

- (22) Potgieter, M., *J. Am. Dietetic Assoc.*, **16**, 898-904 (1940).
- (23) Rather, J. B., *J. Am. Chem. Soc.*, **39**, 2506-15 (1917).
- (24) Reddi, P. B. V., Murti, K. S., and Feuge, R. O., *J. Am. Oil Chemists' Soc.*, **25**, 206-11 (1948).
- (25) Sherman, H. C., "Chemistry of Food and Nutrition," 6th ed., New York, Macmillan Co., 1941.
- (26) Simpson, I. A., Chow, A. Y., and Soh, C. C., *Bull. Inst. Med. Research, Federation Malaya*, New Series No. 5, 1-28 (1951).
- (27) Spitzer, R. R., and Phillips, P. H., *J. Nutrition*, **30**, 183-92 (1945).
- (28) Sreenivasan, A., *Nature*, **143**, 244-5 (1939).
- (29) Subrahmanyam, V., Sreenivasan, A., and Das Gupta, H. P., *Indian J. Agr. Sci.*, **8**, 459-86 (1938).
- (30) Tani, T., et al., *Rept. Food Research Inst. Tokyo*, No.3, 11-25 (1950).
- (31) Thompson, A. R., *J. Agr. Research*, **3**, 425-30 (1915).
- (32) U. S. Agr. Marketing Service and Regulatory Announcement No. 147, rev. 1941.
- (33) West, A. P., and Cruz, A. O., *Philippine J. Sci.*, **52**, 1-76 (1933).
- (34) Wolff, E. T., "Aschenanalysen Von Landwirtschaftlichen Produkten," Bd. 1, p. 29; Bd. 2, p. 22. Berlin, Wiegandt and Hempel, 1871.
- (35) Yampolsky, C., *Wallerstein Lab. Commun.*, **7**, 7-26 (1944).

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## PECTIN PRODUCTION

# Pilot Plant Production of Low-Methoxyl Pectin from Citrus Peel

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Low-methoxyl pectins are versatile materials which make possible the preparation of many new food products and the preparation of old food products in new or easier ways. Use of various modified pectins is restricted, however, by limited availability. Suitable grades and types of low-methoxyl pectin can be prepared in good yield from citrus peel. Care is required throughout the manufacturing operation to avoid excessive loss of pectin quality. Procedures have been developed on a pilot plant scale. Three stages are involved: preparation of peel for extraction, extraction of pectin and clarification of the extract, and chemical modification and isolation of low-methoxyl pectin. All operations are conducted in an aqueous phase, in contrast to present production methods. An integrated, practical process for making low-methoxyl pectin from citrus peel has been developed. Operating conditions, procedures, and equipment are discussed in a manner designed to assist prospective users of the process.

LOW-METHOXYL PECTIN is a comparatively recent development, although pectin has been manufactured in the United States for more than 35 years (15). The many methods reported in the literature for the preparation of low-methoxyl pectins have been based on laboratory scale experiments. Little if any information has been published on production methods used by the several manufacturers who have made low-methoxyl pectin on a commercial scale.

Pectin is largely the partial methyl ester of anhydrogalacturonic acid and, as extracted from common source materials, contains ordinarily from 9 to 12% by weight of methoxyl groups. When a substantial portion of the methyl ester groups is removed by hydrolysis, the modified pectin attains the ability

to form uniform strong gels in the presence of bivalent cations over a wide pH range. This property makes low-methoxyl pectin useful for numerous applications in which ordinary pectin cannot be used. Applications suggested for low-methoxyl pectin include jellied fruit cocktail (14), tomato aspic (19), low-solids gels (3, 5, 19, 28), milk puddings (19, 37), candy centers (10), and coatings for various food materials (27). Numerous patented applications are not reviewed here.

This paper summarizes the development of a process for making low-methoxyl pectin from citrus peel. The process is divided into three parts: preparation of the peel, extraction of pectin from the peel and clarification of the extract, and processing of the extract to prepare the dry, low-methoxyl pectin prod-

uct. Processing of the pectin extract involves the controlled de-esterification of the pectin, isolation of the low-methoxyl pectin by acid precipitation, dewatering and neutralizing of the low-methoxyl pectin, and drying and grinding to make the final product.

Pectin is a long-chain molecule which is de-esterified and degraded (reduced in chain length) by heat, acidity, alkalinity, and enzyme action. The quality—i.e., the gelling ability—of a pectin product is controlled largely by the chain length of the molecule—the longer the chain, the higher the quality. Most of the pectin in fresh citrus peel is fairly insoluble in cold water. Heat and acid are used to render the pectin extractable, but these agents also degrade the pectin. The amount of degradation must be limited, so that satisfactory quality is

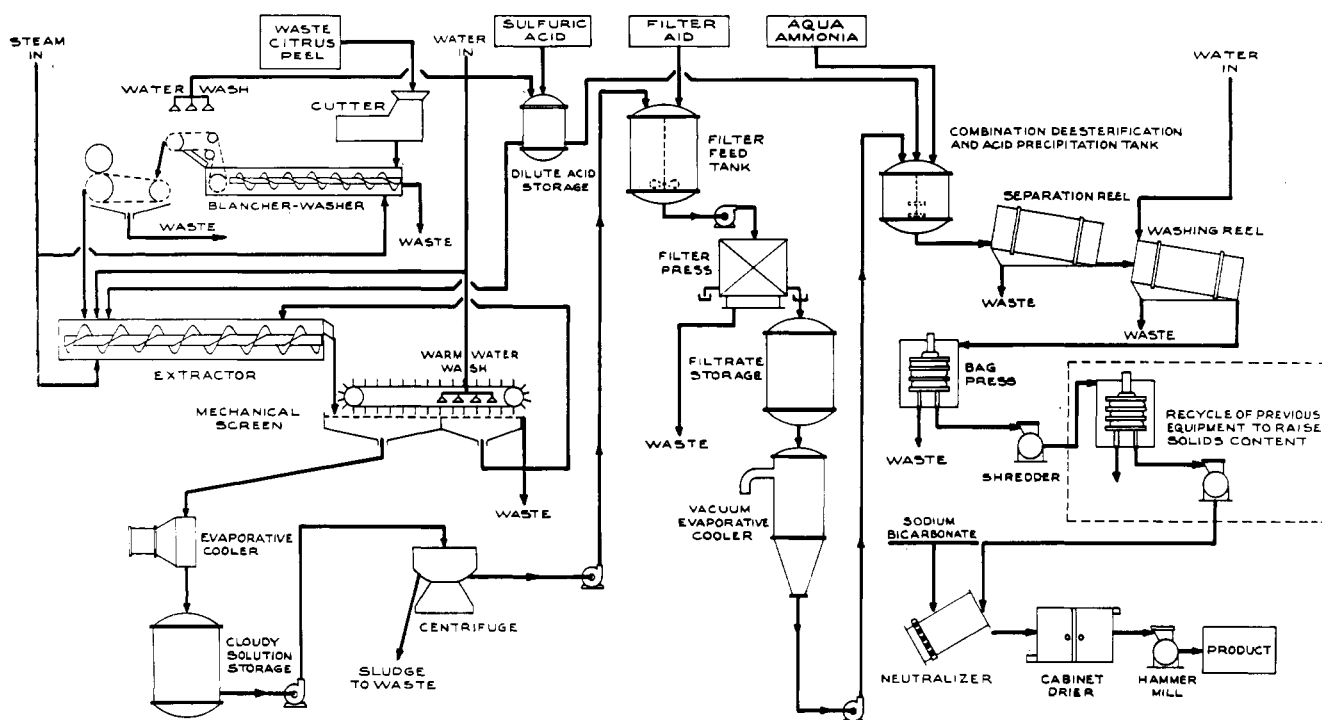


Figure 1. Flow sheet for manufacture of low-methoxyl pectinic acid from waste citrus peel

maintained in the low-methoxyl pectin product. Further degradation occurs during the processing of the extract to low-methoxyl pectin. The process employed, therefore, is a compromise, designed to accomplish the necessary physical and chemical operations in a reasonable manner with a minimum of degradation.

The steps involved in making low-methoxyl pectin have been investigated to varying degrees, at this laboratory and other places. Pectin has been de-esterified experimentally by ammonia (18, 19), acid (2, 4, 23, 33), and enzymes (12, 22, 25). The products obtained differ somewhat in properties (11, 24, 29, 32); the product from the aqueous-phase, ammonia de-esterification of pectin is readily adapted to the applications outlined above. Low-methoxyl pectin may be precipitated from solution by the addition of solvents, polyvalent cations, or acid (20, 21). The first two methods impose solvent handling and recovery problems on a process, and/or need for concentrating the solution before isolation of the low-methoxyl pectin. Acid precipitation offers the advantage of simplicity; excess acid is easily removed by washing the precipitated low-methoxyl pectin with water.

Studies on the de-esterification of an aqueous solution of pectin with ammonia (18, 19) and the isolation of the low-methoxyl pectin with acid (20, 21) were a part of the background work leading to the development of the process described in this paper. However, as various process steps were studied in the laboratory, it became apparent that meaningful results could be obtained only by starting with the extraction of

citrus peel. As finally developed, the process covers the entire production of low-methoxyl pectin from citrus peel, rather than its production from commercial pectin as a starting material.

The flow sheet of the process is shown in Figure 1.

#### Preparation of Citrus Peel For Extraction

Orange, lemon, or grapefruit peel can be used as the starting material in this process. Peel as produced by any of the commercial juicing machines is suitable. The presence or absence of the flavedo is immaterial.

**Cutting** Several types of cutter have been used successfully. The most suitable type for a particular case depends upon whether the peel is dried for storage or extracted in its wet state. For making dried peel, a kraut cutter producing slices from  $\frac{1}{8}$  to  $\frac{3}{16}$  inch thick is suitable. If the peel is to be extracted immediately, a finer cut is preferable. A suitably cut peel was obtained from a high-speed comminutor fitted with a screen perforated with round holes  $\frac{1}{4}$  inch in diameter. Dicing-type cutters were too low in capacity to be useful in preparing either type of cut peel.

**Blanching and Water Washing** Blanching of raw peel immediately after cutting is necessary to minimize the action of naturally occurring enzymes upon the pectin. Washing of the cut peel removes substantial quantities of soluble solids such as sugars and acids, and colloidal materials such as pigments, glycosides, and finely divided material produced during the cutting operation. Removal of these

materials makes the pectin extract easier to filter, less colored, and substantially tasteless. Refining of the extract in this manner is reflected in the quality of the final low-methoxyl-pectin product.

Blanching and washing of  $\frac{1}{8}$ - to  $\frac{3}{16}$ -inch slices of peel are effected in a single continuous unit (9). The unit shown in Figure 2, used in a field pilot plant, comprises a wooden trough, 12 feet long and 12 inches wide, with a semicylindrical bottom. The trough is fitted with an interrupted-flight screw and a perforated metal ramp and drag for removing peel. At the peel feed end of the trough, steam is injected over a 3-foot section to maintain a water temperature of 194° F. Freshly cut peel is introduced into the heated section to accomplish blanching, and is conveyed by the screw through the trough. Cold water, added as a spray above the drag, flows counter to the peel and out an overflow pipe. Valencia orange peel has been washed successfully in this unit at a rate of 400 pounds per hour with a retention time in the trough of 30 minutes. About 3 pounds of water per pound of peel was required to wash the peel adequately. The blanching was sufficient to inactivate the pectin enzymes without seriously reducing the quality of the pectin.

The blancher-washer described above has not been tested on finely cut peel such as that produced with a high-speed comminutor. Minor changes in operating conditions would probably be necessary for finely cut peel, particularly for reduction of blanching time to correspond to the smaller piece size. No serious problems are anticipated in using the continuous blancher-washer for handling finely cut peel.

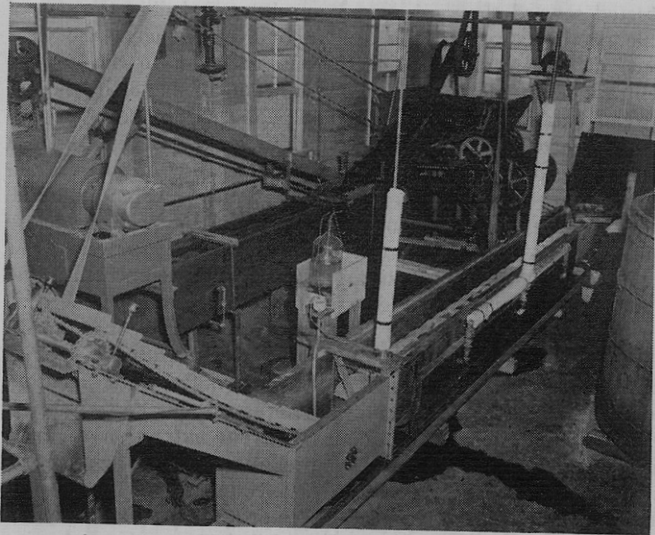


Figure 2. Section of field pilot plant  
 Countercurrent blancher-washer with roller press in rear and countercurrent extractor in foreground

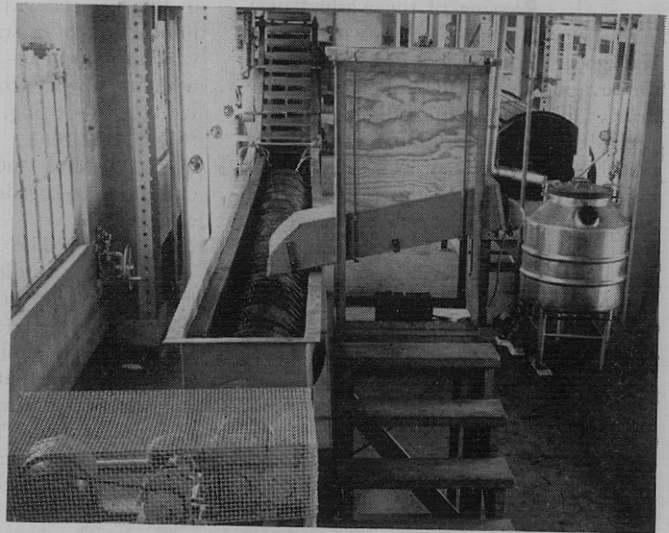


Figure 3. Extractor setup for countercurrent extraction, showing feeder for dried peel

Figure 5. Vacuum evaporative cooler  
 Showing first stage ejector and intercondenser

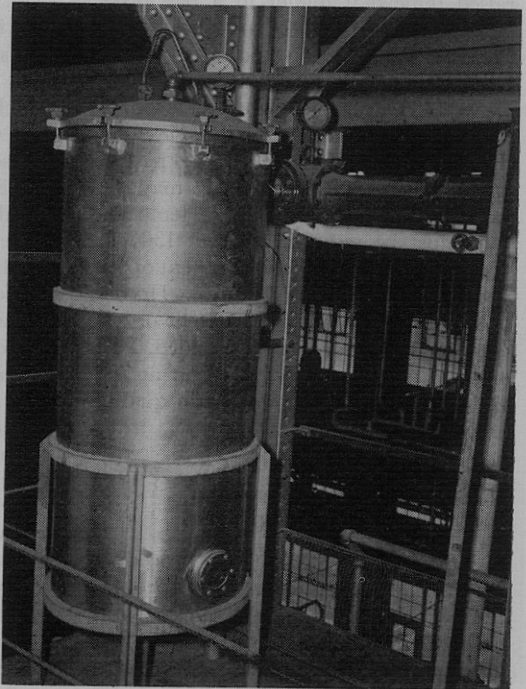


Figure 4. Evaporative cooler, overhead

Cloudy solution storage tank under cooler and filter feed tank

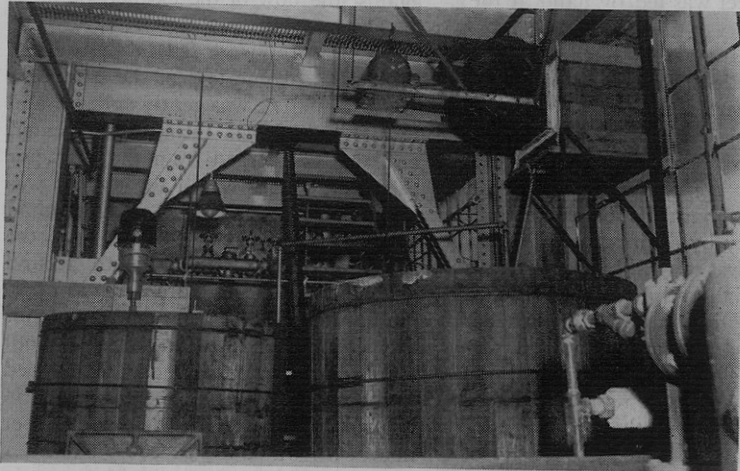


Figure 6. Processing tanks used for de-esterification and acid precipitation

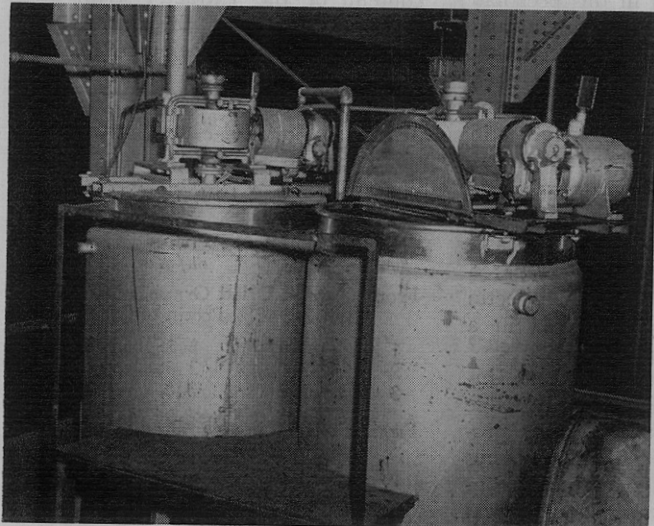
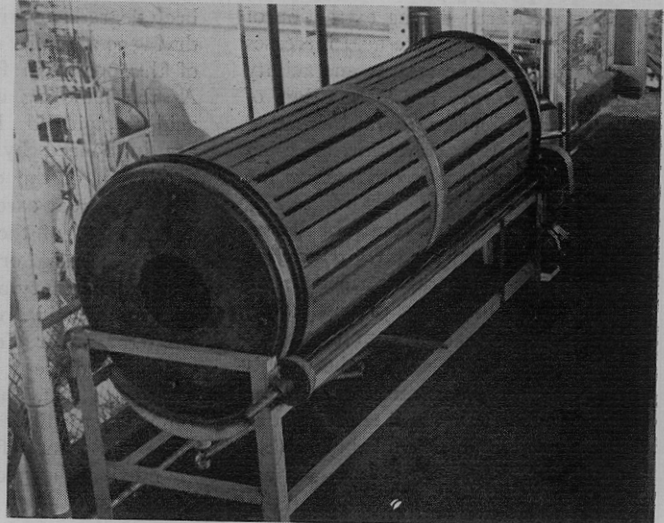


Figure 7. Loose-cloth-lined reel



**Pressing** Pressing of the washed peel is desirable for two reasons. If the peel is to be dried, pressing reduces the evaporative load imposed on the dryer. If extracted immediately, pressed peel absorbs the acidulated water more readily than unpressed peel, thus hastening the extraction of the pectin. The pilot plant has been operated, however, on both pressed and unpressed fresh peel.

A roller press of simple construction, as shown in Figure 2, is adequate for pressing the peel. Two solid rubber belts, both 3 feet in width, are run one above the other at the same linear speed. Washed peel is fed between the belts, and is pressed during passage through the 4-foot length of the press. At a capacity of 400 pounds of washed peel per hour, the press raises the solids content of the peel from about 7% to about 13%. Solids content of the washed peel can be raised somewhat higher in a bag press without serious loss of pectin. However, the gain is not sufficient to justify the additional time and labor involved.

**Drying** Citrus peel for use as a source of pectin must be carefully dried to avoid damage to the pectin. Initial phases of the drying may be carried out at 200° F., but the temperature must be reduced to about 130° F. when the average moisture content of the peel is reduced to about 30%. Drying of the peel to a moisture content of less than 10 to 12% should be avoided. Peel so dried can be stored for a year at 70° F. without serious loss of pectin quality (30).

#### Extraction of Pectin from Peel And Clarification of Extract

Factors related to the best procedure for extracting pectin from citrus peel include quality of pectin in the peel initially, amount of pectin recovered, quality of pectin in the extract, and concentration of pectin in the extract. The extraction of high-quality pectin from citrus peel is more difficult than extraction of low-quality pectin. The amount of pectin recovered during extraction of citrus peel with hot dilute acid increases as the extraction temperature and acidity increase. The rate of degradation of the pectin also increases with increasing temperature and acidity, thus affecting the quality of the pectin obtained in the extract.

The concentration of pectin in the extract is highly important. Handling characteristics of the de-esterified pectin obtained in subsequent processing require that the extract contain 0.6 to 1% pectin, depending upon the quality of the pectin. Production of a more dilute extract is easier and concentration of the dilute extract is technically feasible. However, the procedure is expensive. A large portion of the extraction studies

in this project was directed toward the production of a suitable pectin extract which could be used directly—i.e., without further concentration—in subsequent processing. Proper balance between the many relevant factors required careful and extensive study of extraction procedures in pilot plant equipment.

**Extracting** The unit used in these studies (Figure 3) was constructed for countercurrent extraction of the peel (9). Its general construction was similar to the blancher-washer, except that all portions contacting the peel or extract were fabricated of stainless steel. The screw was 16 feet long and 16 inches in diameter with a 4-inch pitch. The trough was steam-jacketed in three sections, up to shaft-center level. Spray nozzles for the introduction of hot water were provided above the perforated ramp and drag. Sulfuric acid, pumped by a glass metering pump, was introduced into the water between the screw and ramp. At the opposite end, the sol was discharged over a weir which controlled the tank level. Dried peel was fed at a point about 3 feet from the sol discharge end by means of a vibrating feeder. Fresh peel or reconstituted dried peel was fed by hand at the same point.

This apparatus was modified for parallel-flow extraction. The rotation of the screw was reversed and bars were provided between the flights, to increase agitation. A mechanically cleaned screen was placed at the discharge end of the extractor for separation of the extract from the slurry by drainage. The pulp on the screen was washed by water sprays after substantially all of the extract had drained, and the wash water was returned to the extractor near the discharge end. The feed point for peel was adjusted to give desired retention time.

In order to eliminate the variables associated with raw material, a quantity of grapefruit peel which had been dried as a pectin source material was used in many of the pilot plant operations. Unfortunately, the pectin in this peel had been degraded during the processing and drying to a quality much lower than that of the pectin in fresh grapefruit peel. As the handling characteristics of the acid-precipitated low-methoxyl pectin are influenced by the pectin quality, extraction of the dried grapefruit peel required great care to minimize further degradation of the pectin. Fresh and dried Valencia orange peel, prepared at the laboratory and containing pectin of high quality, were also used in a limited number of experiments.

Countercurrent extraction was investigated first, and found to be usable only when dilute extracts were produced. Because of mechanical problems in moving the peel and extract in opposite directions, a working limit was

imposed on the permissible fineness of the peel (and in consequence the yield of pectin obtained), or on the permissible concentration of pectin in the extract. The pectin in the extractor was also exposed to an undesirable amount of degradation because of the characteristics of the countercurrent method. Until extracted from the peel, the pectin traveled upstream in the extractor while being exposed to high temperature and acidity. After extraction from the peel, the pectin retraced its course while traveling downstream with the extract under the same unfavorable environment. Attempts were made to improve the countercurrent extraction of dried peel by preliminary cold acid reconstitution or pickling of the peel. This procedure improved the extraction in general, but failed to permit production of an extract at the desired concentration of pectin. Thus, the countercurrent extraction method was proved to be inherently unsuited to the process requirements, and was abandoned.

In order to obtain pectin solutions of higher concentration, the theoretically more efficient countercurrent extraction method had to be abandoned. To avoid the difficulty of forcing the peel to travel against the flow of the extract, parallel-flow extraction was used. The peel and extractant were fed in the desired proportion at one end of the extractor, and the mixture was gently agitated and heated as it flowed through the treater, and was discharged from the extractor as a slurry from which the extract was continuously separated. This method was much easier to control than the countercurrent method. With ground grapefruit peel, the extractor was operated at 194° F., with a retention time for the peel of 20 minutes, and a pH of about 1.8 to 1.9 in the extract. The dried peel, ground to pass a 40-mesh screen in burrstone mill, was fed at 40 pounds per hour with water fed at 180 gallons per hour to yield an extract with a pectin content of about 0.9%. Variations in the feed rate were used to alter the pectin content of the extract. With fresh Valencia orange peel, the retention time was increased to 30 minutes, blanched

Table I. Parallel-Flow Extraction of Pectin

Run	Extract		Recovery of Pectin, %
	Pectin content, %	( $\eta$ ), dl./g.	
From Ground Dried Grapefruit Peel Containing 4.2% Pectin of ( $\eta$ ) 4.1			
1	0.9	3.8	68
2	1.0	3.6	60
3	1.2	3.8	62
From Fresh Valencia Orange Peel Containing 3.6% Pectin of ( $\eta$ ) 9.0			
4	0.7	6.7	50

and washed peel was fed at 400 pounds per hour, and the water at 100 gallons per hour.

Typical data for several parallel-flow extractions are given in Table I. It is difficult to compare these recoveries with those obtained in commercial practice, because pectin contents of raw materials are not usually determined. The methods for determining pectin content and intrinsic viscosity, ( $\eta$ ), of the pectin are those used previously (30).

Because parallel-flow extraction is really batch extraction on a continuous basis, the influence of variables on the extraction process may be readily studied on a laboratory scale. In Tables II and III, the influence of variables upon the yield and quality of extracted pectin is shown. These are results obtained from small batch extractions, from which the extract was separated and cooled rapidly. Results given may be used directly to predict operation of the parallel-flow extractor.

**Cooling** It is important that the extract be cooled to about room temperature as rapidly as possible after its separation from the extracted peel. In this manner, degradation of the pectin is arrested.

The cooler used was a forced-draft evaporative cooler, shown in Figure 4. It comprised a distributor pan discharging on a series of staggered wooden slats to break up the liquid flow, and a collector pan to receive the cooled extract. Forced draft was provided by a blower. The size of the unit, exclusive of the extract-collecting pan and the blower, was 24 × 30 × 24 inches. This unit was capable of cooling extract from 190° to 90° F. at a rate of about 3 gallons per minute.

**Centrifuging** The extract from the extractor contains solid material which can be readily removed by centrifugation. Removal of this material is desirable to increase capacity and rate during subsequent filtration. The centrifuge used was an overflow type with a 17-inch, stainless-steel, solid basket. The extract was fed directly from the cooler to the centrifuge; 2 to 4% of the weight of extract was removed as sludge by the centrifuging operation. The sludge was discharged from the basket after 150 to 200 gallons of extract had been processed.

**Filtering** The filtering of pectin extracts at ambient rather than elevated temperature represents a minor departure from commercial practice. Extracts filtered at elevated temperatures decrease in clarity as they cool to room temperature. Consequently, extracts filtered at lower temperatures are clearer than those filtered at higher temperatures. The improved clarity is carried through to a solution prepared from the low-methoxyl pectin product.

Filtration was carried out in a conventional plate-and-frame filter press precoated with a medium-slow filter aid. One half per cent of the same filter aid was used in the extract. Filtration rates of 5 to 10 gallons per square foot per hour were obtained with solutions containing 0.8 to 1% pectin by weight.

**Optional Processing Of Pectin Extract** The process as described up to this point is the simplest and most straightforward that has been used with success in the course of the pilot plant work. If, however, particular circumstances should dictate the production of a dilute extract, concentration of the extract prior to further processing would be desirable. The reason for this is that the reagent cost for de-esterifying pectin and acid-precipitating the low-methoxyl pectin is almost directly proportional to the volume of extract handled, regardless of the concentration of pectin in the extract. Dilute pectin extracts have been concentrated successfully in vacuum on a pilot plant scale, provided that adequate con-

trol was maintained over the concentration of bivalent ions in the extract. The procedures used for ion exchanging and vacuum concentrating the dilute extracts, and for de-esterifying the modified extract will be described in another paper.

#### Processing of Pectin Extract to Prepare Dry Low-Methoxyl Pectin

Processing of the pectin extract to make dry, powdered, low-methoxyl pectin involves de-esterification of the pectin with aqueous ammonia at reduced temperature, isolation of the low-methoxyl pectin by acid precipitation, dewatering and neutralizing of the low-methoxyl pectin, and drying and grinding to make the final product.

Preceding the pilot plant studies, laboratory studies were made on the de-esterification of pectin with ammonia (19) and acid precipitation of the low-methoxyl pectin (21). In most of these, solutions prepared from dry commercial pectin were used. Consequently, results of many of the laboratory studies were

Table II. Batch Extraction of Ground Dried Grapefruit Peel

Wt. Water/ Wt. Peel	Pectin Content of Sol at 100% Theoretical Recovery, %	Extraction Conditions			Theor. <sup>a</sup> % Recovery	Extract Pectin ( $\eta$ )
		Temp., °C.	pH	Time, min.		
42	1.0	85	1.7	15	73	4.1
				30	78	3.6
				45	82	3.5
				60	85	3.2
42	1.0	90	1.7	10	74	4.0
				20	83	3.7
				30	86	3.3
				40	90	3.1
42	1.0	90	1.85	10	70	4.1
				20	82	3.8
				30	86	3.5
42	1.0	90	2.15	20	69	3.7
				30	69	3.6
				40	77	3.3
42	1.0	95	1.7	5	72	3.7
				10	82	3.6
				20	88	3.2
33.5	1.25	90	1.85	20	82	3.8
				20	69	4.1
				2.0	20	77
28	1.5	90	1.85	20	77	4.0
				2.0	20	72

<sup>a</sup> Calculated assuming that all water added contains pectin at concentration measured in sol and all is removed from peel. Actual recoveries will be about 80% of these figures.

Table III. Batch Extraction of Ground Fresh Valencia Orange Peel

Wt. Water/ Wt. Peel	Pectin Content of Sol at 100% Theoretical Recovery, %	Extraction Conditions			Theor. <sup>a</sup> % Recovery	Extract Pectin, ( $\eta$ )
		Temp., °C.	pH	Time, min.		
3	1.0	80	1.9	10	40	9.7
				20	55	8.8
				30	64	8.7
3	1.0	90	1.9	10	58	8.4
				15	67	8.1
				20	76	7.7
				30	83	6.8
				40	96	6.4

<sup>a</sup> Calculated assuming that all water added contains pectin at concentration measured in sol and all is removed from peel. Actual recoveries will be about 80% of these figures.

**Table IV. Changes in Methoxyl Content of Pectin with Time During Pilot Plant De-esterification**

Run No.	Elapsed De-esterification Time, Min.	Observed Methoxyl Content, %
1790	120	5.9
	165	5.0
	210	4.2
	240	3.8
1793	120	6.0
	165	5.1
	210	4.2
	240	3.6
1807	60	8.3
	125	6.2
	180	5.1
	240	3.7

not directly applicable to the pilot plant because impurities in the peel extracts, not present in the solutions prepared from commercial pectin, greatly affected the de-esterification and acid-precipitation steps. Calcium, an important impurity in the peel extracts, caused the formation of strong gels during the alkaline de-esterification. Much of the process development in this phase of the work involved methods and equipment for handling large quantities of strong gel, in both the de-esterification and acid-precipitation steps.

The following discussion assumes that the pectin extract has been produced at the proper concentration (0.6 to 1.0% by weight) for subsequent processing. More dilute extracts may be adjusted to the desired concentration by vacuum evaporation.

**Cooling** The sol is cooled to reduce the amount of degradation occurring during de-esterification of the pectin. The temperature to which the sol is cooled represents a compromise between decrease in the amount of degradation and increase in the time required for de-esterification.

Cooling was accomplished by vacuum evaporation. The unit used for cooling was a cylindrical stainless-steel vacuum body (shown in Figure 5). The unit was 30 inches in diameter and 6 feet high, and contained a series of baffles de-

signed so that pectin extract fed at the top flowed in thin films, in and out, in proceeding toward the bottom. The vacuum was provided by a four-stage steam ejector capable of operating at an absolute pressure of 4 mm. of mercury. Extract was cooled from room temperature to 50° F. at a rate of 150 to 200 gallons per hour in this unit. The temperature to which the extracts were cooled varied from 43° to 58° F. in various experimental runs.

**De-esterifying** The de-esterification operation was carried out in a batch manner. Two stainless-steel cylindrical tanks of about 180-gallon capacity were used alternately (Figure 6). The tanks were insulated by an air jacket. A slow-speed, opposing agitator was provided in each tank for mixing in reagents and breaking gels. The tanks were covered to minimize loss of ammonia and were provided with a 3-inch center drain for discharging gel.

To conduct a de-esterification, about 165 gallons of cooled extract were collected in one of the de-esterification tanks. About 5 gallons of concentrated aqueous ammonia were metered into the tank and mixed into the extract, making the ammonia concentration about 0.75% by weight in the extract. During treatment with ammonia, methyl ester groups of the pectin are hydrolyzed to ionized carboxyl groups, and the pectin solution slowly sets to a gel. Gel formation is the result of a reaction between the carboxyl groups of the low-methoxyl pectin and calcium or other polyvalent cations in the extract. The gel must be broken up periodically by agitation during the de-esterification to avoid formation of a solid mass in the tank.

In some instances the methoxyl content of the pectin was followed during the de-esterification to determine the proper time for stopping the reaction. Methoxyl content was determined by saponification of a small sample drawn from the batch. Since saponification involves measurement of alkali consumed, the conventional method for determining methoxyl content had to be modified to avoid loss of ammonia from the sample during the determination.

Otherwise, alkali (ammonia) lost from the sample could not be distinguished from alkali consumed in the saponification. Typical data showing the change in methoxyl content with time as determined by this method are given in Table IV.

With experience gained from observing many samples of various methoxyl contents, the end point of the de-esterification was usually determined from the physical handling characteristics of a small grab sample precipitated with acid.

The results of several de-esterification runs made in the pilot plant are shown in Table V. Commercial dried grapefruit peel was used in all these runs except 1804 and 1807. Valencia orange peel prepared at the laboratory was used in these runs. Run 1804 was made on washed peel that had been pressed but not dried. Run 1807 was made on part of the same peel dried prior to extraction. The intrinsic viscosity of the pectin was considerably higher in the extracts in runs 1804 and 1807, and the intrinsic viscosity of the final low-methoxyl pectins was correspondingly higher.

Table V includes data from extracts of varying pectin contents, run at different temperatures and undoubtedly containing different concentrations of cations because different extraction methods were used. Even with all these variables, the time required for de-esterification in most of the samples was in the range of 3.5 to 4.5 hours. Almost all of the samples could have been stopped in this time range and still have had a methoxyl content within the desired range of 3.0 to 3.5%. In a plant where extraction is made in a uniform manner to yield a sol of reasonably constant pectin content, and where the de-esterification is carried out under fixed conditions of temperature and ammonia concentration, control of the time for de-esterification would be relatively easy.

**Increasing De-esterification Rate (Optional Procedure)** The rate of de-esterification of pectin under alkaline condi-

**Table V. Pilot Plant De-esterifications**

Sample No.	Method of Extraction	Pectin in Extract		Concn. after Evap., %	De-esterification Conditions				Final Product			
		Concn., %	( $\eta$ ), dl./g.		Temp., ° C.	NH <sub>4</sub> OH, gal./100 gal. extract	Init. pH	Time, min.	MeO content, %	( $\eta$ ), dl./g.	pH of 1% sol	Ca content, %
1753	Pickled and countercurrent	0.5	3.4	1.2	10	3.0	10.8	195	3.2	2.7	4.3	0.03
1767	Pickled and countercurrent	0.5	3.8	1.4	10.5	2.85	10.5	200	3.6	2.9	4.5	0.06
1772	Pickled and countercurrent	0.6	3.9	1.2	6	3.0	10.8	255	3.2	2.9	4.5	0.05
1790	Pickled and countercurrent	0.4	3.8	1.3	9	3.0	10.6	240	3.8	3.1	4.2	0.06
1793	Pickled only	1.0	3.6	No evap.	10	3.0	10.7	240	3.5	3.0	4.3	0.04
1794	Pickled only	0.9	3.7	No evap.	9	3.0	10.6	240	3.4	3.0	4.4	0.04
1804	Countercurrent, no pickling	0.5	6.1	1.2	11	3.0	10.4	350	2.7	3.5	4.6	0.05
1807	Pickled and countercurrent	0.2	7.5	0.9	12	3.0	10.5	275	3.2	4.2	4.5	0.07
1827	Parallel flow	0.9	3.6	No evap.	9.5	3.0	10.6	210	3.4	2.8	4.4	0.04
1830	Parallel flow	1.0	3.6	2.4	9.5	4.0	10.5	255	3.4	2.9	4.2	..
1832	Parallel flow	1.0	3.8	No evap.	11.5	3.0	10.6	180	3.8	3.0	4.4	..

**Table VI. Effect of Sodium Sulfate Concentration upon Time Required for De-esterification**

(Details in text)

Sample	Concn. of Na <sub>2</sub> SO <sub>4</sub> , N	Time Required to Produce Low-Methoxyl Pectin of 3.5% MeO, Min.
1	0	250
2	0.04	195
3	0.1	135
4	0.2	100

tions is greatly influenced by the presence of cations, particularly bivalent cations (16, 17). The cation content of pectin extracts is usually high enough so that de-esterification proceeds at a reasonable rate. With extracts which have been treated to replace bivalent with monovalent cations, the de-esterification rate of the pectin in the extract is only about three fourths that of pectin in the natural extract.

Laboratory tests were made in which monovalent cations were added to a pectin solution to increase the de-esterification rate. Only monovalent cations were used in order to facilitate their removal during subsequent washing of the low-methoxyl-pectin gel. The tests were made on a solution containing 1.5% by weight of commercial pectin which had a very low ash content. The de-esterifications were conducted at 45° to 46° F. in the presence of 30 ml. of 28% aqueous ammonia per liter of solution and sodium sulfate at four concentration levels. The decrease in de-esterification time with increasing salt concentration is shown in Table VI.

**Acid Precipitating** The de-esterification is terminated by the acid precipitation of the low-methoxyl pectin.

This is accomplished by breaking the gel by thorough agitation and introducing acid slowly, so that it is incorporated as uniformly as possible. Sufficient acid is added to neutralize the ammonia and reduce the pH to 1.5 or slightly lower. Upon acidification, the gel is converted from the calcium form to the hydrogen form without any apparent change in structure. For completion of the transition, the batch was allowed to stand for 20 to 30 minutes following the incorporation of the acid.

Considerations of cost and corrosion indicate the use of sulfuric acid for the precipitation step. Because of the nature of low-methoxyl pectin, concentrated technical sulfuric acid with a high lead content cannot be used directly. Low-methoxyl pectin binds bivalent cations very effectively. At low pH, of course, the hydrogen ion concentration is sufficient to displace these ions largely from the low-methoxyl pectin. This is the mechanism by which the calcium con-

tent is reduced in the final low-methoxyl pectin product. However, the amount of lead that can be tolerated is so low that this method does not reduce the lead content sufficiently.

Introduction of lead must be avoided in so far as possible. No lead-containing materials should be used at any point in the plant where lead might be introduced into the product, and reagents should be scrutinized with respect to their lead content. The reagent of major concern is the sulfuric acid used for precipitation. The use of ACS grade sulfuric acid would assure a very low lead content in the low-methoxyl pectin, but the acid is expensive. Although concentrated technical sulfuric acid has a high lead content, a satisfactory method has been found for reducing its lead content to an acceptable range by dilution and settling of the lead sulfate precipitate. It was found that the minimum lead content per unit of sulfuric acid was reached at about 55 weight % sulfuric acid. The lead content of sulfuric acid diluted to varying degrees from concentrated technical acid is shown in Table VII. For comparison, ACS grade sulfuric acid must contain not more than 0.0001% heavy metals (as lead) or 1 p.p.m. (7). For use in the pilot plant, concentrated technical sulfuric acid was diluted with ice in glass vessels. The dilute acid was allowed to stand 18 to 24 hours, and was then decanted from the precipitated lead sulfate as needed for acid-precipitation of the low-methoxyl pectin.

**Draining** The acidified gel was discharged from the tank into a loose-cloth-lined reel where the acidulated water was drained (see Figure 7). The solids content reaches about 6% in this step. Details of construction and the action of this type of reel have been described (7).

**Washing** When the draining was completed, a spray of water was played on the gel as it was reeled. The washing was continued until the pH of the wash water draining from the gel had risen to about 1.8. The rate at which the wash water was added was controlled so that time of application was about 0.5 hour. Washing in this

manner was extremely effective because the wash water tended to displace rather than to dilute the acidulated water. After washing, reeling was continued until the solids content of the gel reached about 8%.

These operations of draining and washing may be carried out in separate reels if desired.

**Pressing** The water-washed gel was removed from the reel and pressed in a rack-and-frame hydraulic press. Pressure was applied at a rate such that the maximum pressure of about 100 pounds per square inch on the "cheese" was reached in 5 to 10 minutes. This pressure was maintained as long as the drainage rate was reasonable, usually about 15 minutes.

**Shredding** The pressed gel was shredded by passing it through a hammer mill equipped with knives in place of the customary hammers. A screen with 3/8-inch round perforations was used.

The gel was recycled through the pressing and shredding steps until the solids content of the material reached the range of 30 to 35%. This usually required a total of three pressings. Repeated pressings may be avoided, if purity of the product is not important, by partially drying the gel to a solids content of 30 to 35% after the first pressing. If this method of raising the solids content were used, a rotary or continuously mixing dryer would be preferred to maintain a uniform moisture content in the product. Low-methoxyl pectin is very sensitive to degradation under these pH conditions, and should the drying be uneven, the overdried and overheated portion would be liable to damage.

After the solids content was adjusted to the desired range, the gel was finally ground through a screen with 1/4-inch round perforations. The final grinding prepared the gel for neutralization and permitted easier grinding of the final dried product.

**Neutralizing** To render the low-methoxyl pectin stable for storage and to increase its rate of solution, the product must be partially neutralized. Any excess sulfuric acid remaining in the gel as well as a portion of the free carboxyl groups of the low-methoxyl pectin must be neutralized.

Neutralization must be carried out carefully to avoid local excessively alkaline conditions. Sodium bicarbonate has been found to be ideal for this purpose (8) because of the relatively low pH produced even at high concentrations. To have the reaction proceed uniformly throughout the material, a high moisture content of the gel is desirable. However, neutralization at too high a moisture content causes some solution of the pectin; the material becomes sticky and lumpy, and is difficult to dry. The optimum range for

**Table VII. Lead Content of Diluted Technical Sulfuric Acid Removed from Precipitated Lead Sulfate**

Sulfuric Acid Content Wt./Wt. %	Lead Content of Diluted Acid, P. P. M.	Lead Content Calcd. to Sulfuric Acid, P. P. M.
100	127	127
75	2.96	4.0
70	1.42	2.0
60	0.31	0.9
50	0.44	0.9
40	0.79	2.0

neutralization is between 60 and 65% moisture (30 to 35% solids). After the sodium bicarbonate has been thoroughly mixed into the finely ground low-methoxyl pectin, a holding period of several hours is required to allow complete equilibrium to be established, even to the centers of the pieces. During the drying step to follow, any local conditions of high pH would cause degradation and further de-esterification of the low-methoxyl pectin.

The amount of sodium bicarbonate required for neutralization is about 18 to 20% of the dry weight of the low-methoxyl pectin. The actual amount necessary was determined by checking the pH of a 1% solution prepared from the low-methoxyl pectin. This pH should be between 4 and 4.5.

The batch to be neutralized was spread out on a sheet of canvas and a portion of the sodium bicarbonate was sprinkled on the surface from a sieve. The batch was partially mixed to present new surface and again sprinkled with sodium bicarbonate. When all of the bicarbonate had been added in this manner, the batch was mixed thoroughly by rolling and tumbling on the canvas and then placed in a covered vessel to equilibrate. For convenience the material was usually held overnight before drying. A tumble barrel or similar piece of equipment would be satisfactory for this mixing step.

**Drying** Drying was accomplished in thin layers on trays in a forced-draft oven operating at 130° F. Starting temperatures as high as 150° F. were used, but were of short duration. The product was rabled at least once during the drying period. Heating beyond the point when the sample contained 10 to 15% moisture was avoided because it causes unnecessary damage to the product. Drying required about 3 hours.

**Grinding** The dried low-methoxyl pectin was ground in a hammer mill in two stages. It was first passed through a screen with 1/8-inch round perforations, and was finished using a screen with 0.024-inch round perforations. Again care is required to avoid overheating of the low-methoxyl pectin, with attendant product damage.

#### **Characterization of Low-Methoxyl Pectin Prepared by Ammonia De-esterification in Aqueous Solution**

Working specifications for low-methoxyl pectin are set forth by each manufacturer of the product at present, largely because the method of production affects somewhat the characteristics of the product. For this reason, the characteristics of low-methoxyl pectin prepared by ammonia de-esterification in aqueous solution are described. The characteristics are those of the product which has been found most desirable

for general applicability. The specifications for intrinsic viscosity and methoxyl content were established by H. S. Owens of this laboratory. Details of the analytical methods used are published elsewhere (26).

**Moisture Content** An entirely dry product is not desirable. Excessive drying causes degradation and a completely dry product would absorb moisture in an opened package. In consequence, the weight required for a specific purpose would vary, depending upon the amount of water absorbed. A product with a moisture content in the range of 10 to 15% is reasonably stable in this respect. The moisture content was determined by drying the product for 16 hours at 70° C. in a vacuum oven.

**Ash Content** The ash content of this type of low-methoxyl pectin is high, because the product is partially neutralized for stability. The ash content as determined by heating at 600° C. is in the range of 10 to 14% (moisture-free basis), largely because of the carbonate which is produced when sodium pectinate is ashed. An ash content in the range of 5 to 8% is obtained when a correction is made for the carbonate. The correction is made by titrating the ash for alkalinity, calculating the alkalinity as carbonate, and correcting the ash by this amount. The value so obtained is referred to as ash-minus-carbonate.

**Methoxyl Content** The methoxyl content of the low-methoxyl pectin product should be in the range of 3.0 to 3.5% on a moisture-free, ash-minus-carbonate-free basis. The methoxyl content is determined by saponification.

**Intrinsic Viscosity** The intrinsic viscosity of the low-methoxyl pectin product should be at least 3.0 dl. per gram on a moisture-free, ash-minus-carbonate-free basis. The intrinsic viscosity is measured by determining the efflux time of an 0.1% pectin sol containing sodium chloride and sodium hexametaphosphate at pH 6.

This method of measuring quality has the advantage over any jelly-strength measurement, in that no control is necessary over the calcium content. In fact, the influence of calcium is removed by the presence of the sodium hexametaphosphate.

**Calcium Content** The calcium content of the low-methoxyl pectin should not exceed 0.1% on a moisture-free and ash-minus-carbonate-free basis. Calcium is determined volumetrically, using permanganate. Corrections are made for moisture and ash-minus-carbonate. No difficulty has been experienced in maintaining the calcium content below this value.

**Lead Content** The Federal Food and Drug Administration has not established any tolerance for

the lead content of low-methoxyl pectin. No difficulty should be experienced in maintaining a lead content under 10 p.p.m., provided the necessary precautions are taken. Lead content is determined by a spectrographic (13) or dithizone method (6).

**pH** The pH of a 1% solution of the final low-methoxyl pectin should be between 4.0 and 4.5. This is near the point of maximum stability, and the low-methoxyl pectin has a reasonably high rate of solution in this range. A 1% solution of the product is prepared on a moisture-free basis and the pH is measured with a glass-electrode pH meter.

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#### **Literature Cited**

- (1) AM. CHEM. SOC., "Reagent Chemicals," Am. Chem. Soc. Specifications, p. 369, 1950.
- (2) Baker, G. L., and Goodwin, M. W., Del. Agr. Expt. Sta., *Bull.* 234, *Tech. No.* 28 (1941).
- (3) *Ibid.*, 246, *Tech. No.* 31 (1944).
- (4) Baker, G. L., and Goodwin, M. W., U. S. Patent 2,233,574 (1941).
- (5) Baker, G. L., Pollari, V. E., and Murray, W. G., *Fruit Products J.*, 24, 356-60 (1945).
- (6) Bricker, L. G., and Proctor, K. L., *Ind. Eng. Chem., Anal. Ed.*, 17, 511-12 (1945).
- (7) Graham, R. P., and Shepherd, A. D., Gel Dewatering Reel, to be published.
- (8) Graham, R. P., and Shepherd, A. D., U. S. Patent 2,503,258 (1950).
- (9) *Ibid.*, 2,548,895 (1951).



- (10) Hall, H. H., and Fahs, F. J., *Confectioner* (October 1946).
- (11) Hills, C. H., Mottern, H. H., Nutting, G. C., and Speiser, R., *Food Technol.*, **3**, 90-4 (1949).
- (12) Hills, C. H., White, J. W., Jr., and Baker, G. L., *Proc. Inst. Food Technol.*, **3**, 47-58 (1942).
- (13) Jeppesen, C. R., Eastmond, E. J., and Logan, H. G., *J. Opt. Soc. Amer.*, **34**, 313-18 (1944).
- (14) Kaufman, C. W., Fehlberg, E. R., and Olsen, A. G., *Food Inds.*, **14**, 57-8, 109 (1942); **15**, 58-60 (1943).
- (15) Kertesz, Zoltan, "The Pectic Substances," New York, Interscience Publishers, 1951.
- (16) Lineweaver, H., *J. Am. Chem. Soc.*, **67**, 1292-3 (1943).
- (17) Lineweaver, H., and McCready, R. M., U. S. Patent 2,386,323 (1945).
- (18) Maclay, W. D., and McCready, R. M., *Ibid.*, 2,478,170 (1949).
- (19) McCready, R. M., Owens, H. S., and Maclay, W. D., *Food Inds.*, **16**, 794-6, 864-5, 906-8 (1944).
- (20) McCready, R. M., Owens, H. S., and Maclay, W. D., U. S. Patent 2,448,818 (1948).
- (21) McCready, R. M., Owens, H. S., Shepherd, A. D., and Maclay, W. D., *Ind. Eng. Chem.*, **38**, 1254-6 (1946).
- (22) Mottern, H. H., and Hills, C. H., *Ibid.*, **38**, 1153-6 (1946).
- (23) Olsen, A. G., Stuewer, R. F., Fehlberg, E. R., and Beach, N. M., *Ibid.*, **31**, 1015-20 (1939).
- (24) Owens, H. S., McCready, R. M., and Maclay, W. D., *Food Technol.*, **3**, 77-82 (1949).
- (25) Owens, H. S., McCready, R. M., and Maclay, W. D., *Ind. Eng. Chem.*, **36**, 936-8 (1944).
- (26) Owens, H. S., McCready, R. M., Shepherd, A. D., Schultz, T. H., Phippen, E. L., Swenson, H. A., Miers, J. C., Erlandsen, R. F., and Maclay, W. D., U. S. Dept. Agr., Bur. Agr. Ind. Chem., *Mimographed Circ. Ser. AIC-340* (June 1952).
- (27) Owens, H. S., and Schultz, T. H., U. S. Patent 2,517,595 (1950).
- (28) Pollari, V. E., Murray, W. G., and Baker, G. L., *Fruit Products J.*, **25**, 6-8 (1945).
- (29) Schultz, T. H., Lotzkar, H., Owens, H. S., and Maclay, W. D., *J. Phys. Chem.*, **49**, 554-63 (1945).
- (30) Shepherd, A. D., and Graham, R. P., *Food Technol.*, **6**, 411-13 (1952).
- (31) Shepherd, A. D., McCready, R. M., and Owens, H. S., *Food Eng.*, **23** (7), 44-5, 180 (1951).
- (32) Ward, W. H., Swenson, H. A., and Owens, H. S., *J. Phys. & Colloid Chem.*, **51**, 1137-43 (1947).
- (33) Woodmansee, C. W., and Baker, G. L., *Food Technol.*, **3**, 82, 85 (1949).

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## Factors in Destruction of Alfalfa Carotene Evaluated

# CAROTENE

## Factors Affecting Destruction in Alfalfa

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The rapid destruction of carotene in alfalfa during field curing has been attributed to the summation of the losses by enzymatic and photochemical processes. Accurate evaluation of the extent of destruction under field conditions is confused by continual changes in temperature, moisture, light intensity, and physical state of the tissue. By incubating aqueous suspensions of macerated alfalfa leaves under controlled conditions it was possible to measure separately the effects of temperature, pH, heat treatments, and cyanide upon the enzymatic, photochemical, and autoxidative losses of carotene. The enzyme system was found to have a temperature optimum of about 43° C., to be more active at pH 4 to 5 than at higher pH values, to be heat labile, and to be partially inhibited by cyanide. Photochemical destruction was not markedly affected by pH changes between 4 and 8, by temperature changes between 10° and 45° C., or by prolonged heat treatments after enzyme inactivation. The loss of carotene in the absence of enzymatic and photochemical destruction was attributed to autoxidation. This mechanism appears to be of minor importance below 40° C., and is little affected by pH changes between 4 and 8. It is difficult to conclude that one mechanism of carotene destruction predominates over the other during field curing. Both contribute to carotene loss and both must be controlled to reduce the total loss.

ALFALFA IS POTENTIALLY A RICH SOURCE OF CAROTENE for livestock, but 45 to 90% of the carotene is lost during the field curing process (3, 9,

13, 14). This loss is attributed chiefly to the rapid destruction of carotene by oxidative processes which are catalyzed by enzymes (5, 6) and light (5), and to a slower destruction, probably by autoxidation in the absence of light and enzyme activity.

Attempts have been made to determine the relative importance of enzymatic and photochemical destruction of carotene during field curing by comparing the losses of carotene in samples incubated at similar temperatures in the light and dark (4, 12). However, it is difficult

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