
CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF PATULIN CONTAMINATION IN APPLE JUICE AND APPLE JUICE INGREDIENTS IN OTHER BEVERAGES

CAC/RCP 50-2003

INTRODUCTION

1. Patulin is a secondary metabolite produced by a number of fungal species in the genera *Penicillium*, *Aspergillus* and *Byssosclamyces* of which *Penicillium expansum* is probably the most commonly encountered species. Patulin has been found as a contaminant in many mouldy fruits, vegetables, cereals and other foods, however, the major sources of contamination are apples and apple products.
2. Alcoholic fermentation of fruit juices destroys patulin and, therefore, fermented products such as cider and perry will not contain patulin. However, patulin has been observed in apple cider where apple juice was added after fermentation. Ascorbic acid has been reported to cause the disappearance of patulin from apple juice, although the optimal conditions for inactivation have not been fully established. Patulin is relatively temperature stable, particularly at acid pH. High temperature (150 °C) short-term treatments have been reported to result in approximately 20% reduction in patulin concentrations. However, thermal processing alone is not sufficient to ensure a product free of patulin.
3. There is no clear evidence that patulin is carcinogenic, however, it has been shown to cause immunotoxic effects and is neurotoxic in animals. The IARC concluded that no evaluation could be made of the carcinogenicity of patulin to humans and that there was inadequate evidence in experimental animals. Patulin was evaluated by the JECFA in 1990 and re-evaluated in 1995. The latter evaluation took into account the fact that most of the patulin ingested by rats is eliminated within 48 hours and 98% within 7 days. A study on the combined effects of patulin on reproduction, long-term toxicity and carcinogenicity pointed to a harmless intake of 43 µg/kg body weight per day. On the basis of this work and using a safety factor of 100, the JECFA set a provisional maximum tolerable daily intake of 0.4 µg/kg body weight.
4. Patulin occurs mainly in mould-damaged fruits although the presence of mould does not necessarily mean that patulin will be present in a fruit but indicates that it may be present. In some instances, internal growth of moulds may result from insect or other invasions of otherwise healthy tissue, resulting in occurrence of

patulin in fruit which externally appears undamaged. However, it can also occur in bruised fruit after controlled atmosphere storage and exposure to ambient conditions both with and without core rot being present. Washing of fruit, or removal of mouldy tissue, immediately prior to pressing will not necessarily remove all the patulin present in the fruit since some may have diffused into apparently healthy tissue. Washing apples with ozone solution is reported to contribute substantially to the control of patulin during processing.

5. Although the spores of many of the moulds capable of producing patulin will be present on fruit whilst it is still on the tree, they will generally not grow on fruit until after harvest. However, mould growth and patulin production can occur in fruit pre-harvest if the fruit becomes affected by disease or damaged by insects or where fallen fruit is gathered for processing. The condition of the fruit at harvest, the way in which the fruit is handled subsequently (especially during storage) and the extent to which storage conditions are inhibitory to the growth of moulds, will all affect the likelihood of patulin contamination of juice and other products prepared from fresh and stored fruit.
6. The recommendations for reducing patulin contamination in apple juice in this document are divided into two parts:
 - I) Recommended practices based on Good Agricultural Practice (GAP).
 - II) Recommended practices based on Good Manufacturing Practices (GMP).

I. RECOMMENDED PRACTICES BASED ON GAP

Preharvest

7. During the dormant season cut off, remove and destroy all diseased wood and mummified fruits.
8. Prune trees in line with good commercial practice producing a tree shape which will allow good air movement through the tree and light penetration into the tree. This will also enable good spray cover to be achieved.
9. Measures should be taken to control pests and diseases which directly cause fruit rots or allow entry sites for patulin-producing moulds. These include canker, eye rot (*Botrytis* spp and *Nectria* spp), codling moth, fruitlet mining tortrix moth, winter moth, fruit tree tortrix, blastobasis, sawfly and dock sawfly.
10. Wet weather around the time of petal fall and of harvesting is likely to increase the risk of rot and appropriate measures, such as application of fungicide to prevent spore germination and fungal growth should be considered.

11. Apples of poor mineral composition are more likely to suffer physiological disorders in store and hence are more susceptible to particular types of rot especially by *Gloeosporium* spp and secondary rots such as *Penicillium*. Consignments of apples for the fresh fruit market which do not meet the recommended mineral compositional standards, as determined by fruit analysis, should therefore be excluded from long-term storage i.e. storage for longer than 3–4 months.
12. Where levels of minerals in the fruit for the fresh fruit market are outside optimum ranges, improving calcium and phosphorus levels in the fruit, particularly increasing the calcium/potassium ratio by controlled fertiliser usage, will improve cell structure, which will then reduce susceptibility to rotting.
13. Records of rot levels should be kept each year for individual orchards since historical data is the best guide, at present, to potential rot levels, which will indicate the need for fungicide application and the storage potential of the fruit from that orchard.

Harvesting and transportation of fruit

14. Apples for processing are from two different origins:

a) Mechanically harvested fruit

15. Mechanically harvested fruit is obtained by shaking the tree and collecting the fruit from the ground with appropriate mechanical machinery.
16. All fruit should be handled as gently as possible and every effort made to minimize physical damage at all stages of the harvesting and transportation procedures.
17. Before shaking the trees, deteriorated fallen fruit (rotten, fleshed etc.) should be removed from the ground in order to make sure that only fresh and/or sound fruit is collected.
18. Mechanically harvested fruit has to be transported to processing plants within 3 days after harvest.
19. All containers used to transport harvested fruit should be clean, dry and free of any debris.

b) Fruit for the fresh fruit market

20. Fruit from orchards with a history of high levels of rot should be harvested separately and not considered for storage.

21. Ideally all fruit should be picked in dry weather conditions, when the fruit is mature, and placed in clean bins or other containers (e.g. boxes) suitable for transportation directly to store. Bins or boxes should be cleaned, ideally by hosing with clean water or preferably by scrubbing with soap and water, and fruit and leaf debris should be removed. Cleaned bins and boxes should be dried prior to use. Avoid exposure of fruit to rain.
22. Adequate training and supervision should be provided to ensure good damage-free picking practice.
23. All fruit in which the skin is damaged, or with the flesh exposed, as well as all diseased fruit, should be rejected in the orchard at the time of picking and fruit bruising should be minimised as far as possible.
24. All soil-contaminated fruit, i.e. rain splashed fruit or fruit on the ground, should be rejected for storage purposes.
25. Care must be taken to avoid the inclusion of leaves, twigs etc. in the picked fruit.
26. Fruit should be placed in cold storage within 18 hours of harvest and cooled to the recommended temperatures (see Table 1) within 3–4 days of picking.
27. During transport and storage, measures should be taken to avoid soil contamination.
28. Care must be taken during handling and transport of the bins or boxes in the orchard, and between the orchard and store, to avoid soil contamination of the container and the fruit and to minimize physical damage e.g. bruising of the fruit.
29. Harvested fruit should not be left in the orchard overnight but moved to a hard standing area, preferably under cover.

Post-harvest handling and storage practices of fruit for the fresh fruit market

30. All fruit, whether for the fresh market or for later processing, should be handled as gently as possible and every effort made to minimise physical damage e.g. bruising at all stages of post-harvest handling prior to pressing.
31. Apple growers, and other producers of juice who do not have controlled storage facilities, need to ensure that fruits for juicing are pressed as soon as possible after picking.

32. For controlled atmosphere storage ensure that stores are checked for gas tightness, where appropriate, and that all monitoring equipment is tested before harvesting commences. Pre-cool stores thoroughly before use.
33. Where appropriate post harvest fungicide treatments may be applied in accordance with authorized conditions of use.
34. Stored apples should be examined regularly, at least once a month, for rot levels; a record of the levels should be maintained from year to year. The sampling procedure used should minimize the risk of atmospheric changes occurring in the store (see para. 37).
35. Random samples of fruit should be placed in suitable containers (e.g. net bags) situated close to the inspection hatches to permit monitoring of fruit condition during the storage period (see para. 36). Samples should be examined for rots, general fruit condition and shelf life at least every month. Shorter intervals may be recommended in stores where the fruit storage conditions are less than optimum and/or the fruit has a predicted storage life of less than 3 months, because of adverse growth and/or harvesting conditions.
36. Where samples indicate problems with fruit condition appropriate action should be taken to remove the fruit for use before extensive damage occurs.
37. Mould growth normally occurs in a warm environment. Rapid cooling and maintenance of store atmosphere conditions will improve fruit condition. Ideally fruit should be loaded and cooled to less than 5 °C in 3–4 days and to optimum temperatures within a further 2 days. Controlled atmosphere conditions should be achieved within 7–10 days from the start of loading, and ultra-low oxygen regimes (i.e. less than 1.8% oxygen) should be established within a further 7 days.

Post-storage grading of fruit for the fresh market or juice manufacture

38. All rotten fruits, even those with only small areas of rot, should be eliminated as far as possible and wholesome fruit should be kept in a clean bulk container.
39. When containers are removed from storage to select fruit for retail distribution, the containers of fruit remaining for juicing should be specifically marked and returned to cold store within 12 hours of sorting. The time the fruit is at ambient temperatures should be kept to a minimum. Ideally fruit for juicing should be kept at < 5 °C between withdrawal from store and juicing and should be utilized as soon as possible.

40. Fruit which is to be sent for juicing should be utilized as soon as possible and within the normal shelf life which would be recommended for fruit from the same store. Any bruising will encourage patulin formation hence bruising should be kept to a minimum, especially if fruit is to be stored for longer than 24 hours at ambient temperature before juicing.

II. RECOMMENDED PRACTICES BASED ON GMP

Transportation, checking, and pressing of fruit

Mechanically harvested fruit and fruit for the fresh market

a) fruit for the fresh market

41. Stored fruit should be transported from the cold store to the processor in the shortest time possible (ideally <24 hours to pressing unless cold stored).
42. Varieties with an open calyx are particularly susceptible to core rots. These varieties should be examined for internal rots by regular checks immediately prior to pressing. An appropriate random sample of apples should be preferably taken from each separate batch of fruit. Each apple is then cut across its equator and examined for signs of mycelial growth. If the frequency of core rots exceeds an agreed level the consignment should not be used for juicing. The processor should specify the maximum proportion of supplied fruit which can have any sign of rotting, taking into account the capacity of the processor to remove the rotting fruit during pre-process inspection. If this proportion is exceeded the whole consignment of fruit should be rejected.
43. On arrival at the factory the fruit should be checked for quality, particularly for evidence of both external and internal mould damage (see para. 44).

b) mechanically harvested fruit and fruit for the fresh market

44. During processing and prior to pressing, the fruit should be sorted carefully to remove any visually mouldy fruit (check randomly and routinely for internal mould by cutting some fruit as indicated in para. 42) and washed thoroughly, using potable or suitably treated water.
45. Juice presses and other manufacturing equipment should be cleaned and sanitised in accordance with industry "best practices". Juice presses and other equipment will generally be washed down with pressured water hoses and sanitised by application of a suitable sanitiser, followed by a further rinse with potable cold water. In some

plants, which operate almost continuously, this should preferably be a once per shift or once per day cleaning operation.

46. After pressing samples of juice should be taken for analysis. A representative bulk production sample should be analysed for patulin by an appropriate method in a laboratory which is accredited to carry out such analyses.
47. The juice should preferably be chilled to $<5^{\circ}\text{C}$ and maintained chilled until it is concentrated, packaged or pasteurised.
48. Juice should only be sent for packing on a positive release basis after patulin analysis has been confirmed as being below the maximum agreed limit. Specifications for the purchase of apple juice should include an appropriate limit for patulin subject to confirmation by the recipient.

Packaging and final processing of juice

49. Moulds which are capable of producing patulin may occur, together with other moulds and yeasts, particularly in NFC juice. It is essential to prevent the development of such organisms during transport and storage to prevent spoilage of the product and by the same means prevent the production of patulin.
50. If juice is to be held for a period prior to use the temperature should preferably be reduced to 5°C or less, in order to reduce microbial development.
51. Most juice will be heat processed to ensure destruction of enzymes and spoilage organisms. It must be recognized that whilst such processes will generally destroy fungal spores and vegetative mycelium the process conditions will not destroy any patulin which is already present.

Quality assessment of juice

52. Specifications for the purchase of apple juice or apple juice concentrates should include a maximum limit for patulin based on an appropriate method of analysis.
53. A sampling plan should be developed for random sampling of product to assure that the finished product is within the maximum limit for patulin.
54. The packer must satisfy himself that the juice supplier is able to control properly his own operations to ensure that the recommendations given above are carried out.

55. Assessment of the quality of apple juice by the packer will include °Brix, acidity, flavour, colour, turbidity, etc. The microbiological quality should be carefully monitored since this indicates not only the risk level of potential organisms for the production of patulin but also the hygienic aspects of the previous stages in the production cycle.
56. Further checks should be carried out on the packaged product to ensure that no deterioration has taken place during the packaging stage.

Table 1: Recommended temperatures for storage of apples in air

| Variety | Temperature | | Variety | Temperature | |
|---------------------|-------------|-------|---------------|-------------|-------|
| | °C | °F | | °C | °F |
| Bramley | 3.0-4.0 | 37-39 | Idared | 3.0-4.0 | 38-39 |
| Cox's orange pippin | 3.0-3.5 | 37-38 | Jonagold | 0.0-0.5 | 32-33 |
| Discovery | 1.5-2.0 | 35-36 | Red delicious | 0.0-1.0 | 32-34 |
| Egremont | 3.0-3.5 | 37-38 | Spartan | 0.0-0.5 | 32-33 |
| Golden delicious | 1.5-2.0 | 35-36 | Worcester | 0.0-1.0 | 32-34 |
| Crispin | 1.5-2.0 | 35-36 | | | |