

PATULIN IN APPLE FRUITS:

II. EFFECT OF APPLE TREATMENTS WITH SOME CHEMICAL SANITIZERS ON PATULIN PRODUCTION BY *Penicillium expansum*

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ABSTRACT

Penicillium expansum is a wide spread fungi found on apples that causes fruit decay and may lead to production of a toxic secondary metabolite, basically patulin. This study was carried out to evaluate the effectiveness of several chemical sanitizers against *P. expansum* NRRL 2304 and to establish sanitizing washing treatments that would inhibit *P. expansum* growth and subsequent patulin production on *Anna* apples destined for cider. Wash treatments with chemical sanitizers including NaOCl (100 to 200 ppm) , Potassium sorbate (0.1 to 0.5%) , SO₂(100 to 300 ppm) and acetic acid (0.5 and 3%) .

The results showed that washing with NaOCl (200 ppm) delayed growth of *P. expansum* on inoculated apple discs but failed to completely inhibit patulin production. Acetic acid solutions (2 to 3%) were the most efficient chemical against *P. expansum* and subsequent patulin production on apple juices. While no significant effect in patulin production was found by SO₂ and potassium sorbate.

The effect of pressing on molded *Anna* apple discs fruits was studied. The pressing resulted in a partially elimination of patulin in apple juices. Patulin in the final juices is dependent on initial the concentrations.

Keywords: Patulin, secondary metabolite and chemical sanitizers .

INTRODUCTION

The mycotoxin patulin is produced by several species of *Penicillium*, *Aspergillus* and *Byssochlamys*. Originally, this drew researchers attention due to its antibiotic properties. However, it was rapidly known to be a highly toxic to plants and animals (Gaucher, 1979).

The LD₅₀ for rats and mice ranged from 5 to 30 mg/kg b.w. (Busby and Wogan, 1981). Patulin causes gastrointestinal distress and neurotoxic effects in rodents (Hopkins, 1993), immunotoxic effects in mice and rabbits (Sharma, 1993), and genotoxic effects on mammalian cells (Wouters and Speijers, 1996). Patulin has been found in apples, pears, their juices and jams (Burda, 1992; Machinski and Midio, 1996; Meyer, 1982 ; Plessi, 1998 and Sylos and Rodriguez-Amaya, 1999) , grapes and grape juices (Scott *et al.*, 1997), and beets (Wisniewskaabd Pirsorska ,1982). Brown rot was found in fruits, such as bananas, pineapples, grapes, peaches, and apricots, indicating that the use of unsound fruits for processing would lead to the presence of the toxin in the products (Frank *et al.*, 1977). Patulin was also found in peaches, apricots, bananas, strawberries, melons, tomatoes,

cucumbers and carrots inoculated with *P. expansum*, *P. urticae* and *Byssochlamys nivea* (Frank *et al.*, 1977) and in barley inoculated with *Aspergillus clavatus* (Lopez-Diaz and Flanningan, 1997).

The effect of processing on patulin has been extensively studied in apples. The toxin was shown to be resistant to heat and to the presence of acids, however, alcoholic fermentation will destroy it (Scott, 1984). The World Health Organization (WHO) recommends a maximum permitted level of 50 µg/L for apple juice and also the European Commission recently adopted a maximum permission of a level of 50 µg/kg for patulin in some foodstuffs mainly derived from or containing apples.

Lovett and Peeler (1974) reported that patulin is quite stable in aqueous solution at 105-125°C in the 3.5–5.5 pH range (McIlvaine's buffer). Patulin degradation increases as the patulin concentration decreases and the pH value increases to 6. Brackett and Marth (1979) found by using Sorensen's phosphate buffer at 25°C, the half-life of patulin to be 64 h at pH value of 8 versus 1310 h at pH 6 (apparent 1st-order reaction). Therefore, heat treatment (pasteurization) and storage cannot completely inactivate patulin in apple juice or ciders (Harrison, 1989 and Kadakal and Nas, 2003). Kadakal and Nas (2003) reported that, the thermal treatment of apple juice at 100°C for 20 min followed by evaporation leads to 30% degradation. Aytac and Acar (1994) have shown patulin to be unstable in the presence of 100 ppm SO₂ (decrease from 6000 to 200 ppm patulin after 4 months at room temperature). According to Burroughs (1977), more than 200 ppm sulfite is required to reduce patulin efficiently in apple juice. Levels as high as 2000 ppm are necessary to degrade 90% of the patulin in 2 days (initial level = 150 ppm). Addition of 500 ppm ascorbic acid, sodium ascorbate or both at pH value 7.5 has emerged as another advantageous way to degrade patulin in apple juice contaminated with *Penicillium expansum* (decrease from 6000 to 60 ppm patulin after 4 months at room temperature) (Aytac and Acar, 1994 and Brackett and Marth, 1979).

Therefore, the objective of this study was to screen the commonly used sanitizers for effectiveness against *P. expansum* on apples and to establish effective sanitizing wash treatments that both inhibits *P. expansum* and prevents the formation of patulin on stored apples destined for cider. Wash treatments included NaOCl (100 to 200 ppm), potassium sorbate (0.1 to 0.5%), SO₂ (100 to 300 ppm) and acetic acid (0.5 and 3%).

MATERIALS AND METHODS

Materials

Anna apples were obtained from Cairo local market in Cairo, Egypt.

Methods

Culture preparation

Penicillium expansum (NRRL 2304) was obtained from the United States Dept. of Agriculture, Agricultural Res. Service (Natl. Center for Agricultural Utilization Research, Peoria, Ill., U.S.A.). Strain *P. expansum* (NRRL 2304) is a known patulin producer. The Culture preparation of *P.*

expansum (NRRL 2304) was obtained from the United States Dept. of Agriculture Agricultural Research Service (Natl. Center for Agricultural Utilization Research, Peoria, Il., USA).

Strain NRRL 2304 is a known patulin producer. The culture was maintained on potato dextrose agar (PDA; Difco, Sparks, Md., USA) at 25 °C. Spore suspensions were prepared by flooding PDA plates (1 wk old with visibly abundant spore formation) with 10 mL of sterile water and swirling gently to release the spores. The number of spores in suspension was enumerated by the Iso-Grid ® sys-maintained on potato dextrose agar (PDA; Difco, Sparks, Md., USA) at 25 °C .

Apple inoculation

Sound apples were dipped for 5 min into various dilutions of different wash treatments by (106 spore /mL) of spore suspension, allowed to air-dry, and then stored at 25 °C for 14 days .

Effect of sanitizing wash treatments

The following 4 wash solutions were screened for their effectiveness against *P. expansum*: sterile distilled water; 50 ,100 , 150 and 200 ppm NaOCl ; 0.1 , 0.2 , 0.3 , 0.4 and 0.5% (w/v) potassium sorbate; 100, 150 , 200 , 250 and 300 ppm SO₂ (prepared by dissolving sodium metabisulfite in sterile distilled water); and 0.5 , 1.0 , 1.5 and 2.0 , 2.5 and 3% acetic acid (v/v). All previous of experiments were conducted in triplicate.

Preparation of apple juice

A study on the effect of pressing of Anna apples was carried out .Molded Anna apple discs fruits were blended in blender then the pure apple juice was hydrolic by pressing through cheese close until using .

Extraction and determination of patulin

Patulin was extracted from apple tissues or juice and then determined according to the method was mentioned in details in the first part of this study (Amra *et al.*,2009).

Statistical analysis

The obtained results were analyzed by ANOVA using Excel 2003 Microsoft Corp.

RESULTS AND DISCUSSION

Patulin was found in apples inoculated with *Penicillium ssp.*, where Schieberle (2001) noted that patulin production at 20 °C in Golden Delicious apples began 2 days after the inoculation with a strain of *P. expansum*, whereas Sydenham *et al.* (1997) found that patulin levels in juice freshly pressed from deck-stored apples increased dramatically, from 90 µg/g on day 7 to 2445 µg/g after 33 days of storage .

McCallum *et al.* (2002) showed that patulin production in mold-inoculated juice prepared from Empire apples, after an initial lag, increased over 14-days of incubation period and was preceded by a drop in juice pH value to a value more favorable to toxin production. Therefore, it appears that patulin content by inhibited mold growth by the effectiveness of several chemical sanitizers against *P. expansum* (NRRL 2304).

Effect of different concentrations of acetic acid on patulin production

The results in Table (1) indicate that production of patulin was significantly inhibited by acetic acid from 1.0 % to 3.0% and the results also indicate that acetic acid was an effective sanitizer for apple tissue, which could inhibit production of patulin in apple tissue, This increasing of effectiveness may be due to acid residue remaining on the inoculated apple discs after the wash treatment. When apple discs were washed with less than 2%acetic acid, mold growth was not completely eliminated and patulin production was detected at the end of 14 days. These results indicate that an apple wash treatment with 1% acetic acid could be an effective sanitizer against *P. expansum*.

Table (1): Effect of different concentrations of acetic acid on patulin production in Anna apple discs by *P. expansum* NRRL 2304 during storage for 14 days at 25°C.

Concentrations of acetic acid (%)	Patulin content (µg/g apple)
0	210a
0.5	202a
1.0	15b
1.5	5c
2.0	0c
2.5	0c
3.0	0c

Numbers labeled with different letters indicate significant differences between treatments at p <0.05. Measurements are means of 3 separate trials.

Effect of different concentrations of NaOCl on patulin production

Chlorine and a number of hypochlorites compounds are widely used as sanitizers in the food processing industry. Good Agricultural Practice (GAP) guidelines currently recommend a postharvest wash treatment of fresh product in 50 to 200 ppm total chlorine solution, at a pH of 6.0 to 7.5, with a contact time of 1 to 2 min (USFDA 1998).

Data in Table (2) indicate that no significant inhibition ion of patulin production by *P. expansum* NRRL at 50 and 100 ppm NaOCl, while it had significant effect at 150 and 200 ppm. Okull and LaBorde (2004) noted that 200 ppm chlorine and acid-adjusted 200 ppmchlorine (pH value adjusted to 6.9) were effective in inactivating spores of *P. expansum* over a 5-min contact time in aqueous suspension .The effect was more pronounced and more rapid with pH value adjusted(neutralized) chlorine .

Table (2):I Effect of different concentrations of NaOCl on patulin production in Anna apple discs by *P. expansum* NRRL 2304 during storage for 14 days at 25°C.

Concentrations of NaOCl (ppm)	Patulin content (µg/g apple)
0	210a
50	200a
100	180a
150	170b
200	120c

Numbers labeled with different letters indicate significant differences between treatments at p <0.05. Measurements are means of 3 separate trials.

Effect of different concentrations of SO₂ on patulin production

Table (3) show that no significant inhibition of patulin production by *P. expansum* (NRRL 2304) during storage for 14 days at 25 °C in 100 to 300 ppm of SO₂ .While, Podgorska (1992) reported that SO₂ at 300 ppm has been shown to effectively inhibit the growth of *P. expansum*, and subsequent patulin secretion, when present in a broth of agar medium for an extended period of (12 days).

Table (3): Effect of different concentrations of SO₂ on patulin production in Anna apple discs by *P. expansum* NRRL 2304 during storage for 14 days at 25 °C.

Concentrations of SO ₂ ppm	Patulin content (µg/g apple)
0	210a
100	210a
150	200a
200	200a
250	198a
300	195a

Numbers labeled with different letters indicate significant differences between treatments at p <0.05. Measurements are means of 3 separate trials.

Effect of different concentrations of potassium sorbate on patulin production

Table (4) indicate that potassium sorbate (from 0.1 to 0.5%) did not significantly inhibit patulin production by *P. expansum* NRRL 2304 during storage for 14 days at 25 °C. whereas 150, 200 ppm NaOCl and 300 ppm SO₂ decreased significantly the inhibition of patulin production. Several studies have described the effectiveness of sorbic acid and its potassium salts as preservatives against fungi in culture media. Sorbic acid, when added at concentrations up to 0.025% as part of the culture medium, was reported to stimulate the growth of *P. expansum* and secretion of patulin, at higher concentrations leading to a decrease in toxin production relative to the control Podgorska (1992). Lennox and McElroy (1984) also showed that potassium sorbate added to nutrient broth at 0.3% reduced growth of *P. expansum* by 57% and patulin synthesis by 67%. Increasing the concentration of potassium sorbate to 1.5% in the growth medium inhibited patulin production by 98% (Lennox and McElroy, 1984).

Also, no inhibitory effect was observed at 0.5% potassium sorbate, this may be due to the short contact time; 5 min for wash solution versus up to 12 d in culture media .

Generally, Potassium sorbate (at 0.1 to 0.5%) did not significantly inhibit the patulin production by *P.expansum* (NRRL2304) during storage for 14 days at 25 °C .Where it was significantly decreased the inhibition of patulin production by 1.0 to3.0 % of acitic acid , 150, 200 ppm NaOCl and 300 ppm SO₂ .

Table (4) Effect of different concentrations of potassium sorbate on patulin production on Anna apple discs by *P. expansum* NRRL 2304 during storage for 14 days at 25°C .

Concentrations of potassium sorbate (ppm)	Patulin content (ug/g apple)
0	210a
100	210a
150	200a
200	200a
250	198a
300	195a

Numbers labeled with different letters indicate significant differences between treatments at p <0.05. Measurements are means of 3 separate trials.

Effect of press on patulin in Anna apple discs

The present data in Table (5) indicate that although the pressing resulted in markedly destruction of patulin in molded Anna apple discs, the final juice still containing of patulin concentrations. The concentrations of patulin in the final juices depended on the initial concentrations. The percent of destruction ranged from 30 to 60 %, in the initial concentration ranged from 100 to 200 ppm .

Physical methods such as centrifugation, filtration ,absorption with activated carbon in a static or flow through system have been evaluated by Huebner *et al.*(2000) and Gokmen *et al.*(2001).They added that adoption of these types of methods by the process is hindered due to their limited effectiveness and also to the adverse quality changes produced in the juice.

Table (5): Effect of pressing on patulin in Anna apple discs .

Concentrations of patulin (ppm)	Concentrations of juices (ppm)	Destruction %
200	140	30
180	115	36
150	90	40
100	50	50
50	20	60

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الباتيوولين في ثمار التفاح:

٢ . تأثير معاملات الغسيل لثمار التفاح بواسطة بعض المركبات على انتاج

الباتيوولين بواسطة فطر *Penicillium expansum*

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يعتبر فطر *Penicillium expansum* من الفطريات الواسعة الانتشار والتي تتواجد على ثمار التفاح وهي تسبب فساد التفاح وربما انتاج الباتيوولين كنواتج تمثيل لهذه الفطريات. وقد صممت هذه الدراسة لتقدير بعض المركبات الكيميائية فى تثبيط نمو فطر *P. expansum* NRRL 2304 وكذلك الحد من انتاج الباتيوولين على التفاح من نوع Anna. فى هذه الدراسة تم استخدام (١٠٠ الى ٢٠٠ جزء فى المليون) من هيبوكلوريت الصوديوم و (١٠ الى ٥٠%) من سوربات البوتاسيوم و (١٠٠ الى ٣٠٠ جزء فى المليون) من ثانى اكسيد الكبريت (٥ و ٣ %) من حامض الخليك . افادت النتائج يمكن تثبيط نمو فطر *P. expansum* NRRL 2304 باستخدام تركيز ٢٠٠ جزء فى المليون من محلول الصوديوم هيبوكلوريت ولكن لم يتخلص كليتا من الباتيوولين بينما اعطى تركيز حامض الخليك من ٢% الى ٣% احسن النتائج من حيث تثبيط انتاج الباتيوولين . بينما لم تعطى معاملات سوربات البوتاسيوم و ثانى اكسيد الكبريت التأثير الملحوظ . تم دراسة تأثير العصر باستخدام الخلاط والفلتره , حيث وجد ان تكسير الباتيوولين يعتمد على تركيزه فى التفاح قبل استخدام الخلاط والفلتره .