COMMUNICATIONS

Clarifying Properties of Pectin Fractions Separated by Ester Content

Commercial citrus pectin, commercial low methoxyl pectin, and two pectinesterase-treated pectins were fractionated according to degree of esterification. Fractions varying in degree of esterification from 6 to 70% were tested for the critical esterification level at which pectin destabilizes orange juice cloud. Juice was clarified by fractions of 6–14% esterification, but not by fractions of 21% or higher esterification.

Insoluble pectates are formed in fresh citrus juices by the action of pectinesterase (PE) on soluble juice pectins. Precipitation of these pectates destabilizes cloud, causing juice clarification (Stevens et al., 1950). However, the critical ester content associated with this destabilization has not been clearly established (Krop et al., 1974). Orange juices differ widely in their susceptibility to clarification, presumably because of differences in PE level, pectin content, and ester content of the pectin (Stevens et al., 1950). Although PE and pectin content can be readily determined, lack of knowledge about the critical ester content limits their usefulness in indicating storage stability of processed juice. If this value was known, then juice pectin content, extent of pectin esterification, and PE level could more accurately reflect clarifying potential. Juice in which a substantial portion of the soluble pectin had an ester content near the critical value would be very susceptible to even low levels of PE (Primo et al., 1965) and precautions could be taken to prevent any further deesterification.

This critical ester value cannot be determined by measurement of overall juice pectin esterification, as values so obtained are averages of the ester content of all pectin molecules present. Pectin molecules are naturally heterogeneous with respect to ester content. In addition, PE activity is not random, but removes methoxyl groups in a blockwise pattern on an individual pectin molecule (Deuel and Stutz, 1958). Thus, in a substrate-rich system like orange juice, some molecules would be extensively deesterified while others would be largely unaffected (Krop et al., 1974). Therefore, ester content of the cloud destabilizing fraction would be masked by the higher ester contents of more fully esterified fractions.

For determination of the critical ester content, pectins must be fractionated according to ester content and tested in a system which will measure clarifying potential. Fresh juice is not a sensitive test system, because soluble pectin present inhibits the natural coacervation of cloud and pectate (Baker and Bruemmer, 1972). However, if juice cloud is removed from fresh juice by centrifugation, pectin precipitates as an insoluble pectate floc in the aging serum and can be removed. Resuspension of aged cloud in aged depectinized serum provides a sensitive test system for testing clarifying potential of pectins of known ester content (Baker and Bruemmer, 1972).

This paper reports the separation of a commercial citrus pectin, a commercial low methoxyl pectin, and two PE-treated pectins into fractions differing in degree of esterification. The fractions were then tested in aged depectinized orange juice, and the ester content necessary for clarification was established.

MATERIALS AND METHODS

Materials. Commercial citrus pectin and low methoxyl pectin were obtained from Sunkist Growers Inc., Corona, CA. Two PE-treated pectins, differing slightly in average esterification value, were used because of their high content of fractions very low in methoxyl groups. These pectins were prepared by partial deesterification of citrus pectin with PE derived from orange (Baker, 1976b).

Aged cloud and depectinized serum were prepared from orange juice as previously described (Baker and Bruemmer, 1972).

Column Preparation. A 40-g sample of DEAE–cellulose (Cellex-D, Bio-Rad Laboratory, Richmond, CA; exchange capacity 0.65 mequiv/g) was washed in three 1-L changes each of 0.5 M HCl alternating with 0.5 M NaOH (Neukom et al., 1960). The column packing was then washed with 1 L of 0.1 M NaH_2PO_4 and packed into a 25 × 500 mm column fitted with a sintered glass plate. Extraneous material was eluted from the column with 1 L of 0.1 M NaH_2PO_4 prior to application of the pectin sample.

Pectin Fractionation. Pectin samples (300 mg) were dissolved in 50 mL of 0.1 M NaH_2PO_4 and applied to the column. Fractionation was at room temperature with 300-mL portions of NaH_2PO_4 solutions, increasing in concentration from 0.1 to 0.6 M in 0.1 M increments. The eluant reservoir was elevated 130 cm above the column, providing a flow rate of about 3 mL/min. Final column elution was with 0.3 and 1.0 M NaOH. The pH of the 0.3 M NaOH eluate was about 6 and that of the 1.0 M NaOH eluate was about 12 and was adjusted to 7 with HCl immediately after elution. However, some demethylation of this sample could have occurred during elution. Eluate volumes were recorded, anhydrogalacturonic acid (AGA) content of each fraction was determined (Bitter and Muir, 1962), and percent recovery of AGA was calculated. Eluates were reduced from 300 mL to about 50 mL on a vacuum rotary evaporator, dialyzed overnight against distilled water, and vacuum evaporated to 10–15 mL. Concentrated fractions were assayed for AGA content before ester content, and clarifying activity was determined.

Determination of Ester Content. Ester contents of unfractionated pectins were measured by the titration procedure of Hinton (1940). Quantity of sample required for this method precluded its use on pectin fractions. Ester contents of fractions were determined by a modification of the gas chromatographic method of Bartolome and Hoff (1972), using a Loenco Model 160 gas chromatograph (Loenco, Inc., Altadena, CA.). Methanol liberated by hydrolysis with NaOH was converted to methyl nitrite with HNO_2 and quantitated by use of propyl nitrite as internal standard. The following modifications were employed: (1) ethanol was used as the internal standard, (2) samples were saponified with 1 M NaOH, and (3) prior to each use, the GC column was heated 3–4 h at 180 °C, cooled to 50 °C,

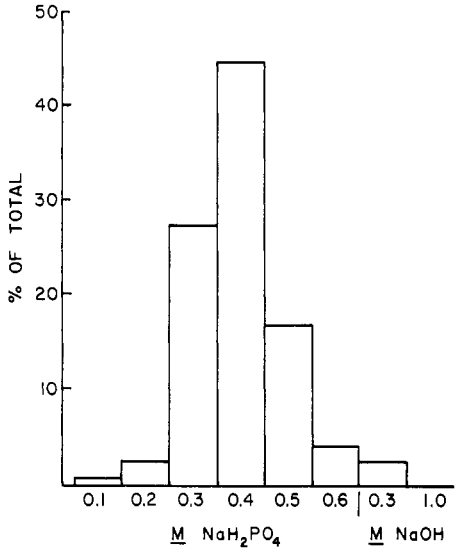

Figure 1. Fractionation pattern of commercial citrus pectin.

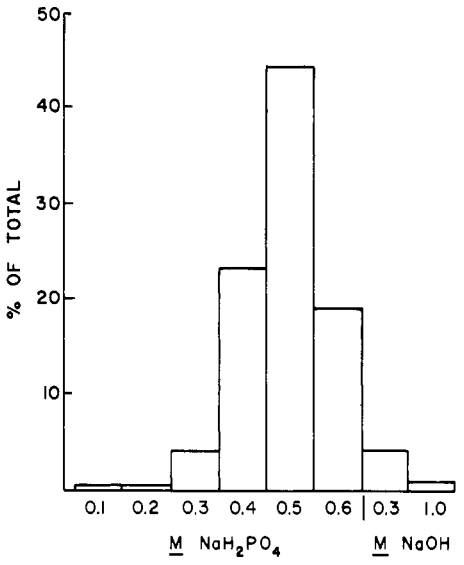

Figure 2. Fractionation pattern of commercial low methoxyl pectin.

and conditioned with several 1-mL injections of air, followed by several injections of methyl/ethyl nitrite standard. Results are expressed as degree of esterification (DE) based on pectinic acid content [AGA content + 17/31 methoxyl content (Kertesz, 1951)]. DE is defined as the percentage of available ester sites occupied by methoxyl groups.

Determination of Clarifying Properties. Pectin fractions differing in DE were added at 400 ppm (AGA basis) to aged, deflocced orange juice serum in which aged cloud was resuspended at the normal level. After 1 h, cloud densities were determined as previously described (Baker, 1976a). Densities were converted to gram/liter bentonite values (Senn et al., 1955) and expressed as percentages of the control.

RESULTS AND DISCUSSION

When pectin is fractionated on DEAE–cellulose with an increasing NaH_2PO_4 concentration gradient, highly esterified fractions are eluted first, followed by less esterified fractions (Heri et al., 1961). Application of this fractionation procedure to four different pectins yielded a number of fractions varying in esterification level and reflecting the treatment history of the pectins (Figures 1, 2, and 3).

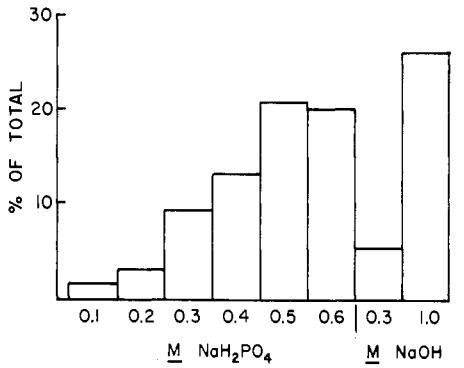

Figure 3. Fractionation pattern of PE-treated pectin.

Table I. Source and Average Degree of Esterification of Pectins Used for Fractionation[a]

pectin	source	DE, %
A	commercial citrus	60
B	commercial low methoxyl	36
C	PE treated	38
D	PE treated	35

[a] Abbreviations used: DE, degree of esterification; PE, pectinesterase.

Table II. Degree of Esterification and Clarifying Activity of Pectin Fractions

M NaH_2PO_4 eluant	pectin	DE, %	cloud % of control
0.3	A	70	98
0.3	C	59	86
0.3	B	57	112
0.4	A	56	103
0.4	C	50	99
0.5	A	46	125
0.4	B	42	87
0.5	B	36	102
0.5	C	33	125
0.6	B	29	118
0.6	C	26	161
0.6	D	21	132
0.3 M NaOH	B	14	48
0.3 M NaOH	C	13	33
0.3 M NaOH	D	13	8
1.0 M NaOH	D	6	3

Fractions of the commercial citrus pectin increased in size with increasing concentrations of NaH_2PO_4 up to 0.4 M and then decreased. The major fraction accounted for 45% of the total pectin (Figure 1). The commercial low methoxyl pectin, which had been chemically deesterified with ammonia, had an elution profile similar to that of the untreated citrus pectin; but the major fraction was eluted with 0.5 M NaH_2PO_4 (Figure 2). This similarity in profile with shift in elution maximum reflects the random action of chemical deesterification, which removes methoxyl groups equally from all pectin molecules. The DE values for the peak fractions shown in Figures 1 and 2 were 56 and 36%, respectively, agreeing well with the overall DE values of 60 and 36% for the two pectins (Table I).

Unlike the elution profiles of the two commercial pectins, the profile of PE-treated pectins did not show a sharp peak (Figure 3). Because of the nonrandom action of PE, some fractions were far more demethylated than others. One fraction (27%) of the pectin was so extensively deesterified it could be eluted from the column only with 1.0 M NaOH.

Table II gives the DE values for fractions of the four citrus pectins studied. Fractions containing less than 30 mg of pectin were not assayed for ester content. DE values

were consistently higher for the citrus pectin fractions than for corresponding fractions of the commercial low methoxyl pectin or the PE-treated pectins. Although NaH_2PO_4 elution chromatography separates pectin by ester content (Smit and Bryant, 1967), results of the present study show that a given molarity of NaH_2PO_4 would not invariably elute pectin of a particular ester content. This phenomenon may be due to differences in the molecular weights of the parent pectins.

When pectin fractions were tested for clarifying properties, fractions having DE values of 21% or higher did not destabilize orange juice cloud, but fractions with DE values of 14% or less did. Prior work relating esterification level to clarification measured juice pectin ester content; thus, the results may well have been biased because of nonuniformity in the DE of the pectins. For example, Rouse (1949) observed a 25% "separation" of cloud in 15 min when the methoxyl contents of juice pectins declined to between 4.56 and 5.87%, expressed on a calcium pectate basis. Expressed on a pectinic acid basis, his data show clarification was initiated as average DE values declined to between 30 and 38%. In the present work, PE-treated pectins and commercial low methoxyl pectin had average DE values between 35 and 38%. Of the fractions derived from these pectins, only those with DE values of 14% and below clarified the test juice.

A further difficulty in relating the DE of unfractionated pectin to clarification becomes evident when Figures 2 and 3 are compared. Both low methoxyl pectins had similar DE values (36 and 38%), yet only 8% of the commercial low methoxyl pectin could clarify juice, as compared with 32% of the PE-treated pectin. Krop et al. (1974) obtained an 85% cloud reduction in juice when juice pectin DE was reduced to 27.4%. The present work showed that cloud was not precipitated by a fraction consisting predominantly of pectins that were only 21% esterified. Therefore, in any study relating pectin esterification to clarification, pectins as homogeneous as possible with respect to ester content should be used.

Table II shows that as the esterification level of pectin fractions approached the critical level, they could support more cloud than the amount normally present in juice. A similar phenomenon has been reported by a number of investigators, who found that cloud density in whole juice increased shortly before the onset of clarification (Joslyn and Pilnik, 1961). The increase in turbidity may be due to the initial precipitation of pectate floc prior to its complexing with cloud particulates.

In conclusion, several pectins differing in ester content were fractionated on DEAE-cellulose and were assayed both for degree of esterification and juice clarifying potential. Pectin fractions with degrees of esterification as low as 21% did not destabilize cloud in the test system employed. Clarification was initiated by pectin fractions with DE values of 14% or less, indicating that the critical DE for clarification is between 14 and 21%.

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Daminozide Residues on Orchard-Treated Apples

Terminal residues of daminozide were determined on apples after application of Alar as a dilute or concentrated spray. Thirty-five days after treatment, uncorrected results for daminozide residues were 5.2 and 7.1 ppm for the dilute and concentrated sprays, respectively, and after 71 days the concentrations were 5.0 and 5.8 ppm, respectively. Control apples showed apparent residues of 2.9 and 4.4 ppm on the early and late harvest dates, respectively. No significant differences (t test, <98%) were found between the residue from either spray type and control apples at the later harvest date.

Daminozide (Alar; succinic acid 2,2-dimethylhydrazide) is used in Ontario (Publication 360, 1978) and in other apple-producing areas mainly as a growth regulator to control preharvest drop of apples. There has been an indication that daminozide-treated fruit shows a higher incidence of physiological disorders than untreated fruit

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