Intl. J. of Food. Ferment. Technol. 3(1): 15-32, June, 2013

DOI Number: 10.5958/j.2277-9396.3.1.002

Review Paper

Occurrence of Patulin its Dietary Intake through Consumption of Apple and Apple Products and Methods of its Removal

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Paper no: 58 **Received**: 11 Jan, 2013 **Received in revised form**: 17 May , 2013 **Accepted**: 10 June, 2013

Abstract

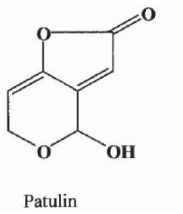
Patulin (4-hydroxy-4H-furo [3,2c] pyran-2[6H]-one), a mycotoxin, is a secondary metabolite produced by a number of fungi, primarily *Penicillium* and *Aspergillus* species common to fruit and vegetable based products. Most notably apple is of major concern from food safety considerations. Patulin 1st discovered as an antibiotic but later on scientific community realised its negative health effects. It is classified as a group-3 carcinogen as there is no evidence of its carcinogenicity in humans but based on a long-term investigation in rats, the World Health Organization (WHO) has set a tolerable weekly intake of 7 ppb (µg/Kg) body weight. The maximum limit of patulin in foods is restricted to 50 ppb in many countries of the world. Conventional analytical detection methods involve chromatographic analyses, such as HPLC, GC and more recently techniques such as LC/MS and GC/MS. The risk associated with the patulin necessitates its control and ultimately removal from the food products. It has been detected in several apple products viz., apple juice, apple puree, apple wine, apple cider and baby foods. The quantities range from 0.5 to 732.8 µg/L. Effort to understand the basic chemical and biological nature of patulin as well as interaction with the other food components are being made. It may occur in apples during harvest and postharvest stages. The principal risk arises when unfit/ rotten fruit is used for the production of juices and other processed products. Process stages and conditions of process may effect concentration of patulin. Nature of processing such as fermentation, heat treatment and clarification applications or additional steps of production line such as the use of binding material can help in removing patulin from products or at least reduce its concentration employing low cost technology.

Keywords: Patulin, apple, HPLC, WHO, risk, removal

Introduction

Food safety is of major concern these days since more and more chemicals are present in our environment. Food is an important route of exposure to contaminants like dioxins, mycotoxins, heavy metals, pesticides, polycyclic aromatic hydrocarbons, drugs and hormones. Mycotoxins are a class of highly toxic compounds, secondary metabolites, produced under particular environmental conditions by certain fungi or moulds, developing in many foodstuffs. Despite various efforts made in prevention, mycotoxins remain a problem of human health concern in several parts of the world including developed countries. Mycotoxins are therefore a food quality and safety issue of top priority. Patulin (PAT) is a polyketide lactone (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one) (Figure 1) is a toxic secondary metabolite produced by a

wide range of fungal species (Table 1) of the *Penicillium, Aspergillus and Byssochlamys* species growing on fruit, including apples, pears, grapes and other fruits. The principal risk arises when unfit/rotten fruit is used for the production of juices and other processed products. Apples and apple products are excellent substrates for *Penicillium expansum* which is the causal agent of blue mould rot to produce the patulin. There may be a risk of human exposure to patulin through the consumption of juices and jams manufactured with apples with small rotten areas. Due to the high consumption of apple products during the first year of life, the children are more exposed to patulin toxicity when compared to the adults (Moake *et al.*, 2005). Children are a vulnerable part of the population due to, in part, their physiology, a fairly restricted diet and a higher consumption relative to their body. In this review all the aspects connected to patulin in apple and its product are discussed besides other topics related to it.



Patulin

Fig. 1. Chemical structure of patulin (4-hydroxy-4H-furo [3,2c] pyran-2[6H]-one)

Table 1: Fungi responsible for patulin production

Fungal species	Synonym	Reference
Penicillium urticae Bainier	*Penicillium griseo-fulvum* Dierckx*Penicillium patulum Bainier*	Kent and Heatley, 1945 Chain *et al.*, 1942 Birkinshaw *et al.*, 1943
Penicillium expansum Link	*Penicillium leucopus* (Pers.) Biourge	Anslow *et al.*, 1943
Penicillium cyclopium Westling		Efimenko and Yakimov, 1960
Penicillium granulatum Bainier	*Penicillium divergens* Bainier and Sartory	Barta and Mecir, 1948
Penicillium claviforme Bainier		Bergel *et al.*, 1943
Penicillium melinii Thom		Karow and Foster, 1944
Aspergillus clavatus Desm.		Umezawa *et al.*, 1947
Aspergillus giganteus Wehmer		Florey *el al.*, 1944
Aspergillus terreus Thorn		Kent and Heatley, 1945
Byssochlamys nivea Westling	*Gymnoascus* sp.	Karow and Foster, 1944

2.0 History

Patulin (4-hydroxy-4H-furo [3,2c] pyran-2[6H]-one) is a water-soluble lactone and was 1st isolated as an antibiotic during the 1940s (Stott and Bullerman, 1975) and has been historically known by various names such as clavacin (Anslow *et al.*, 1943), expansine (Van Luijk 1938), claviformin (Chain *et al.*, 1942), clavatin (Bergel *et al.*, 1943), gigantic acid (Philpot 1943) and myocinC (DeRosnay *et al.*, 1952). Initially isolated as a broadspectrum antifungal antibiotic (Korzybski *et al.*, 1976), it was later found to inhibit more than 75 different bacterial species including both Gram-positive and Gram-negative bacteria (Ciegler *et al.*, 1971). After some time, various studies suggested patulin not only to be toxic to fungi and bacteria but also to animals and higher plants, including cucumber, wheat, peas, corn and flax (Iyengar and Starky, 1953; Norstadt and McCalla 1963, 1968; Berestets'kyi and Synyts'kyi, 1973). As continued evidence for the negative health effects of patulin came into light, many regulatory agencies started to limit the patulin content within foods.

3. Biosynthesis

Patulin is produced by 60-plus species of mold of over 30 genera (Lai *et al.*, 2000). Among these are *Penicillium expansum* (*P. leucopus*), *P. patulum (P. urticae, P. griseofulvum), P. crustosum, P. roqueforti, P. claviforme, Paecilomyces spp., Saccharomyces vesicarium, Alternaria alternata, Byssochlamys nivea, B. fulva, Aspergillus giganteus, A. terreus* and *A. clavatus* (Lovett *et al.*, 1974; Rice *et al.*, 1977; Harwig *et al.*, 1978; Draughon and Ayres 1980; Ough and Corison 1980; Roland and Beuchat 1984a; Laidou *et al.*, 2001; Moss and Long 2002). Patulin production in culture has also been shown to occur when growth rate diminishes because of limitations on cell growth such as nitrogen consumption (Grootwassink and Gaucher, 1980). Patulin biosynthesis is well understood and involves a series of condensation and redox reactions. Most of these reactions are enzyme catalyzed.

Patulin is a polyacetate-derived secondary metabolite or polyketide (Turner 1976). Its synthesis is initiated with acetyl coenzyme A (CoA) and 3 units of malonyl CoA, making it a tetraketide (Ciegler *et al.,* 1971; Turner 1976; Steyn 1992). Acetyl CoA and 3 malonyl-CoA are condensed into 6-methylsalicylic acid (6-MSA) by a 760000-Dalton homotetramer enzyme called 6-methylsalicylic acid synthetase (6-MSA synthetase)(Gaucher 1975; Lynen *et al.,* 1978).

The gene encoding for 6-MSA has been cloned and characterized from *P. patulum* and *P. urticae* (Beck *et al.,* 1990; Wang *et al.,* 1991). Inactivation of 6-MSA synthetase has been shown to be the 1st limitation on patulin production (Neway and Gaucher, 1981). 6-MSA synthetase loss is a selective process because the highly similar fatty acid synthetase of *P. urticae* (Lynen *et al.,* 1978) is stable under the same reaction conditions that inactivate 6-MSA synthetase (Lam *et al.,* 1988). This finding further verified by studies in which treatment of 6-MSA synthetase reaction mixtures, containing Nicotanimide Adenine Dinucleotide Phosphate (NADPH) cofactor, acetyl-CoA and malonyl CoA, with the reducing agent, dithiothreitol and proteinase inhibitor, phenylmethylsulfonyl fluoride, stabilized 6-MSA synthetase. This suggests proteolysis and conformational integrity play a role in the regulation of 6-MSA synthetase (Lam *et al.,* 1988).

The next stage of patulin biosynthesis involves the conversion of 6-MSA into *m*-cresol *via* the activity of 6-MSA decarboxylase (Lam *et al.,* 1988). *M*-cresol is then converted into *m*-hydroxybenzyl alcohol by *m*-cresol 2-hydoxylase (Murphy and Lynen 1975).

The next step in patulin's biosynthetic pathway is debated among 2 main mechanisms. Both agree that *m*-hydroxybenzyl alcohol is eventually converted to gentisaldehyde (Forrester and Gaucher, 1972; Zamir 1980). However, the intermediary between these 2 compounds is believed to be either gentisyl alcohol (Sekiguchi *et al.,* 1983; Iijimia *et al.,* 1986) or *m*-hydroxybenzaldehyde (Sekiguchi *et al.,* 1983). Some studies have suggested that both are possible, with *m*-hydroxybenzaldehyde being favorable (Gaucher 1975), whereas others believe that *m*-hydroxybenzaldehyde is not converted to gentisaldehyde but rather to m-hydroxybenzoic acid (Murphy and Lynen 1975). In the 2nd case, *m*-hydroxybenzyl alcohol dehydrogenase converts *m*-hydroxybenzyl alcohol into *m*-hydroxybenzaldehyde (Gaucher, 1975; Murphy and Lynen,

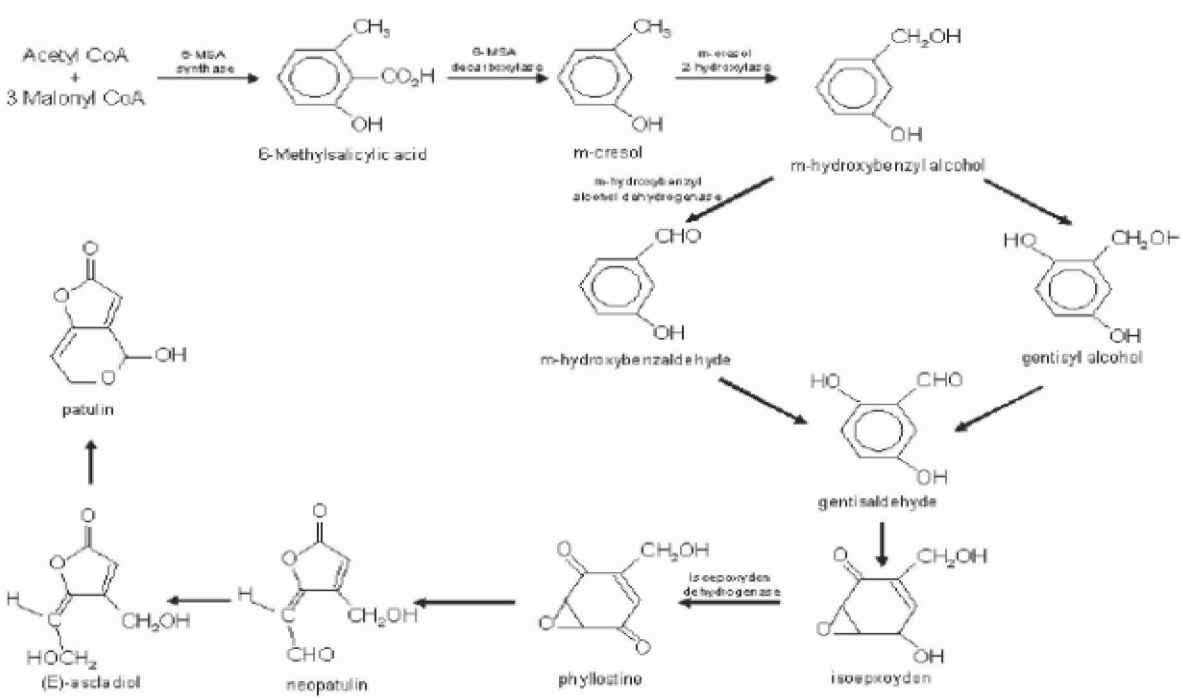


Fig. 2. Patulin biosynthetic pathway. The metabolic intermediaries and relevant enzymes involved in the biosynthesis of patulin are shown (Gaucher 1975; Murphy and Lynen 1975; Sekiguchi and Gaucher 1979; Sekiguchi *et al.,* 1983; Iijimia *et al.,* 1986)

1975). Both this enzyme and *m*-cresol 2-hydroxylase have been shown to require oxygen and NADPH to function (Murphy and Lynen, 1975). Once gentisaldehyde has been formed, it is then, converted to isoepoxydon, phyllostine, neopatulin, E-ascladiol and finally, to patulin (Sekiguchi and Gaucher, 1977, 1978; Sekiguchi and Gaucher, 1979, 1983). The conversion of isoepoxydon to phyllostine is accomplished via an NADP-dependent isoepoxydon dehydrogenase (Sekiguchi and Gaucher, 1979). Conversion of neopatulin to E-ascladiol take place through a reduction by NADPH. The product of this reaction, Eascladiol, is then either oxidized to patulin or non-enzymatically transformed to its isomer Z-ascladiol (Sekiguchi *et al.*, 1983).

4. Detection and Quantification of Patulin

The traditional methods of sample preparation in the analysis of patulin in food samples prior the analysis by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS) and liquid chromatography– mass spectrometry (LC–MS) are Liquid–liquid extraction and solid-phase extraction (SPE) (Songsermsakul and Razzazi-Fazeli, 2008). RP-HPLC, coupled to UV detection, has been found to be the most suitable method in patulin trace analysis since it exhibits strong UV absorption at 275 nm. An accurate HPLC method for determination of patulin in apple based products intended for infants was validated by a collaborative study in order to quantify patulin at levels of 10 µg/kg or lower (Arranz *et al*., 2005). The most common method currently used to quantify patulin in fruit products is high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. This is the official method adopted by AOAC for apple juice (method 995.10) with a detection limit of 5 µg/L. The juice is extracted 3 times with ethyl acetate and cleaned up by liquid-liquid extraction with a 1.5% sodium carbonate solution. The ethyl acetate extract is dried with anhydrous sodium sulfate; the solvent is then evaporated, normally under nitrogen and the dried residue is dissolved with acidified water (pH 4 by addition of acetic acid). This prepared extract is ready for HPLC analysis. The recommended liquid chromatography (LC) systems include analytical reversed-phase LC columns such as octadecylsilane fully end-capped with 5 µm particle stationary phase, 12 to 25 nm pore size, carbon loading of 12% to 17% and a UV detector set at 276 nm (Fig 3), although a photo diode array detector is preferred to aid in the presumptive identification of the patulin peak. The system can be run isocratically at 1 mL/min using 3% to 10% acetonitrile in acidified water (0.095 parts per volume perchloric acid 60%) as long as patulin separates from 5-hydroxymethylfurfural (HMF), a common compound found in apple juice that elutes just before patulin. For cloudy apple juice and apple puree, a collaborative 12 participants from European countries conducted a study to validate the effectiveness of this LC procedure for patulin

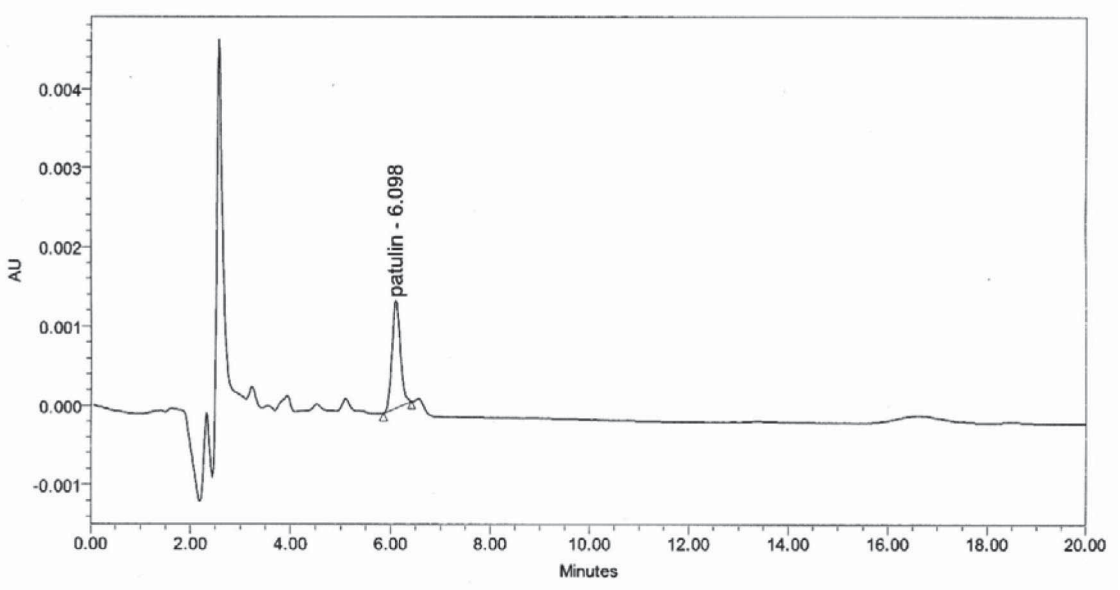


Fig. 3. HPLC separation of patulin (Waters Spherisorb C_{18} 5 µm, 4.6 X 150mm column, flow rate: 0.5 mL/min), isocratic elution, eluent: water–acetonitrile (90:10, v/v)). Detection: UV visible; chromatogram at 276 nm

determination with a slight modification. Prior to the ethyl acetate extraction, the samples were treated with pectinase enzymes and held overnight at room temperature or for 2 h at 40 °C and then centrifuged at 4500 rpm for 5 min. Based on the results, the method is recommended for patulin at concentrartion greater than 50 µg/L in cloudy apple juice and purees (Mac-Donald *et al.,* 2000).

Limit of patulin in different countries

European countries were among the 1st to address the concern and today many countries across the world, particularly those within the EU, limit allowable patulin content within foods at 50 µg/L (Moller and Josefsson, 1980; van Egmond, 1989; Stoloff *et al.,* 1991; Rovira *et al.,* 1993; Forbito and Babsky, 1996; Gokmen and Acar, 1998). While 50 µg/L is now the norm for patulin regulation, several countries have set even lower limits for patulin at 25 to 35 µg/L (van Egmond, 1989). In conjunction with these maximum content standards, a joint Food and Agriculture Organization-World Health Organization (WHO) expert committee established a provisional maximum daily intake of 0.4 µg/kg body weight for patulin (WHO, 1995). The United States has been much slower to set regulation on patulin, but today the U.S. Food and Drug Administration limits patulin to 50 µg/L in single-strength and reconstituted apple juices (USFDA 2004) while in China, the maximum recommended concentration for apple juices is 50 µg/L and for solid apple products is 25 µg/L.

In order to protect infants and young children, the EC regulation introduces a separate limit of 10 µg/kg for patulin in apple juice, solid apple products, including apple puree and baby-foods other than processed cereal-based-foods for infants and young children (Commission Regulation, EC No. 1881/2006). This limit is lower than the maximum permitted levels of patulin in different apple-based-foods established by the European Union (Table 2), like, 50 µg/kg for fruit juices, reconstituted concentrated fruit juices, fruit nectars, spirit drinks, cider and other fermented drinks derived from apples or containing apple juice and 25 µg/kg for solid apple products, including apple compote and apple puree intended for direct consumption. Detection of patulin at a level of 10 µg/kg for patulin concentration in apple-based foods depends on the availability of a more sensitive method enabling the determination of lower levels of patulin.

Table 2: Patulin: EU Limits in Apple Products

Maximum permitted level	Products
50 µg/kg	Fruit juices, concentrated fruit juice as reconstituted and fruit nectars.Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice.
25 µg/kg	Solid apple products intended for adults, including apple compote, apple puree for direct consumption.
10 µg/kg	Apple juice and solid apple products including apple compote and apple puree for infants and young children

Source: European Commission, 2006

5.0 Health related issues

The various risks posed by patulin to humans health are assessed which include acute, chronic and cellular level health effects (Table 3). Patulin (PAT) is genotoxic but no adequate evidence has been reported for its carcinogenic-

Table 3: Effects of patulin on Health of Human beings

Acute symptom	Chronic symptom	Cellular level effect
Agitation, convulsions, dysponea, pulmonary congestion, edema, hyperemia, GI tract distension, Nausea, Epithelial cell degeneration, intestinal hemorrhage, Intestinal inflammation, Ulceration	Genotoxic Neurotoxic Immunotoxic Teratogeneic Immunosuppressive Carcinogenic	Plasma membrane disruption, Protein synthesis inhibition, Transcription disruption, translation disruption, DNA synthesis inhibition, Na-coupled amino acid transport inhibition, Interferon-ã production inhibition, RNA polymerase inhibition, Aminoacyl-tRNA synthetases inhibition, Na-K ATPase inhibition, Muscle aldolase inhibition, Urease inhibition, Loss of free glutathione, Protein crosslink formation, Protein prenylation inhibition

Source: Moake *et al.,* (2005); Speijers (2004); Wouters and Speijers (1995); Riley and Showker (1991); Mahfoud *et al.,* (2002); Hatez and Gaye (1978); Miura *et al.,*(1993); Arafat and Musa (1995); Ueno *et al.,* (1976), Moule and Hatey (1977); Arafat *et al.,* (1985); Lee and Roschenthaler (1987), Cooray *et al.,* (1982); Wichmann *et al.,* (2002)

ity in experimental animals and humans. It is not classified with respect to its carcinogenicity to humans. Accordingly, it is included in Group 3 of the International Agency for Research on Cancer (IARC, 1986).

6.0 Occurrence of Patulin in Foods

6.1 Foods

Besides fruit and vegetable products, patulin has also been isolated from cheddar cheese (Bullerman and Olivigni, 1974) and grain products such as barley and wheat malts, feed silages, cereal stubbles (Escoula, 1974, 1977; Lopez-Diaz and Flannigan, 1997; Pittet, 1998), bread and related flour/ dough products (Reiss, 1976).

In the analysis of 23 homogenised baby-foods Beretta *et al*. (2000) found that the mycotoxin concentration was below the established limits of 10 µg/kg. In a study published in 2003, the authors found patulin in 20% (2/10) of baby food up to the limit of 10 µg/mL, both labelled as ‘‘organic food” (Riteni, 2003). Piemontese *et al.,* (2005) also reported the detection of patulin (<1 µg/kg) in 3/23 fruity baby food samples. According to Majerus and Kapp (2002) a small number of data exists on baby food and 13.8% of the analysed baby-foods were positive indicating the need to perform further studies for reaching a more sure statement about the occurrence data of patulin in baby food. The occurrence of patulin in different food commodities has been the subject of many research investigations in Europe: Spain (Murillo *et al.*, 2008); Belgium (Tangni *et al*., 2003); Italy (Piemontese *et al.*, 2005); Greece (Moukas *et al.,* 2008) and Holland (Boonzaaijer *et al.,* 2005).

Fruits

Patulin-producing strains contaminate a variety of fruits and vegetables (Table 4). Patulin is found in the apple with a higher rate than other fruits. The toxin has been found especially in unprocessed apples. It has been suggested that the patulin content of the apple is not affected by growth of the apple using traditional or organic methods. (Marin *et al*., 2011; Jackson and Al-Taher, 2008; Bandoh *et al*., 2009). Patulin has been identified in apples in several countries of the world.

Table 4: Distribution of patulin and patulin-producing fungi in various fruits and fruit products

Fruits contaminated with patulin-producing fungal strains	Fruit products contaminated with patulin	Countries with apple and juice contamination
Apples	Apple juice	England
Grapes	Apple-acerola juice	New Zealand
Cherries	Pear juice	United States
Crabapples	Grape juice	South Africa
Apricots	Sour cherry juice	Sweden
Persimmons	Orange juice	Turkey
Strawberries	Pineapple juice	Brazil
Nectarines	Passion fruit juice	Austria
Raspberries	Apple cider	Belgium
Black mulberries	Apple puree	Australia
White mulberries	Strawberry jam	France
Peaches	Blackcurrant jam	Canada, Italy
Pear	Blueberry jam	
Plums	Baby food	
Tomatoes	Corn	
Bananas	Cheddar cheese	
Blueberries	Barley Malt	
Black currants	Wheat Malt	
Almonds	Bread	
Pecans		
Peanuts		
Hazelnuts		

Source: Moake *et al*., (2005); Leggott and Shephard (2001); Demirci *et al.,* (2003); Ritieni (2003); Harwig *et al.,* (1973b); Sommer *et al.,* (1974); Brian *et al.,* (1956); Walker (1969); Ware *et al.,* (1974); Scott *et al.,* (1972); Josefson and Anderson (1976); Brown and Shephard (1999); Gokmen and Acar (1998); de Sylos and Rodriquez-Amaya (1999); Steiner *et al.,* (1999a); Tangni *et al.,* (2003)

6.3 Processed apple products

Apple juice and cider were once thought to be the only products to naturally contain naturally patulin (WHO 1995). However, various researchs now have found that patulin occur naturally in many fruit and vegetable products such as apple, apple-acerola, grape, pear, sour cherry, blackcurrant, orange, pineapple and passion fruit juices, pasteurized and unpasteurized apple cider, apple puree, corn, strawberry, blackcurrant and blueberry jams and some types of baby foods (Lovett *et al.,* 1974; Sommer *et al.,* 1974; Frank *et al.,* 1977; Scott *et al.,* 1972; Harwig *et al.,* 1978; Brackett and Marth 1979a; Ehlers 1986; Wheeler *et al.,* 1987; Jelinek *et al.,* 1989; Lin *et al.,* 1993; Prieta *et al.,* 1994; Rychlik and Schieberle 1999; Ake *et al.,* 2001; Leggott and Shephard 2001; Ritieni 2003).

Within the food industry, apples and their respective products are of greatest concern for patulin contamination. While a variety of other food sources and products have demonstrated patulin and/or patulin-producer contamination, the frequency of these events is much less than that of the apple industry. Extensive studies have been conducted around the world that have examined the extent and degree to which apple products have been contaminated by patulin. In Wisconsin, 23 of 40 roadside apple juices were found to contain between 10 to 350 µg patulin/L (Brackett and Marth 1979a). A study showed that 8 of 13 commercial apple juices tested contained between 44 and 309 µg patulin/L juice (Ware *et al.,* 1974). A Turkish study showed 215 of 215 apple juice concentrates examined had between 7 and 375 ppb patulin with 43% being above the

Table 5: Patulin content in apple products

Apple product	Country	Patulin found/total number of samples	The lowest levelfound (LOL)	Range(ìg/L or ìg/kg)
Apple juice, open or closed	South Africa	4/17	5	5–45
fruit juices and nectars	Sweden	5/39	2	2-50
	Turkey	27/45	5	19.1–732.8
	EU	35/43	0.67	2.5–38.8
	Italy	3/8	5	5.8–56.4
	Japan	15/76	1	1.4–45.6
	Iran	13/42	15	15–285.3
	Italy	16/33	0.5	0.5–53.4
	Spain	5/17	0.3	1.5–50.9
	Italy	19/32	1.57	1.57–44.89
	Brazil	4/100	3	3–7
	Greece	66/66	0.23	0.9–36.8
	Spain	66/100	0.7	0.7–118.7
	Spain	30/71	2.08	2.08–15.0
	Iran	150/150	3	6.0–106.0
Organic apple juice, organic	Italy	6/21	1.57	1.57–47.91
open or closed fruit juice	Italy	3/7	5	30.4–33.2
	Italy	12/24	0.5	0.5–69.3
Apple puree	Italy	2/4	5	15.9–16.7
	Italy	0/4	0.5	
	Argentina	4/8	3.8	22–221
	Spain	6/18	0.3	7.7–28.4
	Spain	4/77	2.08	2.08–17.6
Organic apple puree	Italy	7/13	0.5	>0.5
Infant Formula	South Africa	6/17	5	5–20
	Italy	0/6	5	0.7
	Italy	1/11	0.5	2.0
	Spain	42/124	2.08	8–9.6
Organic infant formula	ItalyItaly	2/42/12	50.5	13.1–17.7>0.5
Marmalade	Argentina	6/26	2.8	17–39
Apple wine	South Africa	2/8	5	5–10
	European Union	3/7	0.67	2.8–6.1

Source: Sahin *et al.,* 2011

50-ppb international standard (Gokmen and Acar 1998). Finally, a 1996 to 1998 study in South Africa showed that 5 of 22 juice samples contained between 10 and 45 ppb patulin and 29% of infant apple products showed 5 to 20 ppb (Brown and Shephard, 1999).

7.0 Factors influencing patulin production

The presence of patulin-producing fungi do not necessarily guarantee patulin production. Patulin production is usually associated with apple soft rot and blue mold rot, most commonly caused by *P. expansum* (Pierson *et al.,* 1971; Harwig *et al.,* 1973b; Sommer *et al.,* 1974). Patulin-production within fruits, vegetables and their products is mostly found to be dependent on various factors (Sommer *et al.,* 1974; Northolt *et al.,* 1978; McCallum *et al.,* 2002) as

- Water activity (a_w)
- Temperature
- pH
- Other factors

7.1 Water activity (a_w) and pH

Temperature has been shown to affect pathogen growth and to a greater extent, the production of patulin (McCallum *et al.,* 2002). Patulin production has been observed at all temperatures which permit *P. expansum* growth, at an approximate range of 0 to 30 °C temperature (Sommer *et al.,* 1974). Highest patulin production is observed at 21 °C (Roland and Beuchat, 1984b). However, patulin can be formed at low temperatures like 0-4°C. Keeping fruits in the refrigerator generally does not inhibit patulin formation. It needs little oxygen and grows with a rate of lower than 2% in oxygen in the atmosphere. In conditions where the level of carbon dioxide is higher than 15%, the microorganism grows faster. Therefore, it has been reported that the formation of the toxin can be decreased or prevented by arranging storage conditions of the fruits (Jackson and Al-Taher, 2008).

7.2 Temperature

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7.3 Other factors

Cultivar differences among apples also affect the patulin production by *P. expansum* (McCallum *et al.,* 2002). Patulin formation in apples is affected by geographical localization, the place of growth of the fruit, harvesting, preharvesting practices, method of harvesting, damage formed on the fruit, post-harvesting practices and storage conditions. It is not known which of these factors is mainly involved in the formation of patulin and it is not clear which factors in the growth of apples should be changed and in which way should they be changed to decresase patulin level (Amalaradjou and Venkitanarayanan, 2008). The properties of the fruit and mechanical demage in the fruit also effect patulin formation. Rainy weather during harvesting is another reason which increases fruit infections related to moulds and contamination. Therefore, the fruits should be harvested at dry air and rapidly carried to cold stores. In addition, fruits which are damaged mechanically and have fallen on the ground should not be used for fresh and processing purpose. Keeping the containers used during transport clean is another efficient method to the prevent formation of spores (Bandoh *et al*., 2009; Amalaradjou and Venkitanarayanan, 2008).

Table 6: Temperature and water activity range for growth and patulin production by *P. expansum.*

Growth a_w	Minimum	Maximum
Temperature	0.83, 0.85	35°C
	0.85	33°C
	-6 to 2°C	
	0°C	
Patulin Production a_w		
	0.97	30°C
	0.99	
	0.95	
Temperature	0°C	

Source: Drusch and Ragab (2003); Northolt and Bullerman (1982); Pitt and Hocking (1997); Sommer *et al.,* (1974); Moake *et al.* ,(2005)

8.0 Control of patulin production in apple products

For removal of patulin both during production and after production (Park *et al.,* 1988), effective decontamination/ detoxification procedures must ensure:

- To inactivate, destroy or remove the toxin
- Not to produce or leave new toxic substances
- Retain nutritive value/acceptability of the product
- Not-significantly alter the technological processes associated with the product
- If possible, destroy fungal spores

8.1 Control during Production

There are several methods to control patulin production within standard apple juice production steps. These are summarized here:

- Quality of the fruit
- Juice clarification
- Pasteurization

Quality of the fruit

Processed apple products utilize lower quality fruit that is unsuitable for direct market retail. Removal of decayed/ damaged fruit or trimming of moldy portions can significantly reduce patulin levels in apple products (Lovett *et al.,* 1975b; Taniwaki *et al.,* 1992; Sydenham *et al.,* 1995, 1997; Beretta *et al.,* 2000). Trimming of rotten sections of apple has been shown to remove up to 99% of patulin contamination (Lovett *et al.,* 1975b). However, this process is expensive and labor intensive. Furthermore, patulin can be detected in visibly sound fruit (Jackson *et al.,* 2003) and can spread from rotten areas of apples into sound areas (Beretta *et al.,* 2000). Studies have shown that patulin could diffuse 1 to 2 cm from the rotten core in apples (Taniwaki *et al.,* 1992; Rychlik and Schieberle 2001). Producers' tests on apples have shown that patulin level is often not related to the physical quality of the fruit, with both high-rot fruit and top-quality eating fruit often having high levels of patulin in tests (Corbett 2003), as further demonstrated by the previously mentioned Hudson Valley study on retail apples. While often beneficial, the sorting of decayed apples to the level of being effective is difficult if not impossible, often necessitating the need to reject entire juice loads of apples. If reliant on this method, small-scale producers who cannot afford these losses will be forced to sort by hand, increasing costs to the point that many may cease operating (Rosenberger, 2003).

Juice clarification

The second area of standard juice production capable of reducing patulin levels involves the juice clarification process. But the results from this process have been mixed and those methods most successful at removing patulin often do so at the expense of sensory and authenticity qualities of juice. It has been suggested that standard fruit juice production processes remove only about 20% of patulin (Stray 1978; Harrison 1989). Different methods of juice clarification are:

- Filtration
- Gelatin/Bentonite (fining agent)
- Diatomaceous earth (filter aid)
- Activated charcoal
- Polystyrene-Divinyl Benzene based macro porous resin
- Centrifugation
- Depectinization (Enzyme treatment)
- Ultrafiltration

The traditional apple juice production processes of depectinization, clarification and filtration through a rotary vacuum precoat filter has been reported to reduce patulin levels by 39%. In the same study, use of depectinization, clarification, mixing with gelatin/bentonite and ultrafiltration resulted in a 25% decrease in patulin (Acar *et al.,* 1998). A 2nd study examined the effects of gelatin/bentonite flocculation, filtration, activated charcoal, ultrafiltration, polyvinylpolypyrrolidone and polystyrene-Divinyl Benzene (DVB)–based macro porous resin on apple juice color, clarity, phenolic content, organic acid content and patulin reduction. Activated charcoal treatment had the highest patulin reduction with 40.9% but also significantly reduced color and phenolic content, the two characteristics are associated with juice authenticity. Polystyrene-DVB–based macro porous resin was the 2nd most effective method, reducing patulin by 11%. None of the treatments however altered organic acid content significantly (Artik *et al.,* 2001). Centrifugation and fining of juice pulp have been shown to reduce patulin levels by 89% and 77%, respectively. However, these methods make the removed

cake and/or filter potentially highly toxic and unfit for any further use, such as often is done with juice sediments in animal feed (Bissessur *et al.,* 2001). This control represent a loss of income for many juice producers. Batch absorption with synthetic polymers has also been investigated (Canas and Aranda 1996), as has enzyme treatment with pectinase enzymes used to break down the pectin coat surrounding protein particles, allowing aggregation and sedimentation of protein particles and in the case of patulin contamination, their associated patulin adducts. This method has resulted in a 73% decrease in patulin content within juice (Bissessur *et al.,* 2001). According to Bissessur *et al.,* (2001), paper filtration of apple juice using Whatman number one filter paper with 0.5 % diatomaceous earth as a filter aid, enzyme treatment using pectinase (0.02 %) and use of bentonite (3 %) as a fining agent are also effective clarification processes for the reduction of patulin.

Pasteurization

The another juice production process capable of reducing patulin levels is the pasteurization process but out of earlier discussed methods it is the least effective. The results of several studies on this aspect are summarized in Table 7.

Table 7: Reduction of patulin in apple juice during pasteurization

Pasteurization	Patulin reduction (%)	Reference
20 min at 80°C	50	Scott and Somers (1968)
10 s at 60 and 90 °C	18.8	Wheeler *et al.* (1987)
30 s at 90°C	39.6%	Welke *et al.* (2009)
20 min at 80°C	14.1%	Kadakal and Nas (2003)

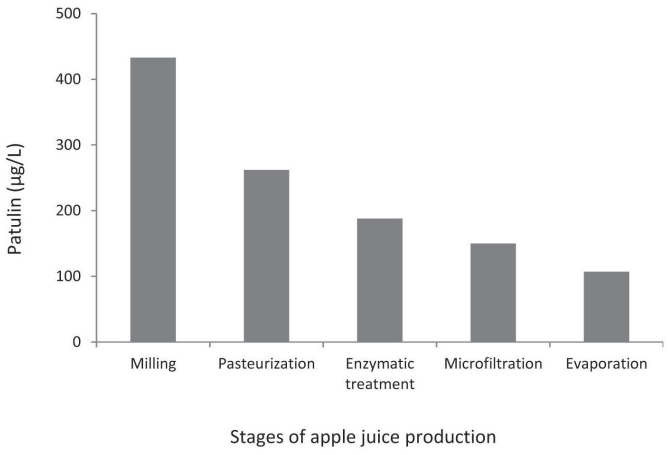

Fig. 4. Effect on Patulin content during apple juice production steps *Source:* Welke *et al*. (2009)

Repeated studies have shown that, while unstable at high pH (McCallum *et al.,* 2002), patulin is relatively stable to thermal degradation in the pH range of 3.5 to 5.5, with lower pH leading to greater stability (Heatley and Philpot 1947; Lovett and Peeler 1973). The half-life of patulin held at 25 °C at pH 6.0 and 8.0 has been shown to be 55 and 2.6 d, respectively (Brackett and Marth 1979c). Evidence also shows that patulin is non volatile and, upon distillation production of apple aroma, patulin remains within juice concentrate (Kryger 2001). Finally, not only is the pasteurization process unable to significantly reduce patulin levels, it often fails to fully remove heat resistant patulin-producing fungi, such as *B. nivea* and *B. fulva* (Ough and Corison, 1980), allowing for potential continued production of patulin within the finished juice. During storage of apple juice, the patulin level either decrease (Scott and Somers 1968; Harwig *et al.,* 1973a) or have no change (Pohland and Allen 1970; Zegota *et al.,* 1988) with refrigerated storage.

8.2 Control after production

Filteration and adsorption

A number of studies have been conducted for the removal of patulin from juice through the use of adsorption filters, columns and agitation treatments using carbon-based material.

- Agitation with 20 mg/mL activated charcoal followed by filtration through a 40 or 60 mesh charcoal column reduced a 30 µg/mL patulin solution to below detectable levels. Further, use of 5 mg/mL charcoal in agitation was able to reduce patulin to below detectable levels in naturally contaminated cider. However, color loss was markedly present in this resulting juice (Sands *et al.,* 1976).
- In a 2nd study, 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3 g/L activated charcoal was added to naturally contaminated apple juice containing 62.3 ppb patulin. Samples were mixed for 0, 5, 10, 20, and 30 min. Three grams/liter activated charcoal was found to be most effective with a time period of 5 min. Clearness of juice increased, color of juice decreased, and small decreases in fumaric acid, pH, and °Brix were also seen (Kadakal and Nas, 2002).
- In another study, ultrafine activated carbon was bound to granular quartz producing a composite carbon adsorbent (CCA). Columns with varying amounts of CCA were prepared and 10 µg/mL patulin were

filtered through at 1 mL/min. Fifty percent breakthrough values for columns with 1.0, 0.5, and 0.25 g CCA were 137.5, 38.5, and 19.9 µg, respectively (Huebner *et al.,* 2000).

- In 4th study designed to compare the effects of different carbon activation methods, steam activated carbon NORIT SA 4 and NORIT SX 4 performed equally, removing 80% and 70%, respectively, of an initial 1 g/L patulin solution in 12 °Brix juice at 55 °C. Chemically activated carbon NORIT CA 1 removed only 45% of the same solution. This study also showed that increased °Brix levels of juice led to decreased patulin removal efficiency, with NORIT SA 4 removing only 20% of patulin from 20 °Brix juice. Within this study, patulin removal was independent of juice temperature between 30 and 65 °C (Leggott and Shephard, 2001).
- In another study, patulin binding to activated carbon compounds was shown to be endothermic, with increased temperatures resulting in improved patulin removal (Mutlu *et al.,* 1997).
- Despite these initial studies where activated carbon was shown to reduce patulin, little work has been done to optimize carbon for this process. Furthermore, the use of activated carbon poses a substantial cost to the juice industry (Leggott and Shephard, 2001), being both time consuming and expensive (Bissessur *et al.,* 2001). Outside of the cost of carbon material itself, activated charcoal treatment creates excess waste that must be dealt with ecologically (Artik *et al.,* 2001). Also, as mentioned within several of the studies, negative affects on color, fumaric acid content, pH and °Brix have been observed with carbon adsorption. These and other modifications made by carbon absorption treatments can negatively alter the taste perception and quality of the juice (Huebner *et al.,* 2000). The use of clays can pose a risk due to removal of essential nutrients from juice (Park and Troxell 2002).

Chemical methods

Chemical decontamination methods have also been shown to be effective and are likely the most easily suitable for industry. Many of the chemicals examined thus far are already considered food grade additives/treatments and therefore, their use would be preferential to many other forms of treatment. Because addition of chemical agents to human foods solely for reducing mycotoxin levels is currently not permitted in the U.S. (Park and Troxell 2002), treatments already accepted for other purposes would allow more rapid integration within industry. Furthermore, many of these same treatments have been shown to be effective in inhibiting patulin-producing molds, making them a double-edged sword in patulin control. Numerous chemical treatments have been utilized to detoxify patulin. Included within these treatments are chemicals designed to oxidize and reduce patulin to hopefully less toxic compounds, as well as treatments to bind up patulin in the form of less toxic thiol-based adducts. The most potent means of chemical detoxification of patulin are ammoniation and potassium permanganate oxidation. Treatment with ammonia has been shown to reduce patulin levels by up to 99.9% in laboratory waste (Fremy *et al.,* 1995) and 99.8% in juice. However, in the 2nd study, the resultant product was unfit for consumption (Ellis *et al.,* 1980). Oxidation by potassium permanganate in acidic and alkali conditions also resulted in better than 99.99% patulin reduction in laboratory waste (Fremy *et al.,* 1995).

Treatment with various sulfur-containing compounds has been another area of intense investigation. Studies involving the use of sulfur dioxide have produced mixed results. Most studies agree that patulin is unstable in the presence of sulfur dioxide (Pohland and Allen 1970), but they vary on its degree of effectiveness.

Table 8: Effect of sulphur dioxide (SO_2) on patulin

SO_2 Concentration (ppm)	Patulin reduction (%)	References
100	50	Ough and Corison (1980)
100	42	Aytac and Acar (1994)
200	12 in 24 h	Burroughs (1977)
2000	90 after 2 d	Burroughs (1977)

The sulfur dioxide inactivation of patulin to be reversible or irreversible depend up on the pH (Steiner *et al.,* 1999b). According to Harrison (1989), filtration through charcoal and addition of 25 to 50 ppm sulphur dioxide (SO_2) essentially removes all patulin from apple juice.

Another chemical method examined for the decontamination of patulin has been treatment with a variety of organic acids and vitamins, many of which are considered to be food-grade additives. In one study, addition of ascorbate and ascorbic acid increased the rate at which patulin was reduced from solution in a concentration-

dependent manner. These reduction rates were found to be slower in juice, but still present. A mechanism was proposed in which a reaction of ascorbate or ascorbic acid with metal ions produced singlet oxygen molecules that attacked and oxidized patulin. However, no evidence for any such reaction products was given (Brackett and Marth 1979b). A study utilizing ascorbic acid found that treatment with 500 mg/kg juice could produce a 50% reduction of patulin (Aytac and Acar 1994). A 3rd study, however, found that degradation by ascorbic acid resulted in only 5% degradation in 3 h and 36% degradation after 44 h (Fremy *et al.*, 1995). Besides ascorbic acid, treatment of apple juice with the B vitamins, thiamine hydrochloride, pyridoxine hydrochloride, and calcium-d-pantothenate, caused statistically significant reductions in patulin content over the control juices (Yazici and Velioglu 2002).

One more chemical detoxification method examined has been the use of ozone, which has now been approved for use in the treatment and processing of foods and has been shown as an effective alternative to heat pasteurization of juice. In a study, treatment of a 32 µM patulin solution with 10% ozone for 15 s reduced patulin to undetectable level and produced no detectable reaction products (McKenzie *et al.,* 1997). The regulatory approval for some of the proposed chemical treatments such as ammoniation and potassium permanganate must be sought prior to use in the food industry.

Biological methods

Biological methods of patulin control involves the degradation of patulin during yeast fermentation. Besides being quite successful, this method is much better understood compared with other decontamination methods. Approximately 90% of patulin can be removed during yeast fermentation (Burroughs, 1977). In 1 study, 6 of 8 yeast strains reduced patulin levels to below detectable levels, while all 8 strains resulted in a 99% or better decrease in total patulin content. Control juice, on the other hand, stored for an equal amount of time (2 weeks), had only a 10% reduction (Stinson *et al.,*1978). In a 2nd study, yeast fermentation reduced patulin levels completely after 2 weeks. This same study also showed that patulin levels failed to decrease significantly in juices that had been yeast fermented and then, filter sterilized to remove yeast, suggesting that active yeast, and not their byproducts, were required for the reduction (Harwig *et al.*, 1973a). Treatments of patulin along with cyclohexamide, a protein synthesis blocker of yeast, completely blocked protein synthesis and prevented the detoxification of patulin. Addition of cyclohexamide 3 h after patulin addition, however, resulted in a reduced, but continued, rate of patulin degradation, suggesting that the proteins synthesized within the 3-h window were catalytically active against patulin and did not just bind it up in adduct formation (Sumbu *et al.,*1983). A later study showed that 3 strains of *Saccharomyces cerevisiae* reduced patulin levels during fermentive growth but not aerobic growth. This reduction resulted in the production of 2 major products: E-ascladiol, patulin's immediate biosynthetic precursor and its isomer Z-ascladiol. These 2 products were also seen in the treatment of patulin with the reducing agent sodium borohydrate (Moss and Long, 2002). E-ascladiol is itself a mycotoxin (Suzuki *et al.,* 1971), which has reduced

Table 9: Biodegradation of patulin using *S. cerevisiae* in sterile apple juice

Incubation(hours)	Apple juice + patulin			
	Residual patulin(ìg/25 ml)		Residual patulin(ìg/25 ml)	
	Control	*S. cerevisiae*	Control	*S. cerevisiae*
0	112.5	112.5	175.0	175.0
8	108.3	105.5	172.3	172.1
19.5	74.6		163.1	
24	56.7		124.7	
30	36.4		116.4	
37	30.1		94.6	
48	108.1	18.8	172.2	74.4
74	9.9		31.2	
97	5.7		19.9	
120	5.2		16.0	
143	106.6	3.7	169.1	n.d.

Source: Coelho *et al.* (2008)

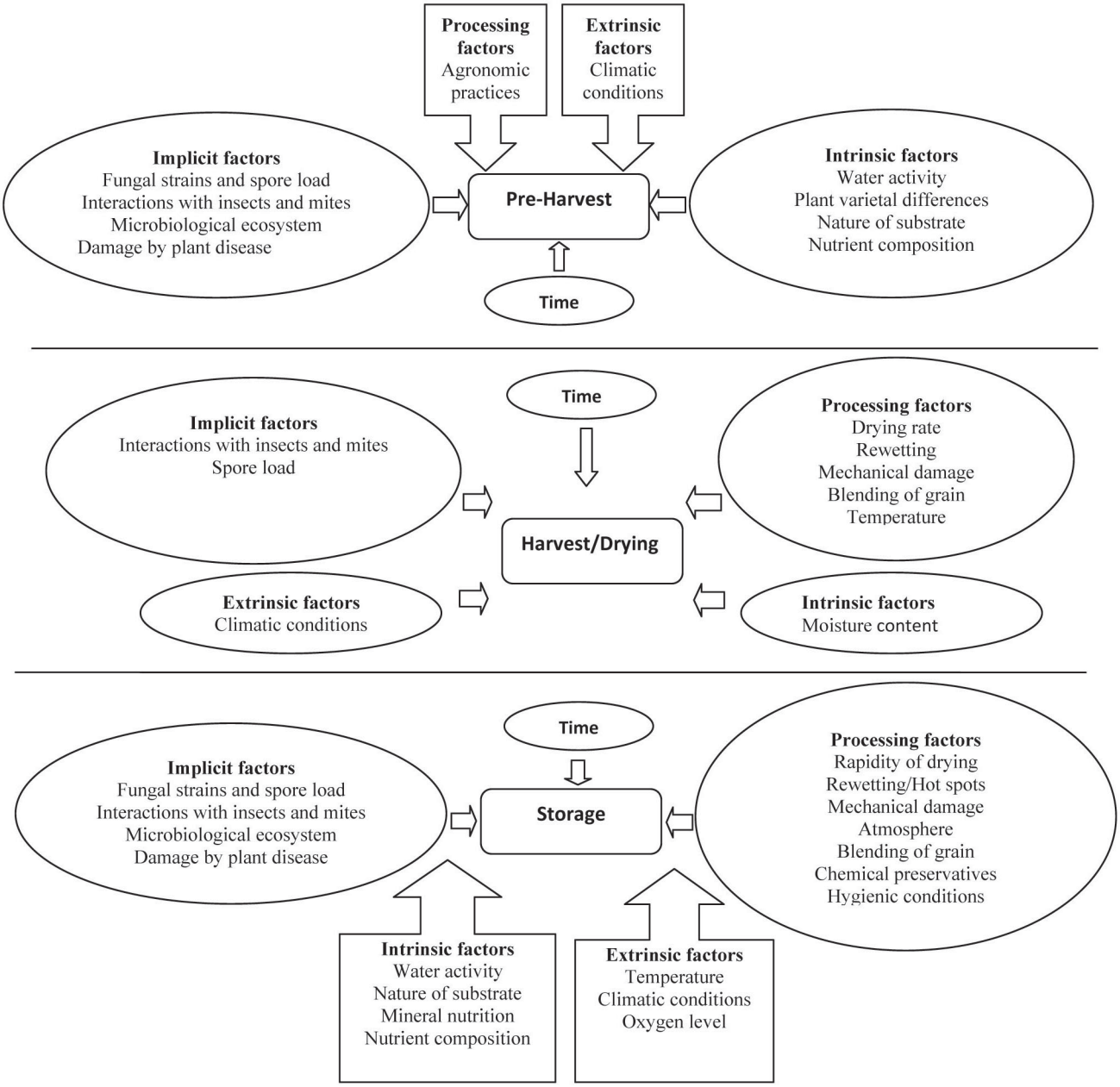

Fig. 5. The interaction between intrinsic and extrinsic factors in the food chain which influences mould spoilage and mycotoxin production in stored commodities (Magan *et al*., 2004)

toxicity compared with patulin and also reacts with sulfhydryl-containing compounds (Sekiguchi *et al.*, 1983). While effective, biological control with yeast is limited to products that can be fermented. Furthermore, yeast are themselves sensitive to patulin and at concentrations greater than 200 µg/mL, yeast have been shown to be completely inhibited, preventing fermentive detoxification (Sumbu *et al.*, 1983).

Conclusion

Patulin is a relatively toxic secondary metabolite, a mycotoxin, which is threatening human health. It can be produced by a variety of molds but *Penicillium expansum* occurring on apples appears to be the most important. Various apple and apple products are found to be contaminated with patulin (0.5 to 732.8 µg/L). However, patulin in

apples or apple products from the Indian market have not yet been studied. It is a matter of concern as consumers of apples and apple products are unaware of the exposure risk to patulin because Indian legislation is silent on this contaminant. The various methods are used for the removal of patulin, such as clarification with diatomaceous earth (0.5 %), pectinase (0.02 %) and bentonite (3 %), filtration through activated charcoal (0.05 to 0.3 %), concentration, apple juice fermentation (with yeast), addition of sulphur dioxide (25 to 2000 ppm), ascorbic acid (0.05 %) and ascorbate and pasteurization (60 to 90°C for 10 sec to 20 min). The food industries should use good quality fruits and should sort out the damaged and rotten fruits during processing to reduce the levels of patulin in the final product below 10 ppb (µg/L). It is necessary to raise the awareness of the food industries to protect the public health. The methods for detecting and quantifying patulin have greatly been improved over the years but the sensitivity of these methods is still often a limiting factor for many aspects of patulin control. These days, no rapid analytical technique exists for the analysis of patulin. A rapid detection method that can be used "in-house" by the food industry without any complicated equipment would be extremely beneficial.

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