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Sonia Marín, Hector Morales, Hassan Ahmed Hassan Hasan, Antonio J. Ramos, Vicent Sanchis

► **To cite this version:**

Sonia Marín, Hector Morales, Hassan Ahmed Hassan Hasan, Antonio J. Ramos, Vicent Sanchis. Patulin distribution in the tissue of *Penicillium expansum*-contaminated Fuji and Golden apples. Food Additives and Contaminants, 2006, 23 (12), pp.1316-1322. 10.1080/02652030600887610 . hal-00577482

HAL Id: hal-00577482

<https://hal.science/hal-00577482>

Submitted on 17 Mar 2011

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Patulin distribution in the tissue of *Penicillium expansum*-contaminated Fuji and Golden apples

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-089.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	01-Jun-2006
Complete List of Authors:	Marín, Sonia; Lleida University, Food Technology Morales, Hector; Lleida University, Food Technology Hasan, Hassan; Assiut University, Botany Ramos, Antonio; Lleida University, Food Technology Sanchis, Vicent; Lleida University, Food Technology
Methods/Techniques:	Chromatography - HPLC, Clean-up, Extraction, Mycology
Additives/Contaminants:	Mycotoxins - fungi, Mycotoxins - other
Food Types:	Fruit, Fruit juices

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1 **Patulin distribution in the tissue of *Penicillium expansum*-**
2 **contaminated Fuji and Golden apples**

3
4 S. Marín, H. Morales, H.A.H. Hasan, A.J. Ramos, V. Sanchis

5
6 Lleida University - Food Technology, Rovira Roure 191 Lleida Lleida 25198 (Spain)

7
8 **Running head:** Patulin accumulation and distribution in apples

For Peer Review Only

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7 10 This work aimed to assess the extent of patulin contamination in *Penicillium expansum*
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9 11 infected apples stored at room temperature for short periods of time and its relationship
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11 12 with apple variety (Golden or Fuji), degree of ripeness, and size of lesions. Inoculated
12
13 13 apples were incubated at 20°C. Cylindrical samples were taken around the lesions and
14
15 14 cut into 0.5 cm thick sections in which patulin was determined in both the sound and
16
17 15 the decayed tissue. Higher accumulation of patulin occurred in Golden apples with less
18
19 16 ripened apples showing higher concentrations. Total accumulated patulin was similar
20
21 17 or higher in 4-cm lesioned apples than in 2-cm ones, although a decrease in patulin
22
23 18 concentration was observed in the older sections of the lesions. Patulin accumulation
24
25 19 occurred in a short period of time at room temperature thus stand-by periods before
26
27 20 processing should be minimised. About 2-6% of total patulin was found to migrate to
28
29 21 the surrounding sound tissue thus trimming apple tissue around the rotten part may be
30
31 22 a good preventive practice for apple derivatives production.
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39 25 **Keywords:** patulin, apple, *Penicillium expansum*, trimming, storage
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27 **Introduction**

28 Patulin is a mycotoxin produced by a number of fungi common to fruit- and vegetable
29 products, most notably apples. Patulin production is usually, but not exclusively,
30 associated with apple soft rot and blue mould rot, most commonly caused by
31 *Penicillium expansum* (Brian et al. 1956). Within the food industry, apples and their
32 respective products are of greatest concern for patulin contamination. Numerous
33 studies around the world have examined the extent and degree to which apple
34 products have been contaminated by patulin, finding high percentages of apple juice
35 samples contaminated by patulin in levels exceeding the 50 ng g⁻¹ limit imposed by the
36 EC (Comission Regulation 1425/2003) (Moake et al. 2005).

37

38 The health risks posed by patulin necessitate its control and removal from apple
39 products, creating a demand for food-processing techniques capable of doing so,
40 preferably at low cost for industry (Moake et al. 2005). In many countries apple juice is
41 typically a by-product produced from culled apples unfit for other, higher quality and
42 higher profit, purposes. Bruises, skin breaks, and other physical damage within these
43 apples provide a perfect entry for *P. expansum* and other patulin-producing species
44 into the fruit (Moake et al. 2005). Low quality apples originating from storage rooms
45 (either controlled atmosphere (CA) or not) are also usually employed for apple juice
46 production. As a result, the use of low quality raw material leads to highly patulin
47 contaminated apple juices. In Spain, juice concentrates are mainly produced from
48 Golden apples, although Fuji apples are sometimes used when they are not suitable for
49 fresh consumption. The exposition of these *P. expansum* infected apples intended for
50 juice production to room temperature for short periods of time during postharvest
51 operations has been shown to be critical for the accumulation of patulin (Marín et al.
52 2006; Morales et al. 2006a).

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7 54 At present, many juice industries rely on high-pressure water application as a means of
8
9 55 removing the rotten parts of apples. However, some previous studies have suggested
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11 56 that patulin may have diffused to the healthy tissue (Taniwaki et al. 1992; Rychlik and
12
13 57 Schieberle 2001). The present work aimed to assess the extent of patulin
14
15 58 contamination in infected apples stored at room temperature (20°C) in both rotten and
16
17 59 healthy tissues, as a function of apple variety (Golden and Fuji) and apple ripeness.
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21 61 **Material and methods**

22 62 *Isolates*

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26 63 A strain of *Penicillium expansum* 3.78 isolated from cold stored apples in Lleida (Spain)
27
28 64 was used. The strain was stored in sloped potato dextrose agar (PDA) tubes at 5°C in
29
30 65 the Fungal Collection of the Food Technology Department of Lleida University.
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34 67 *Apples*

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37 68 Apples var. Golden and var. Fuji were used for the experiment. For each variety, two
38
39 69 lots with different degree of ripeness were used (6 apples per lot). Quality parameters
40
41 70 were determined before inoculation for both Fuji and Golden apples belonging to each
42
43 71 lot, lot A being the less ripened one and lot B the more ripened one (Table I). Firmness
44
45 72 was measured on two opposite peeled sides using a penetrometer fitted with an 11 mm
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47 73 diameter probe.
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51
52 75 Soluble solids concentration (SSC) was determined by measuring the refractive index
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54 76 of the juice resulting from apples, and the data were expressed as percentage (g per
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56 77 100 g fresh weight (FW)).
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4 79 Acidity was measured as follows: 10 ml of pulp juice was diluted with 10 ml H₂O and
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6 80 titrated with a 0.1 mol l⁻¹ NaOH solution. The acidity was expressed in grams of malic
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8 81 acid per liter of juice.
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12 83 Determination of quality parameters revealed that, apart from known differences
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14 84 among varieties, the lots differed mainly in colour, firmness and acidity, Golden apples
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16 85 from different lots being more different.
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22 87 [insert table I about here]
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26 89 Before inoculation, apples were surface disinfected with 2% sodium hypochloride,
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28 90 rinsed with water and excess water allowed to dry.
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31
32 92 *Conidial suspensions, apple inoculation and incubation*
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34 93 Petri dishes of PDA were point inoculated with the strain and incubated at 25°C until
35
36 94 sporulation. A conidial suspension was prepared in Tween 80 (0.005% (v/v)) in sterile
37
38 95 water and adjusted to 10⁶ conidia ml⁻¹. Apples were wounded 2 mm deep with a 2 mm
39
40 96 diameter needle twice in two opposite sides. Both points were inoculated with 20 µl of
41
42 97 the conidial suspension.
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45
46 99 Apples were stored at 20°C. For each variety, half of the apples were removed from
47
48 100 incubation when their lesions were about 2-cm diameters (5 days), and the remaining
49
50 101 ones, when the lesions were 4-cm wide (7-8 days). Incubation at 20°C was carried out
51
52 102 to simulate the interval of time that apples may be at room temperature before
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54 103 processing.
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5 105 *Obtaining sections from apples*

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7 106 For apples with 2-cm lesions, a concentric 3-cm cylinder was taken around each lesion
8
9 107 with the aid of a die-cut. From each cylinder, 0.5 cm sections were cut across starting
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11 108 from the surface of the apple (Figure 1); when no lesion was observed in one section,
12
13 109 one more section was taken in depth. From all the sections, those visible decayed were
14
15 110 divided in two samples: sound and rotten, while the sound sections were considered as
16
17 111 a single sample. The same procedure was applied to 4-cm lesions, although in this
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19 112 case the cylinder had 5.5 cm of diameter.
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24 114 [insert figure 1 about here]

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28 116 *Patulin analyses*

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30 117 Eight ml distilled water were added to the sound/decayed samples. A “Polytron” unit of
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32 118 Kinematica AG (Switzerland) was used to pulp them. Puree samples were
33
34 119 enzymatically treated to remove pectins and allowed to react for 1 h at 40°C. After that,
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36 120 samples were centrifuged at 6000 rpm for 5 min and 5 ml of the supernatant were
37
38 121 analysed following AOAC method 995.10 (Brause et al. 1996). Patulin was extracted
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40 122 with ethyl acetate and then cleaned up by extraction with sodium carbonate solution.
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42 123 Extracted samples were dried with anhydrous sodium sulphate. After evaporation of
43
44 124 ethyl acetate, patulin was re-suspended in water pH 4 (adjusted with acetic acid) and
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46 125 determined by reversed-phase LC with UV detection. The mobile phase was
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48 126 water+tetrahydrofuran (100+0.8) at a flow rate of 1.2 ml min⁻¹. Patulin was detected at
49
50 127 UV at 276 nm wavelength. The recovery rates obtained by spiking apple pieces with
51
52 128 10, 40, and 100 ng patulin g⁻¹ in duplicate were of 96.2, 88.5 and 69.1%, respectively.
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54 129 Regarding repeatability the S_r of five determinations (25 ng g⁻¹) carried out under
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56 130 repeatability conditions was 0.005. Finally the limit of detection of the analysis was
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4 131 between 0.8 and 10 ng g⁻¹ (depending on the initial amount of sample) for the sound
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6 132 samples and between 3 and 100 ng g⁻¹ for the decayed ones.
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11 134 *Statistical analysis of the results*

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13 135 The whole experiment was designed as a full factorial one, with 4 factors to be tested
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15 136 on the patulin accumulated by *P. expansum*: apple variety, degree of ripeness, size of
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17 137 lesion, depth from the surface of the apple. The variables recorded were concentration
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19 138 of patulin both in the decayed and the sound samples from each section. All treatments
20
21 139 were repeated three times. Patulin concentrations for each treatment were evaluated
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23 140 by analysis of variance using the SAS version 8.2 (SAS Institute Inc., Cary, NC, USA).
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25 141 Significant differences (P<0.05) in parameters were tested by a Duncan's Multiple
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27 142 Range Test.
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32 144 Subsequently, total patulin (µg) in each section and for both healthy and decayed
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34 145 fractions was calculated taking into account both patulin concentration and the weight
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36 146 of each sample. Percentage of difused patulin was calculated by adding the patulin
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38 147 contained in the healthy fractions of the sections and dividing by the total patulin found
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40 148 in the decayed fractions of the sections.
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45 150 **Results**

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47 151 An initial analysis of the variance of the whole set of data revealed that the
48
49 152 concentration of patulin in both the decayed and sound portions significantly depended
50
51 153 on lesion diameter, apple variety, and on degree of ripeness for Fuji apples (Table II).
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53 154 Patulin concentrations found in the sections were significantly higher for 2-cm lesions
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55 155 than for 4-cm ones. Variety was the main factor affecting patulin content in the sections
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57 156 analysed, due to the markedly higher concentration found in Golden than in Fuji
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59 157 apples, regardless of the other factors tested. For the patulin content in the sound
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4 158 sections degree of ripeness had some influence on the results, depending on variety
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6 159 and lesion size. Data were thus analysed separately for each lesion size tested (2 and
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8 160 4 cm diameter) (Table III).
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13 162 [insert table II and III about here]
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17 164 For apples with a 2-cm lesion, Golden ones had mean concentrations of 9.67 and 0.28
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19 165 $\mu\text{g g}^{-1}$, in the decayed portion and surrounding one, respectively. For Fuji apples these
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21 166 concentrations were 3.31 and 0.13 $\mu\text{g g}^{-1}$, respectively (Figure 2). No significant
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23 167 difference due to different degree of ripeness was observed as a single factor,
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25 168 however, under ripe Fuji apples yielded significantly higher concentrations of patulin in
26
27 169 both sound and decayed sections. For both sound and decayed sections, most patulin
28
29 170 was found in the upper section, with the second section containing significantly less,
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31 171 and finally levels of patulin found in the remaining sections were not significantly
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33 172 different. Due to the higher patulin levels produced in Golden apples, the distribution,
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35 173 and extent and levels of diffusion of patulin in both sound and decayed tissue
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37 174 depended on the variety.
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43 176 For apples with a 4-cm lesion, Golden ones had mean concentrations of 4.98 and 0.11
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45 177 $\mu\text{g g}^{-1}$, in the decayed portion and surrounding one, respectively. For Fuji apples these
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47 178 concentrations were 1.01 and 0.04 $\mu\text{g g}^{-1}$, respectively (Figure 3). No significant
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49 179 difference due to different degree of ripeness was observed in any of the varieties for
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51 180 patulin in the decayed portions; however, a significantly higher mean level was found in
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53 181 the sound sections of both varieties in the less ripen lot (0.09 vs 0.05 $\mu\text{g g}^{-1}$). In
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55 182 general, there was a decrease in patulin content with depth, the differences among
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57 183 successive sections being more marked for Golden than for Fuji apples.
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5 185 [insert figures 2 and 3 about here]
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9 187 Total patulin contents found in each apple confirmed, besides the high variability of the
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11 188 results, the higher accumulation of patulin in Golden apples, and the lower
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13 189 accumulation in Fuji ones, mainly when they were riper (Table IV). Interestingly, total
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15 190 levels were quite similar for 2 and 4 cm lesions, due to the lower concentrations found
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17 191 in the sections in the 4 cm assay. Looking in detail at the results summarised in Fig. 2
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19 192 and 3, it was observed a decrease in concentration in the first two sections in both
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21 193 sound and rotten tissue, and an increase in the concentration in the subsequent
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23 194 sections, plus the additional sections analysed and found to be positive in the 4-cm
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25 195 assay.
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30 197 Regarding diffusion, patulin found in healthy tissue accounted for 2-6% of the total,
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32 198 more diffusion being observed for Fuji apples, and in under ripe apples for both
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34 199 varieties. In general, patulin was always found in the rings surrounding the lesions, and
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36 200 also in the 0.5-cm section next to the last section where rot symptoms were observed.
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41 202 [insert table IV about here]
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45 204 2-cm diameter lesions resulted in accumulated patulin to a depth of 1.5 cm, or even to
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47 205 2 cm for under ripe Golden apples (Figure 4). For 4-cm diameter lesions, patulin was
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49 206 found at 2 and 2.5 cm depth for ripe and under ripe Fuji apples, respectively, and to 2.5
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51 207 and 3 cm for ripe and under ripe Golden apples, respectively. At a given lesion size in
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53 208 the surface of the apples, the depth of the lesions reached in Golden apples was in
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55 209 general higher than in Fuji ones.
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59 211 [insert figure 4 about here]
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7 213 **Discussion**

8 214 Patulin is a common contaminant of ripened apples used for the production of apple
9 215 juice concentrates (Sydenham et al. 1997). No patulin has been detected in cider
10 216 pressed from tree-picked fruit (culled or not culled). In contrast, cider produced from
11 217 ground-harvested fruit contained up to 374 $\mu\text{g l}^{-1}$, depending on the varieties tested
12 218 (Jackson et al. 2003). No patulin was detected in Golden apples after storage periods
13 219 of 1-2.5 months of storage at 1°C (under CA or not) (Morales et al. 2006a, 2006b).
14 220 Cold stored apples yielded free of patulin ciders if culled, but levels 0-64 $\mu\text{g l}^{-1}$ if not
15 221 culled (Jackson et al. 2003). The removal of fungally decayed and the surrounding
16 222 tissue from apples, prior to further processing, has been reported to reduce significantly
17 223 patulin levels in juiced products (Lovett et al. 1975; Sydenham et al. 1995).

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32 225 In the present study, brown, watery rot increasing depth in rot as the lesion diameter
33 226 increased was observed at 20°C. In agreement with Laidou et al. (2001), an increase in
34 227 lesion diameter corresponded to an increase in depth of penetration of the fungi.
35 228 Stand-by steps at room temperature (e.g. 20°C) prior juice concentrates manufacture
36 229 have been shown to be a crucial point in patulin accumulation prevention (Morales et
37 230 al. 2006a).

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47 232 Patulin concentrations found in the sections were significantly higher for 2-cm lesions
48 233 than for 4-cm ones, although the overall amount of patulin was similar in both cases. In
49 234 Granny Smith apples deck-stored for 7, 15 and 33 days, patulin concentration
50 235 increased from 90 to 2445 ng g^{-1} , but decreased to between 75 and 695 ng g^{-1} ,
51 236 respectively, following a water wash step. Subsequent removal of rotten/damaged fruit
52 237 decreased patulin levels further (to between 55 and 405 ng g^{-1} , respectively)
53 238 (Sydenham et al. 1997).

239

240 Although high patulin concentrations were found in apples after 5 days, a decrease of
241 concentration found in the rotten tissue was observed after 7-8 days, for both varieties.
242 In rotten Golden delicious apples, in the decayed tissue, after a 2-days induction
243 period, patulin increased and, after running through a maximum of 185000 ng g⁻¹ after
244 5 days, decreased to 0.060 ng g⁻¹ after 15 days (Rychlik and Schieberle 2001). Patulin
245 metabolism by fungal enzymes has been proposed (Damoglou et al. 1985). In their
246 study, however, although patulin was significantly lower in the rotten area after 5 days,
247 its content in juice made from the whole fruit remained on a high level of 1700 ng g⁻¹
248 until the tenth day of incubation, before it finally decreased; this is in accordance with
249 the trend observed in this study, because total patulin content in apples was similar for
250 5 and 8 days of incubation. This phenomenon may be attributed to the exponential
251 increase of the rotten part by volume during fungal invasion and to patulin diffusion into
252 the sound tissue (Rychlik and Schieberle 2001).

253

254 Our results showed that between 2 and 6% of total patulin was found in the sound
255 tissues of apples. The total patulin detected in apparently sound tissues of pears
256 inoculated with *P. expansum* was nearly 25% of the total pear tissues (diseased and
257 healthy) (Laidou et al. 2001). Less than 1% of the total patulin was found in sound
258 tissues of apples inoculated with *P. expansum* (Harwing et al. 1973). A similar study
259 was carried out in *P. expansum* inoculated pears kept at room temperature (18-20 °C)
260 till lesions expanded to 10 mm in diameter; in this case 6 mm thick sections were
261 obtained. Patulin was detected in the first three sections at concentrations of 288000,
262 144000 and 144000 ng g⁻¹ from outside to inside of fruits. Patulin could diffuse through
263 6 mm in sound tissues (Laidou et al. 2001). Apples inoculated with *P. expansum*
264 showed diffusion of patulin up to approximately 10 mm into the surrounding healthy
265 tissues; most of the patulin being present in the first 1 cm of the lesion (6.5 to >10 000

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4 266 ng g⁻¹, depending on apples) (Taniwaki et al. 1992). No patulin was found at a distance
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6 267 of more than 2 cm from the infected zone (83700 ng g⁻¹) in Golden delicious apples
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8 268 (1270 ng g⁻¹ at 1 cm, 4.7 ng g⁻¹ at 2 cm and <ld at >3cm) (Rychlik and Schieberle
9
10 269 2001).

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15 271 Fruit cultivars seem to differ in their susceptibilities to *P. expansum* rot and to
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17 272 contamination with patulin. More patulin was always accumulated in Golden apples (2-
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19 273 6-fold) which were softer and more acidic. Regarding degree of ripeness, higher
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21 274 production was observed in less ripen apples, mainly for Fuji apples, suggesting that
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23 275 high acidity, more than softness was the cause for the improved production, as
24
25 276 firmness varied only slightly, and a higher production in more firm apples is unlikely to
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27 277 occur. In fact, pH and patulin production have been shown to be inversely related, with
28
29 278 patulin being unstable at high pH (McCallum et al. 2002). Also, Beer and Amand (1974)
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31 279 reported that the amount of patulin produced in apples artificially inoculated with *P.*
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33 280 *expansum* was inversely proportional to the firmness of the apple.

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39 282 Total patulin in the sections, diseased and healthy, of apples was between 15 and 115
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41 283 µg, depending on varieties and degree of ripeness. Harwing et al. (1973) showed that
42
43 284 one rotten apple could contain 0.02-17.7 mg patulin. Patulin content of pears
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45 285 inoculated with *P. expansum* was nearly 580 µg g⁻¹ (Laidou et al. 2001). Although large
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47 286 variability across literature, and within experiments, can be observed in the reported
48
49 287 patulin levels in both inoculated and non-inoculated fruit, the concentrations are, in
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51 288 general, too high in view of obtaining final apple derivatives with levels < 50 ng g⁻¹, as
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53 289 stated in the EU regulations.

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4 291 Although this study was developed to simulate what it may happen in apples after cold
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6 292 storage, the results may also be applied to ground-picked *P.expansum* infected apples,
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8 293 left in the ground for a certain period of time, and to any other harvested apples which
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10 294 undergo any standby period at room temperature before processing, as long as they
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12 295 have been infected.
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17 297 As a conclusion, patulin accumulation can occur in a short period of time in *P.*
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19 298 *expansum* infected apples left at room temperature and attain dramatically high levels.
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21 299 The concentration of patulin seems to be dependent on apple variety and, in particular,
22
23 300 on acidity, more acidic conditions leading to a higher production of patulin. In addition,
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25 301 trimming just the rotten tissue is not enough to exclude all patulin, but trimming apple
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27 302 tissue around the rotten part may be a good preventative practice for industry using
28
29 303 apple products. Accordingly, Lovett et al. (1975) removed more than 93% of patulin by
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31 304 trimming effective apple tissue from mould-infected rotten apples. One more conclusion
32
33 305 is that washing with a high-pressure water spray, is a good control method, but not
34
35 306 sufficient to totally eliminate the toxin as it may remove just the soft rotten tissue.
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37 307 Consequently, either trimming or culling should be considered as a routine practice in
38
39 308 industries using apples as raw materials.
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45 310 **References**

46
47 311 Beer SV, Amand JK. 1974. Production of the mycotoxin patulin in mature fruits of five
48
49 312 apple cultivars infected by *Penicillium expansum*. Proceedings of the American
50
51 313 Phytopathological Society 1:104-110.
52
53
54 314

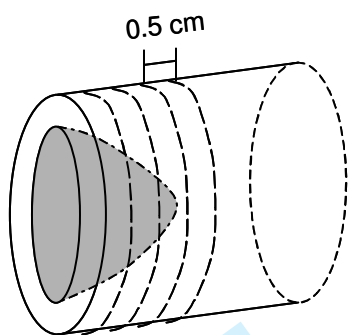
55
56 315 Brause AR, Trucksess MW, Thomas FS, Page WS. 1996. Determination of patulin in
57
58 316 apple juice by liquid chromatography: Collaborative study. Journal of the AOAC
59
60 317 International 79:451-455.

- 1
2
3
4 318
5
6
7 319 Brian PW, Elson GW, Lowe D. 1956. Production of patulin in apple fruits by *Penicillium*
8
9 320 *expansum*. Nature 178:263-264.
10
11 321
12
13 322 Damoglou AP, Campbell DS, Button JE. 1985. Some factors governing the production
14
15 323 of patulin in apples. Food Microbiology 2:3-10.
16
17 324
18
19 325 European Union, Official Journal of the European Communities (EC), *Commission*
20
21 326 *regulation 1425/2003 amending Commission Regulation 466/2001 setting maximum*
22
23 327 *levels for certain contaminants in foodstuffs* (2001). Brussels, Belgium, pp. L203/1-3.
24
25
26 328
27
28 329 Harwing J, Chen YK, Kennedy BPC, Scott PM. 1973. Occurrence of patulin and
29
30 330 patulin-producing strains of *Penicillium expansum* in natural rots of apple in Canada.
31
32 331 Canadian Institute of Food Science and Technology Journal 6:22-25.
33
34 332
35
36 333 Jackson LS, Beacham-Bowden T, Keller SE, Adhikari C, Taylor KT, Chrtel SJ, Merker
37
38 334 RI. 2003. Apple quality, storage, and washing treatments affect patulin levels in apple
39
40 335 cider. Journal of Food Protection 66:618-624.
41
42
43 336
44
45 337 Laidou IA, Thanassouloupoulos CC, Liakopoulou-Kyriakides M. 2001. Diffusion of
46
47 338 patulin in the flesh of pears inoculated with four post-harvest pathogens. Journal of
48
49 339 Phytopathology 149:457-461.
50
51
52 340
53
54 341 Lovett J, Thompson RG, Boutin BK. 1975. Trimming as a means of removing patulin
55
56 342 from fungus-rotted apples. Journal of the AOAC International 58:909-911.
57
58
59 343
60

- 1
2
3
4 344 Marín S, Morales H, Ramos AJ, Sanchis V. 2006. Evaluation of growth quantification
5
6 345 methods for modelling of *Penicillium expansum* growth in an apple-based medium.
7
8 346 Journal of the Science of Food and Agriculture (in press).
9
10 347
11
12 348 McCallum JL, Tsao R, Zhou T. 2002. Factors affecting patulin production by *Penicillium*
13
14 349 *expansum*. Journal of Food Protection 65:1937-1942.
15
16 350
17
18 351 McGuire R. 1992. Reporting of objective color measurements. HortScience 27:1254–
19
20 352 1255.
21
22 353
23
24 354 Moake MM, Padilla-Zakour OI, Worobo RW. 2005. Comprehensive review of patulin
25
26 355 control methods in foods. Comprehensive reviews in Food Science and Food Safety 1:
27
28 356 8-21.
29
30 357
31
32 358 Morales H, Marín S, Rovira A, Ramos AJ, Sanchis V. 2006a. Patulin accumulation in
33
34 359 apples by *Penicillium expansum* during postharvest stages. Letters in Applied
35
36 360 Microbiology (in press).
37
38 361
39
40 362 Morales H, Sanchis V, Rovira A, Ramos AJ, Marín S. 2006b. Patulin accumulation in
41
42 363 apples during postharvest: Effect of controlled atmosphere storage and fungicide
43
44 364 treatments. Food Control (submitted).
45
46 365 Rychlik M. Schieberle P. 2001. Model studies on the diffusion behaviour of the
47
48 366 mycotoxin patulin in apples, tomatoes, and wheat bread. European Food Research and
49
50 367 Technology 212:274-278.
51
52 368
53
54
55
56
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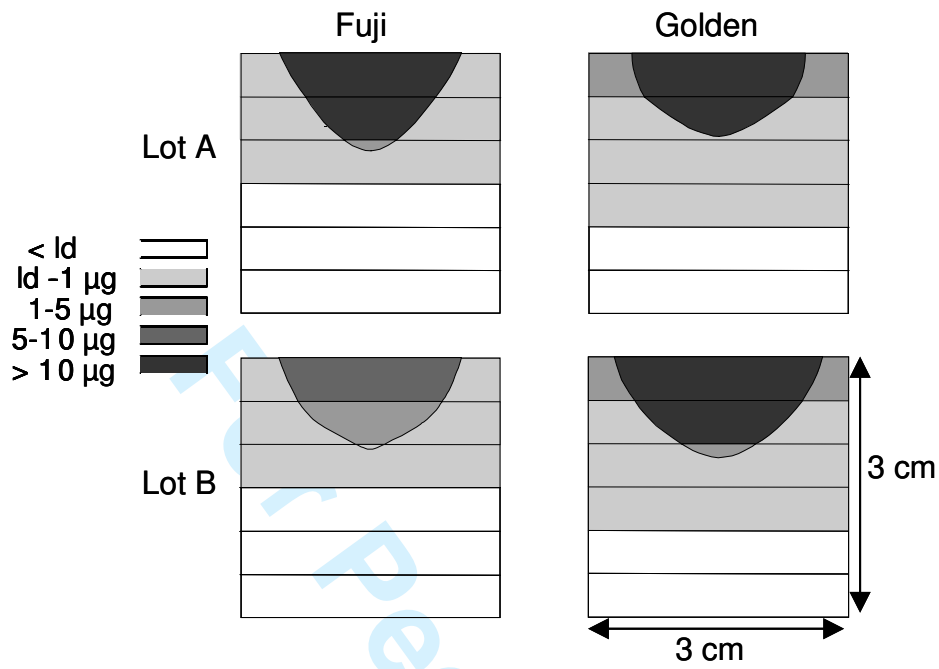
- 1
2
3
4 369 Sydenham EW, Vismer HF, Marasas WFO, Brown NL, Schlechter M, Rheeder JP.
5
6 370 1995. Reduction of patulin in apple juice samples-influence of initial processing. Food
7
8 371 Control 6:195-200.
9
10 372
11
12 373 Sydenham EW, Vismer HF, Marasas WFO, Brown NL, Schlechter M, Rheeder JP.
13
14 374 1997. The influence of deck storage and initial processing on patulin levels in apple
15
16 375 juice. Food Additives and Contaminants 14:429-434.
17
18 376
19
20 377 Taniwaki MH, Hoenderboom CJM, de Almeida Vitali A, Uboldi Eirog ME. 1992.
21
22 378 Migration of patulin in apples. Journal of Food Protection 55:902-904.
23
24
25
26 379
27
28 380
29
30
31
32
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56 2
78 3 Figure. 1. Schematic distribution of the obtained sections from each apple cylinder.
910 4
1112 5 Figure 2. Distribution of patulin ($\mu\text{g g}^{-1}$) in healthy and decayed tissue in apples with 2-
13 cm lesions.
14 615 7
1617 8 Figure 3. Distribution of patulin ($\mu\text{g g}^{-1}$) in healthy and decayed tissue in apples with 4-
18 cm lesions.
19 9
2021 10
2223 11 Figure 4. Patulin ($\mu\text{g g}^{-1}$) found in each section (rotten plus healthy tissue) as a function
24 of depth from the surface of the rotten apples. \square , 2-cm lesion, lot A; \circ , 2-cm lesion, lot
25 B; \blacksquare , 4-cm lesion, lot A; \bullet , 4-cm lesion, lot B.
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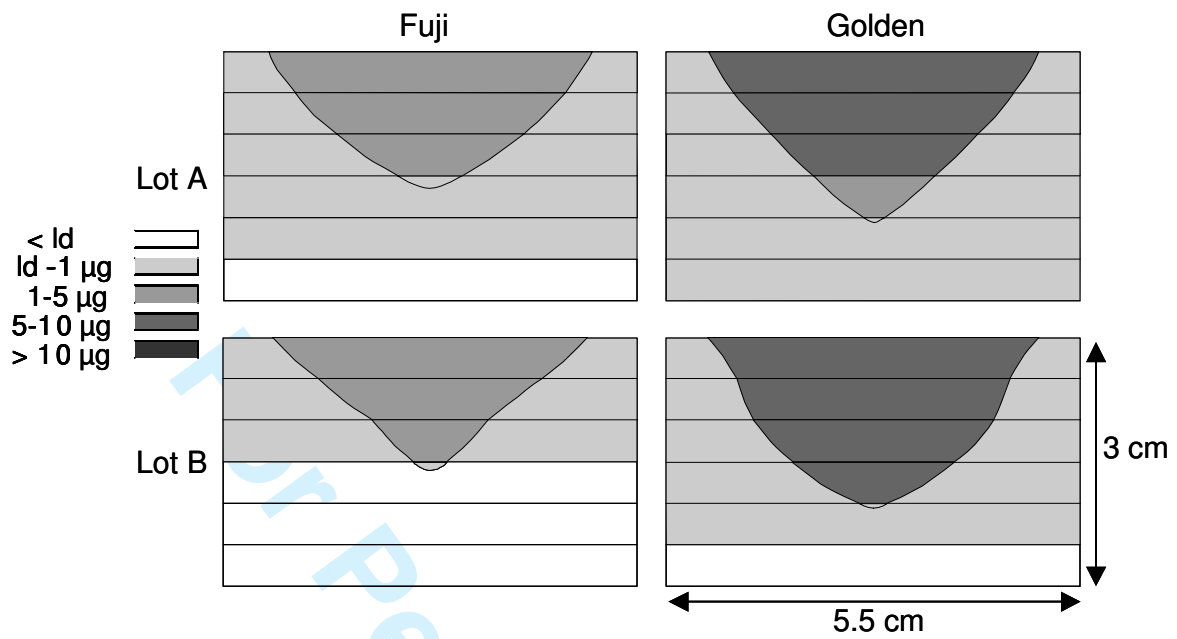


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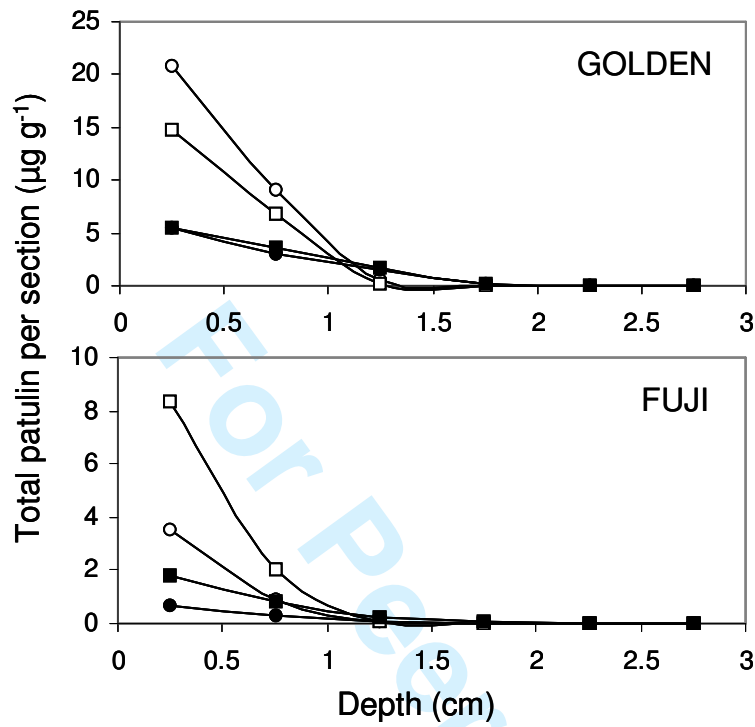
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Table I. Relevant quality parameters for the two different lots of Golden and Fuji apples. Values are the mean of three determinations made for 3 different apples. For each row, different letters mean significant differences among the mean values of the measured parameter.

Quality parameters	Golden		Fuji	
	Lot A	Lot B	Lot A	Lot B
Firmness (kg)	6.77b	4.74c	7.85a	7.55a
Soluble solids concentration (°Brix)	14.8a	14.7a	14.5a	15.17a
Acidity (g malic acid l ⁻¹)	10.68a	9.03b	6.88c	5.36d

Table II. Analysis of variance of patulin concentration found in decayed and sound sections of apples.

Source of variation	Decayed samples			Sound samples	
	DF	MS	F	MS	F
Ripeness (R)	1	18.26	0.53	0.06	1.25
Variety (V)	1	1731.39	50.01**	1.05	23.90**
Depth section (D)	5	1320.05	38.13**	1.92	43.50**
Lesion size (L)	1	1045.36	30.20**	1.87	42.40**
R x D	5	23.23	0.67	0.01	0.23
R x V	1	169.44	4.89*	0.36	8.10**
V x D	5	360.11	10.40**	0.45	10.27**
L x R	1	33.83	0.98	0.00	0.09
L x V	1	99.07	2.86	0.23	5.33*
L x D	4	859.79	24.84**	1.05	23.78**
R x V x D	4	9.81	0.28	0.15	3.39*
L x R x D	3	6.20	0.18	0.00	0.01
L x R x V	1	59.60	1.73	0.40	9.02**
L x V x D	4	210.76	6.09**	0.18	4.09**
L x V x D x R	3	31.60	0.91	0.17	3.96**

**P<0.01

*P<0.05

Table III. Analysis of variance of patulin concentration found in decayed and sound sections of apples; separate analysis are shown for each lesion diameter.

Source of variation	Decayed samples					Sound samples			
	2 cm		4 cm			2 cm		4 cm	
	DF	MS	F	MS	F	MS	F	MS	F
Ripeness (R)	1	33.85	0.79	1.70	0.12	0.02	0.27	0.05	8.59*
Variety (V)	1	1457.5	34.02	554.62	37.55	1.27	18.88*	0.16	26.82*
		3	*			*			
Depth section (D)	5	2346.3	54.77	156.06	10.57	3.06	45.30*	0.15	25.65*
		5	*			*			
R x D	5	24.97	0.58	5.67	0.38	0.01	0.11	0.01	1.05
R x V	1	151.66	3.54*	12.58	0.85	0.51	7.50*	0.00	0.17
V x D	5	559.37	13.06	57.96	3.92*	0.56	8.35*	0.05	9.02*
			*						
R x V x D	5	38.84	0.91	3.59	0.24	0.26	3.91*	0.01	1.29

*P<0.05

Table IV. Mean total patulin (μg) found for each treatment.

Rotten zone	Apple variety	Ripeness	Mean total patulin \pm SD	% in decayed portion	% Diffused
2 cm	Fuji	Lot A	33.44 \pm 10.53	94	6
		Lot B	15.46 \pm 5.07	96	4
	Golden	Lot A	65.10 \pm 24.49	96	4
		Lot B	93.71 \pm 61.32	97	3
4 cm	Fuji	Lot A	39.12 \pm 22.51	94	6
		Lot B	16.20 \pm 7.55	94	6
	Golden	Lot A	115.89 \pm 84.47	96	4
		Lot B	91.75 \pm 63.90	98	2

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