

Sugar composition of reconstituted juices from peeled and sanitized fruits is shown in Table 1 and Fig. 1. The highest sugar concentration in Anna apple juices was fructose. It represented 72.9, 73.3, 62.7, 59.6 and 63.2% of the total sugars found in juices prepared from fresh fruits, peeled fruits and sanitized fruits with chlorine, H_2O_2 and citric acid, respectively. Sucrose content was the lowest sugar. Glucose content of the reconstituted juices ranged between 2.20 and 2.89 g/100ml. These results are in agreement with those reported by Karadeniz and Eksi (2002) who found that sugar content in 51 apple juice samples ranged between 0.85 to 5.51 for sucrose, 0.93 to 3.22 for glucose and 6.61 to 9.60 % for fructose. Moreover, Fuleki *et al.* (1994) found that the range of sugar concentration of varietal juices produced from fresh and stored apples were 1.33 – 4.80; 0.62 – 3.30% for sucrose, 0.70 - 2.27; 0.88 – 2.65% for glucose and 4.12-6.76; 4.27 – 7.43% for fructose, respectively.

Table 2 presents some quality parameters of reconstituted Anna apple juice compared to two reconstituted juices from imported concentrates. Titratable acidity of Anna apple juice was found to be comparable with that prepared from concentrate B, being 0.60 and 0.62%, respectively. Reconstituted juice from concentrate A had lower acidity (0.38%). Similarly, Sugar/acid ratio and pH value of juice from Anna apples and concentrate B were close but differed from that of concentrate A. Vitamin C content of juice from Anna apples was higher than that found in the two imported concentrates. Sucrose content of reconstituted juices prepared from Anna apples, concentrate A and concentrate B was 0.28, 0.07 and 0.28 g/100ml, respectively (Table 2 and Fig. 2). The respective values for glucose and fructose were 2.89 and 8.52; 3.29 and 7.53; and 2.55 and 7.34 g/100ml. The differences between the juices studied can be due to differences between apple fruits or variations in processing, handling and/or storage of concentrates.

Table 1: Effect of Fruit Peeling and Washing with Sanitizing Agents on Some Quality Parameters of Reconstituted Apple Juice

	Fresh fruit	Peeled	Chlorine	H_2O_2	Citric acid
Total Soluble Solids (°Brix)	13.00	13.00	13.00	13.00	13.00
Titratable Acidity% (malic acid)	0.60	0.32	0.40	0.42	0.63
Sugar/Acid Ratio	22.00	40.63	32.50	30.95	20.63
pH	3.94	4.21	4.07	4.06	3.83
Vitamin C (mg/100ml)	0.82	0.49	0.98	0.89	0.82
Sucrose (g/100ml)	0.28	0.21	1.67	1.69	1.52
Glucose (g/100ml)	2.89	2.36	2.20	2.44	2.51
Fructose (g/100ml)	8.52	7.05	6.51	6.10	6.91
Total Sugars (g/100ml)	11.69	9.62	10.38	10.23	10.94

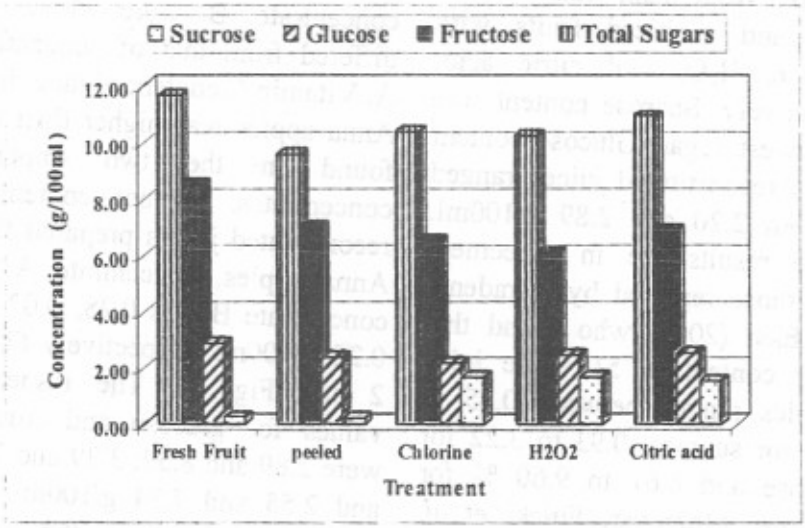


Fig. 1: Effect of fruit peeling and washing with sanitizers on sugar composition of reconstituted apple juice.

(1-Fresh Fruit, 2-Peeled, 3-Chlorine, 4-H2O2, 5-Citric Acid)

Table 2: Some Quality Parameters of Reconstituted Anna Apple Juice Compared to Reconstituted Apple Juices Prepared from Imported Concentrates

	Anna	Concentrate A	Concentrate B
Total Soluble Solids (°Brix)	13.00	13.00	13.00
Titratable Acidity %(malic acid)	0.60	0.38	0.62
Sugar/Acid Ratio	22.07	33.85	20.93
pH	3.94	4.05	3.67
Vitamin C (mg/100ml)	0.82	0.06	0.07
Color Index (A_{420})	1.40	1.25	1.20
Sucrose (g/100ml)	0.28	0.07	0.28
Glucose (g/100ml)	2.89	3.29	2.55
Fructose (g/100ml)	8.52	7.53	7.34
Total Sugars (g/100ml)	11.69	10.89	10.17

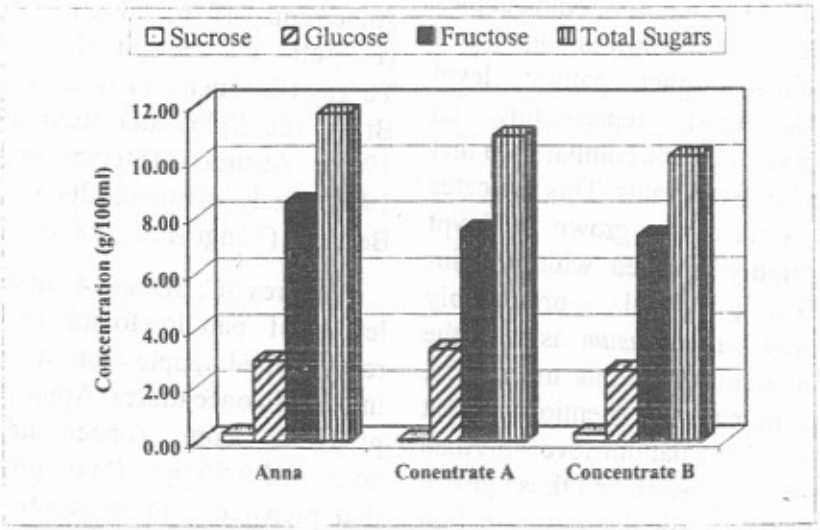
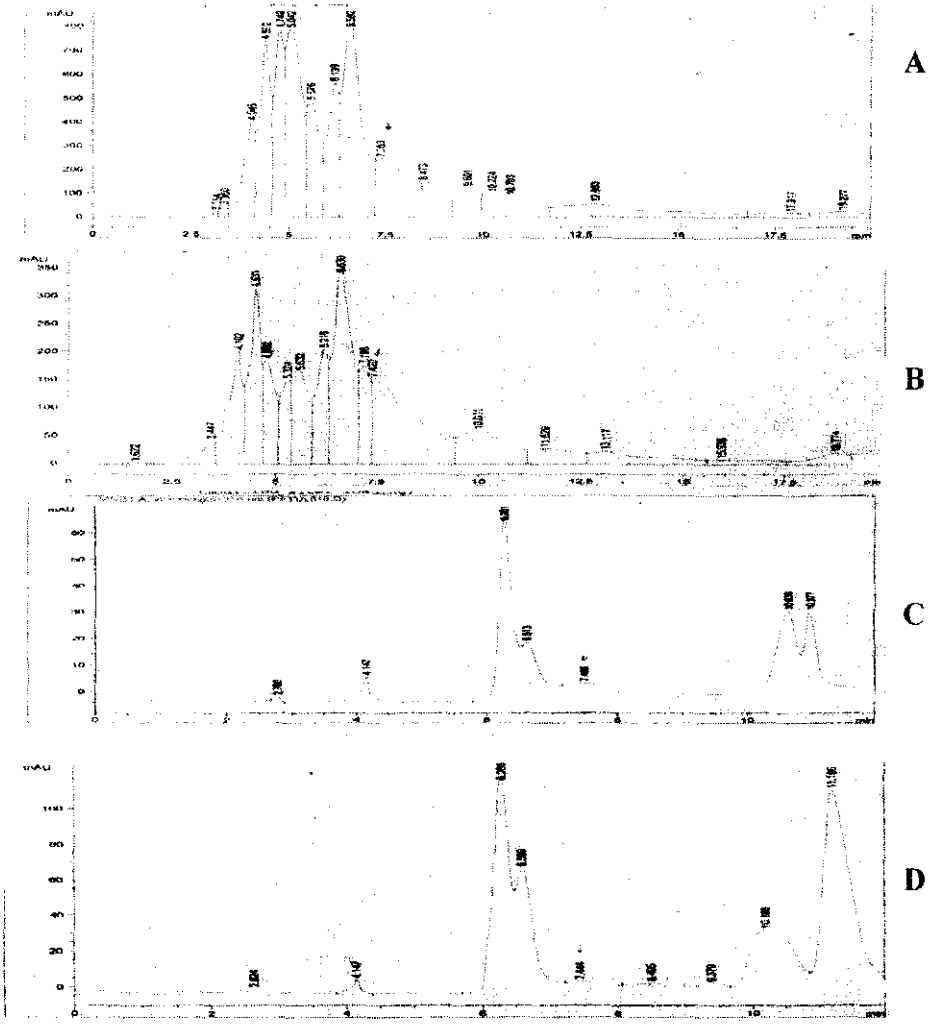


Fig. 2: Sugar Composition of Reconstitued Anna Apple Juice Compared to Reconstituted Apple Juices Prepared from Imported Concentrates

Reduction of Patulin Level

The health risk posed by patulin necessitates its control and reduction from apple products. In this study, removal of patulin from Anna apples grown in Egypt was investigated using peeling step and washing treatment with some sanitizers. Figures 3 and 4 show patulin levels in reconstituted apple juices prepared from fresh and spoiled Anna apples and compared to those prepared from two imported concentrates. Apple juice prepared from fresh apples contained 12.96 ppm patulin (Fig. 3A and 4). Juice from Anna apples which were allowed to spoil contained higher patulin level (16.28 ppm), representing an increase of 25.6% compared to that found in fresh fruits. This indicates that Anna apples grown in Egypt are highly infected with patulin-producing mold, presumably *Penicillium expansum* as it is the main producer of this mycotoxin. It is noteworthy mentioning that the result of patulin level in Anna apples, in general, and those grown in Egypt, particularly, is the first reporting in literature so far. Studies carried out in Egypt by Foaad *et al.* (2002) and Essa and Ayesh (2002) used imported Red Delicious apples which has been reported as one of apple cultivars having the lowest mean contamination of patulin ranging from 3.06 to 5.37 ppm (Kadakal and Nas, 2002). In other varieties such as Richard, Cox's Orange, Pippin, and Bramley the mean contamination of patulin reached as high as 80.50 ppm, whereas golden Delicious was found to be particularly resistant (Corbett, 2003; Kadakal and Nas, 2002 and Northolt, 1978). Moreover, patulin has been identified in apple juices from Canada (Scott *et al.*, 1972), the United States (Ware *et al.*, 1974), Sweden (Josefesson and Andersson, 1976), South Africa (Leggott and Shephard, 2001), Turkey (Gokmen and Acar, 1998), Brazil (de Sylos and Rodrequez, 1999), Australia (Steiner *et al.*, 1999), Italy (Ritieni, 2003) and Belgium (Tangi *et al.*, 2003).

Figures 3C, 3D and 4 show the levels of patulin found in two reconstituted apple juices from imported concentrates. Apple juice prepared from concentrate A contained 0.56 ppm (560 ppb) and that prepared from concentrate B contained 0.334 ppm (334 ppb) patulin. It is clear that these imported concentrates had much higher patulin level than that required by the international standard



A- Fresh Fruit **C- Concentrate A**

B- Spoiled Fruit **D- Concentrate B**

Fig 3: HPLC Chromatogram of the Reconstituted Apple Juices Prepared from Fresh and Spoiled Anna Apples and Imported Apple juice Concentrates

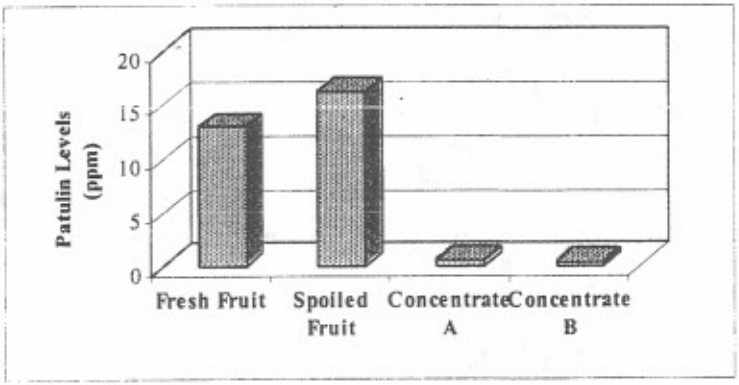


Fig. 4: Patulin Levels in Reconstituted Apple Juices Prepared from Fresh and spoiled Anna Apples and Imported Apple Juice Concentrates

(50 ppb). In the United States, a study showed that 8 out of 13 commercial apple juices contained patulin levels between 44 and 309 ppb, whereas a Turkish study showed that 215 of 215 apple juice concentrates contained between 7 and 375 ppb patulin with 43% of samples being above the international standard (Gokmen and Acar, 1998 and Ware *et al.*, 1974).

The effect of peeling of apples before pressing and the effect of washing of unpeeled apples with sodium hypochlorite solution, diluted hydrogen peroxide and citric acid solution on patulin content of the reconstituted juices are demonstrated in Figures 5 and 6. Peeling of Anna apples led to complete removal of patulin from the resultant juice (Figures 5A and 6). This indicates that in sound unspoiled fruits the mycotoxin is predominantly localized on the peel. Although peeling step would be an extra operation in apple juice processing it proves to be an effective method in the elimination of patulin. Washing of apples with a solution containing sodium hypochlorite lowered the level of patulin in the reconstituted juice to 328.6 ppb (Figs. 5 B and 6). The percentage reduction of patulin compared to unwashed fruits reached 97.5%.

Similarly, washing of apples with H_2O_2 solution resulted in lowering of the patulin level to a value of 258 ppb (Figs. 5C and 6). The percentage reduction of patulin was found to be 98%. In spite of the sharp reduction of patulin by washing with sodium

hypochlorite and H_2O_2 the residual patulin level in the juice remained above the international standard. Sydenham *et al.* (1997) found that the mean patulin level in non-processed fruit ranged between 90 and 2445 ppb, which was dropped to values between 75 and 695 ppb following water wash. High-pressure water spraying removed up to 54% of patulin from apples.

Surprisingly, washing of apple fruits with a solution of citric acid (pH 2.3) showed complete removal of patulin from the reconstituted juice (Figs. 5D and 6). Although the study carried out by Sapers *et al.* (1999) examined the effectiveness of citric acid wash as sanitizing agent in inactivating *Escherichia coli* in Golden Delicious apples, the results presented here is the first mention on the use of citric acid wash for the removal of patulin from apples before juice extraction.

Citric acid has the advantages of being a low price safe GRAS food additive. The fact that citric acid wash completely removed patulin suggests that possible chemical reaction(s) took place which converted the patulin into other derivative(s). Suttajitt (1989) reported on the use several chemicals for the detoxification of mycotoxins, among which are some acids like acetic acid and phosphoric acid. The chemical reactions proposed for detoxification of aflatoxin are primary addition of the double bond of the furan ring and oxidation involving phenol formation and opening of the lactone ring. In the presence of acid aflatoxin B and G will be converted to their 2-hydroxy derivatives. He added that other mycotoxins which are like aflatoxin and have lactone grouping in the molecule, like patulin, can be similarly destroyed. Therefore, the degradation of patulin by citric acid wash at pH 2.3 most probably follows the same route for detoxification. More intensive research need to be done using model systems to examine the degradation of patulin by citric acid and to identify the patulin derivatives formed under this reaction.

In conclusion, Anna apples are shown to be susceptible to growth of patulin-producing mold. It is recommended that for processing of Anna apples into various apple products the fruits must be subjected to washing with citric acid solution (pH 2.3). This treatment is capable of complete elimination of patulin contamination. Peeling, which alters quality parameters of the resultant juice, is not required.

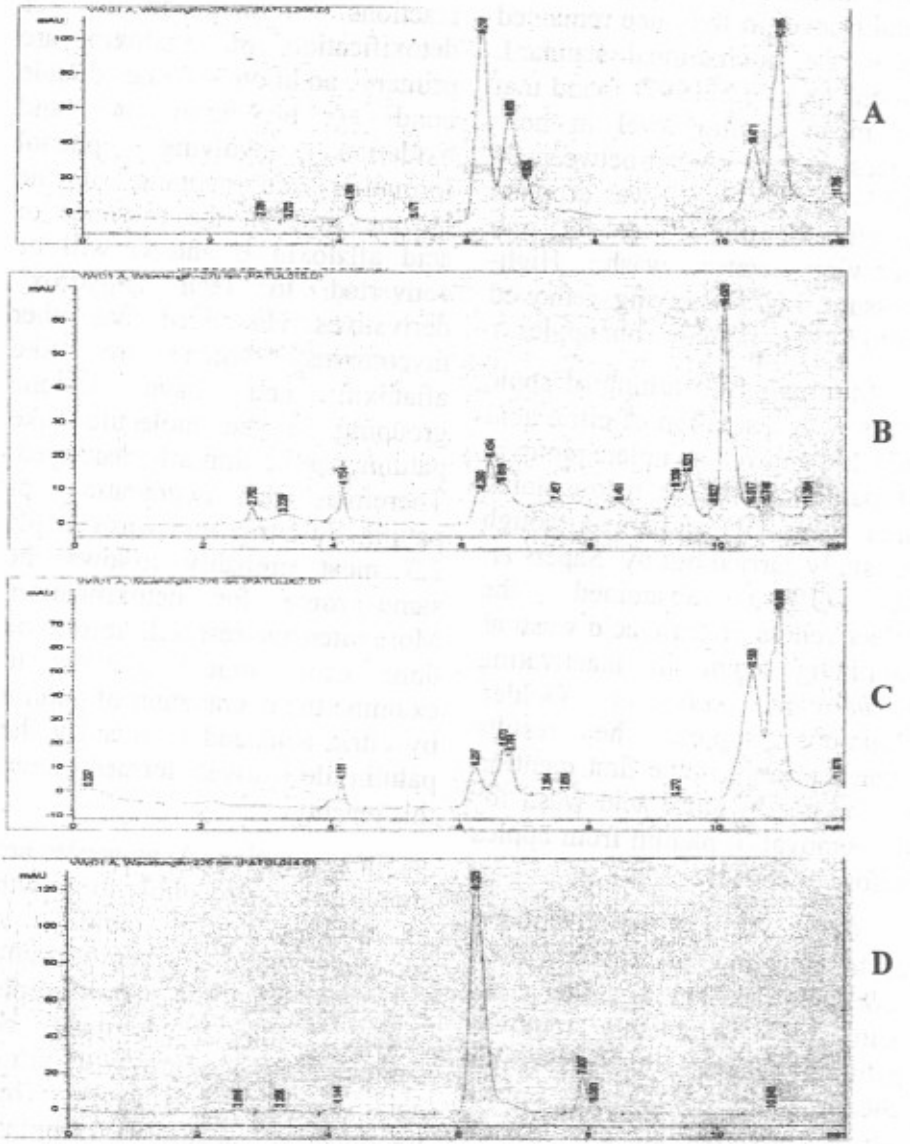


Fig. 5: HPLC Chromatogram of Reconstituted Juices Prepared from Peeled and Sanitized Anna Apples

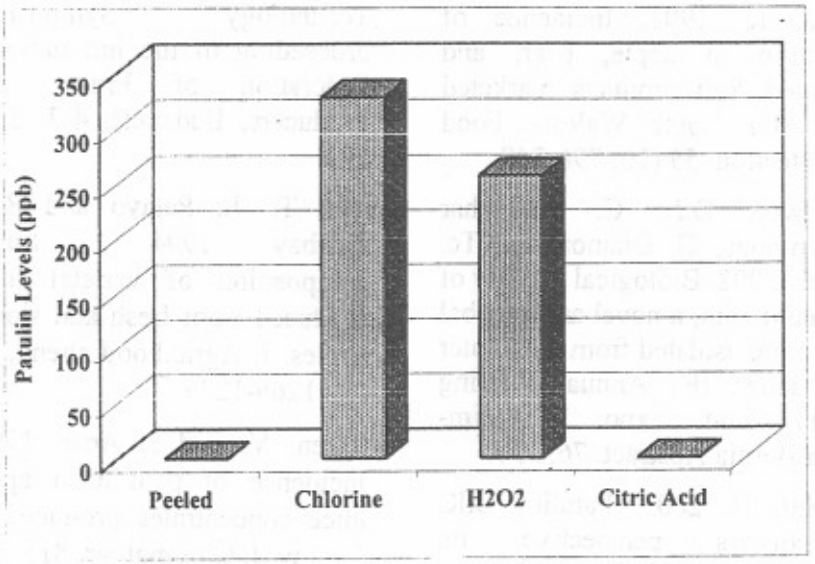


Fig. 6: Effect of Fruit Peeling and Washing with Sanitizing Agents on Patulin Levels in Reconstituted Anna Apples

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تأثير التقشير والتطهير على مستوى الباتيولين في عصير تفاح آنا المسترجع

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يعتبر سم الباتيولين ملوث شائع في منتجات التفاح حيث يمثل خطرا على صحة المستهلك. تم بحث التحكم في مستوى الباتيولين في تفاح آنا المزروع في مصر وذلك باستخدام عملية تقشير ومعاملة غسيل ببعض المطهرات، وقد قورنت نتائج عصير تفاح أنا المسترجع مع تلك الناتجة من عصائر تفاح مسترجعة من مركزات مستوردة.

أدى التقشير قبل التصنيع إلى خفض محتوى الحموضة وفيتامين ج بينما ارتفع رقم الأس الأيدروجيني ونسبة السكر للحامض. وقد تشابهت خواص الجودة لعصير تفاح آنا المسترجع مع ذلك المحضر من أحد المركزات المستوردة بينما اختلفت عن ذلك المحضر من مركز أخر. مستوى الباتيولين في عصير آنا المجهز من تفاح طازج كان ١٢,٩٦ جزء/مليون بينما ذلك المجهز من تفاح عطن احتوى على ١٦,٢٨ جزء/مليون. مركزات عصير التفاح المستوردة أنتجت عصير يحتوي على مستوى من الباتيولين أعلى من المسموح به في المواصفة الدولية (٥٠ جزء/مليون). وأدى تقشير تفاح آنا إلى إزالة الباتيولين تماما من العصير الناتج. غسيل التفاح بمحلول صوديوم هيبوكلوريت ومحلول فوق أكسيد الأيدروجين أدى إلى خفض مستوى الباتيولين بنسبة ٩٧,٥ و ٩٨% على التوالي. وقد تمت إزالة الباتيولين بالكامل من عصير التفاح عند غسيل الثمار بمحلول حمض الستريك ذو رقم أس ايدروجيني ٢,٣. وقد تم مناقشة كيفية ازالة سمية الباتيولين بواسطة حمض الستريك. ويمكن التوصية بأنه لتصنيع تفاح آنا إلى عصير تفاح فإن الثمار يجب أن تتعرض لعملية غسيل باستخدام محلول من حمض الستريك ذو أس ايدروجيني ٢,٣. في هذه الحالة فإنه لا يلزم إجراء التقشير الذي يؤثر على خواص جودة العصير الناتج.