

APRICOT SOFTENING: A PROBLEM OF

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CANNING IS THE PRINCIPAL outlet for California apricots which account for nearly all of the USA pack. Softening of fruit in the can has been recognized as a problem for over 10 years. Annual evaluations by the Cannery League of California of the foreign pack available on the European market indicate that softening is a problem for all major apricot producing countries.

Apricot softening is characterized by loss of tissue integrity. It may occur during the cooking process, or it may not be detectable until some weeks after canning and then continue in the can. It is not directly associated with advanced maturity or excessive cooking, although these two factors can cause the fruit to be soft, and are confusing in borderline cases.

Softening is believed to result from shortening of long chain pectin molecules in the cell wall by hydrolysis. When this occurs the cementing action of pectin in holding cells together is greatly reduced so that the integrity of tissues is lost. There appear to be at least two kinds of softening. In one case softening affects only part of the halves in a can and these do not disintegrate completely when picked up or the container is vigorously

shaken. In the second type, degradation occurs in all the fruit in a can.

Based on the sample cutting of the 1973 California pack, 14% of the cans (2½ size) had two or more soft halves. This compares with a multi-year average of about 20% and a 1972 pack average of 28%. No doubt evaluation procedures and variability between evaluators has been responsible for some of the variation in data obtained from year to year, but such variability does not detract from the total problem. Most of the softening recorded each year involves only part of the units in a can. Foreign pack samples have always had a higher percentage of cans with soft fruit of both types than has the California pack. In the January, 1974 cutout, 29% of the foreign pack cans had softening.

When research on apricot softening was begun by the University of California about 10 years ago, the type of softening affecting only some of the fruit in a can was found to be correlated to a highly significant degree with fruit acidity. Acidity was related to variety, temperature during the ripening period, and the level of nitrogen nutrition of the orchard. It is thought that softening of high acid fruit occurred primarily during the cooking and cooling process as a result of acid hydrolysis of cell wall constituents.

Variability in acidity of different halves within a can could account for some halves softening and others not. Cannery men have attempted to eliminate from their packs certain lots of fruit, based upon acidity information. Their efforts have been only partially successful. Probably eventual elimination of this problem will depend upon the development of new nonsusceptible varieties.

Each year 1 to 3% of the California pack has been affected with the complete breakdown type of softening. This can only be accounted for by assuming the presence of an active enzyme system in the canned fruit. For example, duplicate cans from the same fresh fruit lot may show no softening in one and complete degradation in the other. There is no correlation between this type of softening and acidity. Recent reports from the Union of South Africa and especially from Australia have related fungi to the enzymatic softening problem. *Rhizopus stolonifer* was reported as the likely causal agent. Three University of California scientists conducted independent studies on the possible role of fungi in enzymatic apricot softening. Each researcher studied a different phase of the problem and results are reported in the accompanying articles.

Role of PECTIC ENZYMES on softening in canned apricots

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FUNGAL CONTAMINATION with *Byssoschlamys fulva* has been reported to be responsible for softening of canned apricots in South Africa. In Australia, apricot softening was attributed to the slow action of a heat-resistant pectic enzyme produced by the mold *Rhizopus stolonifer*. The softening was thought to be due to mold contamination in the orchard or following mechanical damage during post-harvest handling.

This study reports the presence of pectic esterase (PE) and polygalacturonase (PG) in apricot fruit at three ripeness levels. The effect of mold PG from *Rhizopus stolonifer*, *R. arrhizus*, and *R. Oryzae* on the texture of canned apricots was also studied.

During the 1973 apricot season, experiments were carried out to study the level of pectin esterase and polygalacturonase in fresh apricots at different ripeness levels. The fresh fruits were harvested on the same day and sorted into under-ripe, canning-ripe and soft-ripe categories. The fruits were washed, halved,

pitted and deep frozen at -26°C in polyethylene bags.

Extraction of enzymes

One hundred grams of frozen apricot tissue was thawed at 1°C, and then blended with 100 ml of water containing 12% polyethylene glycol-6000 and 0.2% sodium bisulfite for 2 min. at 1°C. The mixture was centrifuged for 20 minutes and the supernatant was discarded. The pellet was suspended in 200 ml of cold water and mixed in a Waring blender for one minute. The product was centrifuged again for 20 min., and the super-

THE CANNED FRUIT

natant discarded. The water washing and centrifuging process was repeated once more. The pellet was suspended in 200 ml of cold water and used as a source for PE and PG.

PE assay

PE activity was assayed by measuring the rate of liberation of carboxyl groups of pectin N.F. A 120-ml beaker containing 25 ml of 1% pectin N.F. solution and 10 ml of 1 M NaCl was placed in a water bath at 30°C. The pH of the solution was brought to 8.0 by adding NaOH solution under continuous agitation. Fifteen ml of enzyme suspension was added to the well mixed solution, and the pH was held at 8.0 by adding 0.05 N NaOH. The alkali consumption was measured as a function of time. A blank sample was run in which the added enzyme had been previously inactivated by heating in a boiling water bath for 10 min.

One unit of PE activity (PE_u) gm is defined as one micro mole of $-COOH$ group released per min. per gram of fresh apricot tissue.

PG assay

PG activity was detected by determining the reducing group released from the digestion of polygalacturonic acid by the enzyme. One ml of 1% polygalacturonic acid at pH 4.0 (adjusted by adding NaOH) was put into a 15-ml Pyrex centrifuge tube which contained 0.4 ml of 0.5 M NaCl and 0.1 ml of a mixture containing 0.1% each Chloramphenicol (Sigma Chem Co.) and Actidione (Cycloheximide, Cal-Biochem, Inc.), then 1 ml of enzyme extract was added with thorough mixing. The mixture was incubated for eight hours in a 30°C room with agitation, after which the enzyme reaction was stopped by putting the tubes into boiling water for 3 min. The tubes were then cooled to room temperature. The content of the tube was centrifuged and one ml of supernatant was pipetted into an 18 × 250 mm tube, to which one ml of copper reagents was added for determining the reducing groups. The method used arsenomolybdate as a color reagent for the colorimetric determination of reducing sugar. A spectrophotometer was used to measure the optical density at 500 nm. A blank was run in the same way as the sample except that

the enzyme was inactivated by heating in boiling water. The results are expressed in (PG_u) gm units. One PG_u is one micro mole of aldehyde (reducing group) released per min. per gram of fresh tissue.

PE and PG activity

Both PE and PG activities in apricot samples from different locations of California were assayed. Preliminary tests indicate that the PE activity in the fruit decreased slightly with maturation, dropping from 2.16 in under-ripe Tiltons to 1.74 (PE_u) unit in soft ripe fruit. The PG activity in Tilton apricots increased from 2.62×10^{-4} (PG_u) g in under-ripe fruits to 8.20×10^{-4} in canning-ripe fruit. It appears that during the ripening process, PE may be required for the activity of PG. The PG contributes to the breakdown of pectic materials in the ripening process in which galacturonic acid and soluble oligouronides are formed.

Using polyethylene glycol as a protective agent, it was possible to detect both PE and PG in the fresh apricots. Previous efforts to detect PG in apricots was not successful, due to the inhibition of the enzymes by the natural tannin-like substances present in apricots.

This work is being continued to establish the characteristics of the pectic enzymes in apricots. Equally important to detect in fresh apricots is the PE and PG secreted by molds or other microorganisms when the fruits are mechanically damaged during harvest and handling.

PG mold secretion

Experiments studied the ability of several molds to secrete pectic enzymes into pectin-polypectate liquid medium. The liquid medium contains 1.0 gm asparagine, 2.5 gm $MgSO_4 \cdot 2H_2O$, 2.5 gm KH_2PO_4 , 5.0 gm citrus pectin, 5.0 gm sodium polypectate, and double distilled water to make one liter. It was found that during the growth of the molds *Rhizopus stolonifer*, *R. arrhizus*, (#1381) and *R. oryzae* (#1548) in liquid pectin-polypectate media at 22°C, there was a gradual decrease in pH from 4.4 to 3.8 in 96 hours. The viscosity of the culture medium also decreased from 252.2 sec. to 58.4 sec. showing the secretion of PG by the molds into the medium, causing hydrolysis of the pectin. Pectate lyase was not produced by the molds, as evidenced by the lack of an absorption

TABLE 1. EFFECT OF ADDING PG FROM *R. STOLONIFER* TO THE SYRUP ON TEXTURE OF CANNED APRICOTS IN #303 CANS—90 DAYS AFTER CANNING

Sample code	Treatment	Sensory texture rating*	Lee Kramer shear press readings sq in	Syrup viscosity at 30°C sec.	pH
B	Control	9.3	1.02	55.5	3.8
E	2ml PG/can (<i>R. stolonifer</i> undiluted)	1.0	0.23	12.7	3.8
I	Fruit inoculated with <i>R. stolonifer</i> for 2 days at 22°C prior to canning (20% inoculated + 80% uninoculated)	2.5	0.34	28.7	3.6

* The panel scored the samples on a 1-10 scale for texture. Excellent, 9-10; Good, 7-8; Fair, 5-6; Poor, 3-4; Very poor, (broken completely) 1-2.

peak at 235 nm. It is known that a double bond will be formed between C_4 and C_5 of the galacturonic acid molecule when a polygalacturonide chain is broken down by PTE, with an increase in light absorption at 235 nm in the pectin medium.

The PG activities in the culture media were measured by a viscosimetric method in an Ostwald-Fenske viscosimeter. The activities of the PG in the media were 0.495 viscosimetric units in *R. stolonifer*, 0.563 in *R. arrhizus* (#1381), and 0.615 in *R. oryzae* (#1548) respectively. One viscosimetric unit is the amount of PG activity necessary to give a 50% reduction in relative viscosity of a 0.50% pectic acid in one minute at 30°C.

Effect on texture

When culture filtrate containing PG from *R. stolonifer* was added during canning, the texture of the fruit in No. 2½ cans was destroyed by the enzyme (table 1). In this experiment, the concentration of pectic enzyme added was quite high, thus causing a very severe texture breakdown. The texture breakdown was observable three days after canning. The shape of the halves was still intact but showed no strength when the fruit was handled. The same phenomenon was observed occasionally in commercial samples of canned apricots.

In sample I, (table 1), the fruits were canned 2 days after inoculation with *R. stolonifer*. Besides the effect of the enzymes produced by the mold, maturity level at canning was also responsible in part for the softening.

Texture measurements were also made on the canned samples three months after processing. The control samples had a

good texture even after storage at 37°C for three months, demonstrating the importance of inhibiting PG for better texture retention.

Effect of heat

The PG activities in the liquid media of the mold cultures were quite high. To study the differences between them, the culture filtrate was diluted five times with water. Two ml of the diluted enzyme were added to the 36° Brix syrup of each can during canning.

The cans were processed at 100°C (212°F) and 110°C (230°F) respectively for 10 minutes, with PG from *R. stolonifer* added to the cans. Thirty days after canning the fruit showed some soft-

ening in samples receiving the enzyme treatment (table 2). After storage for 90 days at 68°F (20°C), the texture of the apricot became even softer.

It appears that raising the sterilization temperature from 100°C (212°F) to 110°C (230°F) affected texture more due to heat effect, but much less to the residual enzyme activity. Statistical analyses of the results indicate that the difference in texture between the control and the PG-treated sample was significant at the 95% level by the sensory texture rating as well as by the shear press readings.

Heat resistance of pectic enzymes was checked. Even at a temperature reaching 215°F (101.7°C) for five minutes, re-

sidual PG activity of enzyme from *R. stolonifer* was detected. The PG enzymes produced by *R. arrhizus* and *R. oryzae* were less heat-resistant than that of *R. stolonifer*. The concentration of enzyme other than PG may also be different between the organisms.

Discussion

An important factor in the enzymatic softening problem seems to be intrinsic and/or parasitic-originated pectic enzymes. To solve the problem, the food processing industry should work toward control of molds in apricot orchards, and for methods of handling and processing that result in minimum mechanical damage. Prompt handling and canning of the fruit after harvest may also help.

Much remains to be investigated concerning pectin biosynthesis, activation of pectic enzyme systems, the effect of pectic and cellulase enzymes from microorganisms on fruit texture.

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TABLE 2. EFFECT OF PROCESSING TEMPERATURE ON TEXTURE OF CANNED APRICOTS

Sample code	Treatment	Heat processing temperature (10 min.)		Sensory texture rating*		Lee-Kramer Shear Press readings sq. in.		Syrup viscosity at 30°C sec.	pH
		°C	°F	30 days	90 days	30 days	90 days	90 days	
B-1	Control	100	212	8.5	6.9	2.07	1.45	48.8	3.70
E-1-1	2 ml diluted PG/can (from <i>R. stolonifer</i>)	100	212	7.5	5.5	1.04	0.44	43.8	3.72
B-2	Control	110	230	6.5	5.3	0.83	0.66	82.0	3.90
E-1-2	2 ml diluted PG/can (<i>R. stolonifer</i>)	110	230	5.0	3.8	0.50	0.58	44.2	3.90

* The panel scored the samples on a 1-10 scale for texture. Excellent, 9-10; Good, 7-8; Fair, 5-6; Poor, 3-4; Very poor (broken completely), 1-2.

Implication and chemical testing of two rhizopus fungi in softening of canned apricots

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EVIDENCE FROM THESE tests showed that a single fruit decayed by *Rhizopus arrhizus*, and placed into a no. 10 can of healthy fruit before canning resulted in total disintegration of healthy fruit during six months' storage at room temperature. Addition of a single *Rhizopus stolonifer* decayed fruit also resulted in significant softening within a six-month period in fruit from one out of three orchards. There was little change in rating of fruit after nine months' storage, but in one treatment 48% of the good Tilton fruit showed initiation of

softening, with flesh starting to disintegrate, and soft to the touch.

The addition of Botran (2,6-dichloro-4-nitroaniline) when the fruit was canned did not reduce softening although in one test there was a significant increase in good fruit. There was no correlation between pH and *Rhizopus*-associated softening. Similar results from addition of *R. stolonifer* to canned apricots have been reported from Australia. *R. arrhizus* is also present in Australia but tests with this fungus were not reported. The 1973 California studies showed that pre- and postharvest Botran applications did not control *R. arrhizus* on apricots. Previous studies also have shown that *R. arrhizus* is present in orchards throughout California and that Botran failed to control it.

Chemicals

Chemicals tested were 75% Botran (2,6-dichloro-4-nitroaniline) and 70% Topsin M (1,2-bis(3-methoxy-carbonyl-2-thioureido)-benzene-thiophanate methyl). Botran was applied at the rate of 1½ lb per 100 gallons of water as a field spray and as a postharvest dip. Definite amounts of Botran in acetone were added to empty cans and acetone-evaporated before filling with fruit. Only 4.08 mg of proprietary 75% Botran was added to each can, giving one ppm of active ingredient on a weight-for-weight basis. Topsin M was applied only as a field spray at the rate of 0.5 lb per 100 gallons of water. Field sprays were applied with a hand gun sprayer on June 13 and July 2, 1973, at the rate of about 6 gal per tree.