Fungus and enzyme activity in fresh apricots as related to softening of canned fruits

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CANNED APRICOTS in Australia are believed to be softened by heat-tolerant pectolytic enzymes from the black bread mold, *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind. Australian workers have duplicated softening problems by adding a *Rhizopus*-rotted fruit or juice from a rot lesion to cans. Fungi were killed by heat-processing at 100°C (212°F) for 10 minutes but a portion of their pectolytic enzyme activity remained. The Australian work has now been duplicated independently in two California studies.

This study was done to determine whether fungi are contained in fruits at canning, and, if so, what fungi? A further objective, not yet completed, was to determine whether the isolated fungi produce pectolytic enzymes and, if so, the heat tolerance of fungal pectolytic enzymes.

Apricot samples were collected from four canneries after final sorting but before the fruits were placed in cans. Fruits were preferentially selected that had pronounced growth cracks at the stylar end, sunburn injury at the surface, or heat-injured flesh, since unmistakable fungus lesions could not normally be identified. Fruit samples were carried to Davis in an icebox and held overnight in a refrigerator. Each fruit sample was soaked for 1 to 3 minutes in a 1:10 dilution of commercial laundry bleach to kill surface contaminants. A small piece of tissue from each sample was removed with a sterile scalpel from within the flesh, briefly dipped in hypochlorite solution, and placed without rinsing on a nutrient agar medium in a Petri dish. Many of the suspect fruit did contain fungi, and after several days of incubation, all colonies of filamentous fungi were identified.

Initial studies centered on *Rhizopus* sp. and related fungi because they produce high enzyme activity and because *R. stolonifer* had previously been implicated in the Australian apricot-softening problem. Three isolates of *R. stolonifer* were studied, including an Australian isolate, two of *R. arrhizus*, and one of *Gilbertella persicaria*. The last fungus, though not found in samples from the canneries, was included because it is found in fresh apricots and could be present in fruits placed in cans. Spores of the above fungi were used to inoculate apricot fruits. When almost completely rotted, the fruits were pressed to extract juice, which was sterilized by passage twice through a Seitz filter.

Heat treatments were performed by aseptically transferring 3 ml of juice to a small test tube which was placed in vigorously boiling water. Heating times were 5, 10, 15, 20, 25, 30, 40, and 50 minutes. Thermocouple measurements showed that the juice reached 100°C (212°F) in approximately 1.5 min. Heating was quickly terminated by removing the tube from the boiling water and swirling it in ice water. Heated samples were compared with an unheated sample of juice as to their ability to attack pectin. Controls were a water blank and a juice sample autoclaved at 125°C (257°F) for one hour.

Pectolytic enzyme activity is commonly determined by changes in the viscosity of citrus pectin as measured by the time required for a standard volume of pectin solution to flow through the capillary of a no. 300 Ostwald-Fenske pipette. A 1.5% solution of citrus pectin is viscous and passes slowly through the pipette. As the enzyme reduces the size of pectin molecules, the solution becomes strikingly more fluid and the flow is greatly speeded.

In tests reported here, one ml of juice containing enzyme (or control liquid) was added to 4 ml of 1.5% citrus pectin in a test tube and was incubated at room temperature for 22 hr. Flow rates were measured at 30.5°C (86.9°F).

To ensure that viscosity changes were due to heat-tolerant enzymes and not the consequence of bacterial contamination during the long incubation, the following precautions were taken: 1) the juice sample containing the fungal enzymes was sterilized by passage twice through a Seitz filter; 2) glassware was heat-sterilized and cotton-plugged; 3) the procedures were under aseptic conditions; 4) the pectin substrate was sterilized by exposing the dry citrus pectin to vapors of propylene oxide for 2 days or more and then dissolving it in filter-sterilized citrate buffer; and 5) after tests, aliquots of reaction mixtures were added to nutrient agar in Petri dishes to verify that no bacterial contamination had occurred.

The table shows that juice from apricots rotted by *R. stolonifer*, *R. arrhizus*, and *G. persicaria* contained heat-tolerant pectolytic enzymes. Although there appeared to be minor differences in heat tolerance between various isolates, it is evident that pectolytic enzymes of the Australian isolate of *R. stolonifer* are not more heat-tolerant than those of the California fungi. In prolonged heating of two species, the enzyme activity remained detectable even after 30 or 40 minutes.

These preliminary findings suggest that the presence in fresh apricots of fungal enzymes could result in softening of the canned product. The full extent of the fungus enzyme problem will be known only after the heat tolerance of all fungal species found in apricot fruits has been determined. The fungal enzymes studied thus far seem too heat-tolerant to be inactivated by treatments that the fruit could endure. Therefore, efforts to avoid fungal contamination may be required. If only *Rhizopus* sp. and related fungi produce heat-tolerant pectolytic enzymes, control could be achieved by rapid cooling of the fruit after harvest and refrigerating until processed. If other fungal species are involved, such as *Alternaria tenuis* and *Cladosporium herbarum*, improved control in the orchard may be required.

PECTOLYTIC ENZYME ACTIVITY RETAINED IN JUICE OF FUNGUS-ROTTED APRICOTS AFTER HEATING AS MEASURED BY VISCOSITY CHANGES IN PECTIN

Fungus species	Isolate	Heating @ 100°C	Initial flow	Final flow	Flow-rate change*
	No.	Min.	Min.	Min.	%
Rhizopus stolonifer	55	10	1.77	0.71	60
Rhizopus stolonifer	134**	10	1.70	0.44	74
Rhizopus stolonifer	134**	15	1.70	0.93	45
Rhizopus stolonifer	90	15	1.70	0.59	65
Rhizopus arrhizus	73	10	1.74	0.51	71
Rhizopus arrhizus	91	15	1.70	0.18	89
Gilbertella persicaria	102	10	1.49	0.17	88

* The flow-rate change is a measure of the decreased viscosity of the pectin substrate caused by the action of pectolytic enzymes in juice from apricots rotted by fungal isolates. The greater the flow-rate change, the greater the enzyme activity.

** Australian isolate.

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