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CLARIFICATION WITH LOW METHOXYL PECTINS

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Abstract. **Two series of pectins varying in methoxyl content from 9.83 to 0.33% were prepared by deesterification of citrus pectin. One series was prepared enzymically; the other, chemically. Both series were tested for clarifying potential in fresh orange juice. At pH 3.7, clarification was optimal with the enzymically produced pectins of 2.94 and 4.52% methoxyl contents, and with the base deesterified pectin of 2.42% methoxyl content. The enzymically prepared pectin of 2.94% methoxyl content clarified maximally at pH values between 3.4 and 4.0, whereas the base demethylated pectin of 2.42% methoxyl content was most effective at pH values between 2.6 and 3.4.**

Pectinesterase (PE) can lower the methoxyl content of soluble pectins until they precipitate as pectates. When this precipitation occurs in citrus juice, the pectates occlude and remove from suspension the colloidal particulates which constitute the juice cloud (12). The conditions for optimum clarification of juice—type of pectin, its methoxyl content, and pH of the juice—have not been clearly established. Efficient clarification is important in the manufacture of clarified lime and lemon juices and in the production of very high Brix orange syrups (3).

This paper reports the preparation of two series of pectins of decreasing methoxyl content by PE or NaOH treatment of citrus pectin. These pectins were tested in orange juice for clarifying potential. Those which effectively destabilized cloud were examined further for optimum concentrations and clarifying activity at various pH values.

Materials and Methods

Preparation of pectins. One series (PE series) of low methoxyl pectins was derived from citrus pectin (Sunkist Growers, Inc., Corona, Ca.) by treatment with a PE extract from dried orange flavedo (6). The extract had an activity of 886 x 10^{-4} PEu/ml (9). Pectin solutions (10 g/500 ml water) were adjusted to pH 7.5 with 1N NaOH before addition of 4 ml of PE extract. Solutions were maintained at pH 7.5 by dropwise addition of 0.5N NaOH until an amount of NaOH calculated to provide the desired demethylation had been consumed. Pectin was then precipitated from solution with 500 ml of 95% ethanol, recovered after centrifugation, and washed with two 500 ml volumes of ethanol. Pectins were tumble dried in a vacuum rotary evaporator and then dried to constant weight at 102°C under vacuum.

The other series (NaOH series) of low methoxyl pectins was prepared by direct NaOH demethylation of citrus pectin (6). Pectin solutions (10g/500 ml water) were cooled to 4°C, adjusted to pH 11 with 0.5N NaOH, and held at this pH by addition of 0.5N NaOH until the required volumes of base had been consumed. Solutions were then adjusted to pH 5 with 1N HCl and the pectins precipitated and dried by the same procedure used for the PE derived pectins. The control pectin for both series was precipitated from 500 ml of an untreated 2% pectin solution and dried.

Analysis of pectins. All pectins were analyzed for anhydrogalacturonic acid (AGA) (2) and for methoxyl content (5). Methoxyl content was expressed as percentage of pectinic acid content (AGA content + 17/31 of methoxyl content) (6). Molecular weight of citrus pectin was determined by the method of Smit and Bryant (11).

Cloud loss determinations. The pectins, as 1% aqueous solutions, were added to fresh orange juice to give final concentrations of 200 ppm (w/v). After 1 hr at room temperature, cloud in treated juices was measured by a previously described procedure (1). Transmission values were converted to g/l bentonite values (10); these were subtracted from the bentonite values of the blank and expressed as percent cloud loss.

pH effect. Orange juice samples were adjusted to various pH values with 4N KOH or solid citric acid prior to addition of pectin solutions. Cloud levels of the pH adjusted juices were measured before pectin addition, and any pH influence on turbidity was corrected for in the calculation of percent cloud loss.

Results and Discussion

Six pectins of various methoxyl contents were prepared for each series. The AGA and methoxyl contents are shown in Table 1. Methoxyl contents varied from 9.83% for the control pectin to 0.33% for the NaOH derived pectin No. 6. The methoxyl content of commercial low methoxyl pectins for jelly production range from 2.5 to 4.5% (6).

Table 1. Anhydrogalacturonic acid (AGA) and methoxyl content of pectins prepared by PE or NaOH demethylation.

Pectin Number	PE Treatment AGA (%)	PE Treatment Methoxyl (%)	NaOH Treatment AGA (%)	NaOH Treatment Methoxyl (%)
Control	79.1	9.83	—	—
1	73.8	8.07	78.0	7.52
2	75.6	5.72	79.6	5.28
3	70.7	4.52	82.2	3.52
4	73.8	2.94	81.1	3.23
5	73.1	1.47	78.7	2.42
6	71.8	0.85	78.2	0.33

Pectins of both series effectively clarified fresh orange juice at pH 3.7 (Fig. 1). Of the NaOH pectins, the one with methoxyl content of 2.4% was the most effective clarifier.

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Mention of a brand name is for identification only and does not imply endorsement by the U.S. Department of Agriculture over others which may also be suitable.

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The methoxyl contents of the PE pectins were greater than those of equally effective NaOH pectins. For example, PE pectin No. 2 and NaOH pectin No. 4, which reduced cloud by 56%, had methoxyl contents of 5.72 and 3.23%. The methoxyl content of PE pectin No. 3, which removed 94% of the juice cloud, was 4.52%. This finding agrees closely with that of Rouse (8), who reported cloud separation in juice when the methoxyl content of the pectin was from 5.87 to 4.56%, and with Krop et al. (7), who observed 85% cloud loss when the methoxyl content of juice pectin was 4.48%.

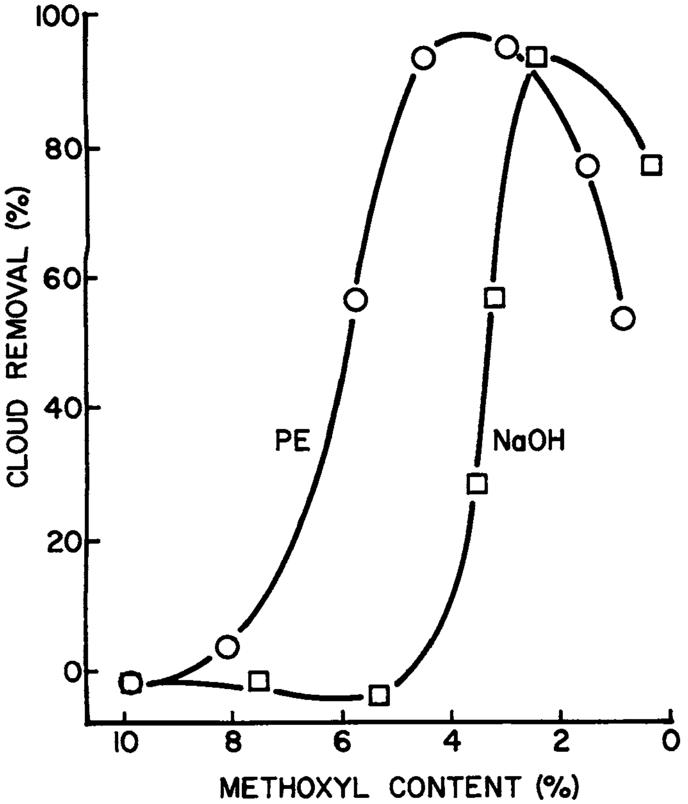

Fig. 1. Effect of methoxyl content on pectin clarifying activity in pH 3.7 orange juice.

Lowering of the methoxyl content beyond 2.94% for the PE series and 2.42% for the NaOH series did not improve the clarifying activity of the pectins. Clarification was optimum when the methoxyl content was near 2.42% for the NaOH pectins, and between 2.94 and 4.52% for the PE pectins.

This information enabled estimation of the average number of free acid units per esterified unit in molecules of the pectins that clarified juice. This number is equal to (total possible methoxyl content—methoxyl content)/ methoxyl content. For the NaOH pectin that was the most effective at pH 3.7, and which contained 2.42% methoxyl, the number was (16.34 – 2.42)/2.42, or 5.75. Since NaOH demethylation is random in its removal of methoxyl groups, those remaining should have been randomly distributed along the molecules between series of free acid units.

Like the most effective NaOH pectin, PE pectin No. 3 also effected 94% cloud removal, but with a methoxyl content of 4.52%. If randomly distributed, the units of free acid would have been distributed among all the pectin molecules in the ratio of 2.62 units per esterified unit. However, PE tends to sequentially demethylate the methoxyl groups within a molecule; thus, some molecules would have been demethylated far more than others (4). That the PE pectin was as effective as the NaOH pectin with 2.42% methoxyl content indicates that the number of free acid units per esterified unit in much of the molecules of the PE pectin approached six also.

At pH 3.2 the activities of those pectins of lowest methoxyl content were much lower than at pH 3.7 (Fig. 2). The pH change to 3.2 shifted the optimum methoxyl content for the PE series to 4.52%, but did not shift the optimum for the NaOH series. Pectin No. 6 of the NaOH series was sufficiently demethylated (0.33% methoxyl content) to be considered as polygalacturonic acid (PGA). Commercial polygalacturonic acids (0.26-0.44% methoxyl content) also clarify citrus juices, and the pH values at which the acids are most effective depend upon their molecular weight (1). Both the PGA I prepared and its parent preparation, Sunkist citrus pectin, had molecular weights of approximately 55000. The PGA preparation clarified juice at pH 3.7, and was inactive at pH 3.2. Yet its closest analogue in the NaOH series (2.42% methoxyl content) clarified juice effectively at both pH values. Apparently, pectins of a low methoxyl content do not necessarily have the same pH optimum for clarification as polygalacturonic acids derived from those pectins.

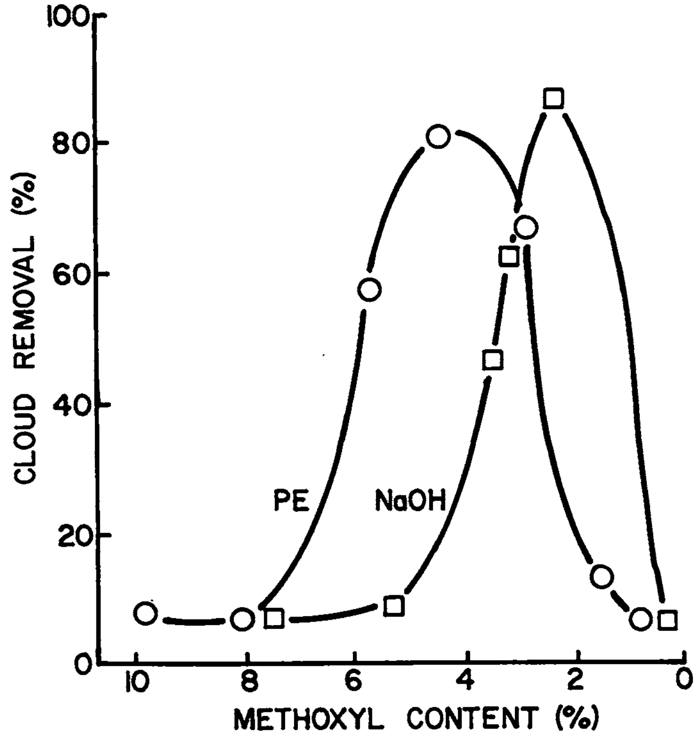

Fig. 2. Effect of methoxyl content on pectin clarifying activity in pH 3.2 orange juice.

The reduction of clarifying activity due to lowered pH was more pronounced for the PE series than NaOH series. This suggested that pectins prepared by the two procedures might differ in pH sensitivity; and differences in pH sensitivity would be important considerations if such pectins are to be used in juices other than orange. Pectins No. 4 of the PE series and No. 5 of the NaOH series were therefore compared for pH sensitivity. Both were similar in methoxyl content and in clarifying activity at pH 3.7 (Fig. 1). When added to a series of pH adjusted orange juice samples, the two pectins caused dissimilar responses (Fig. 3). The PE pectin effectively clarified orange juice at pH 3.4-4.0, but removed only 8% of the juice cloud at pH 2.6. These results suggest the possibility that low methoxyl pectins formed by native or added enzymes are relatively ineffective in low pH juices, such as those of lime and lemon. Such a

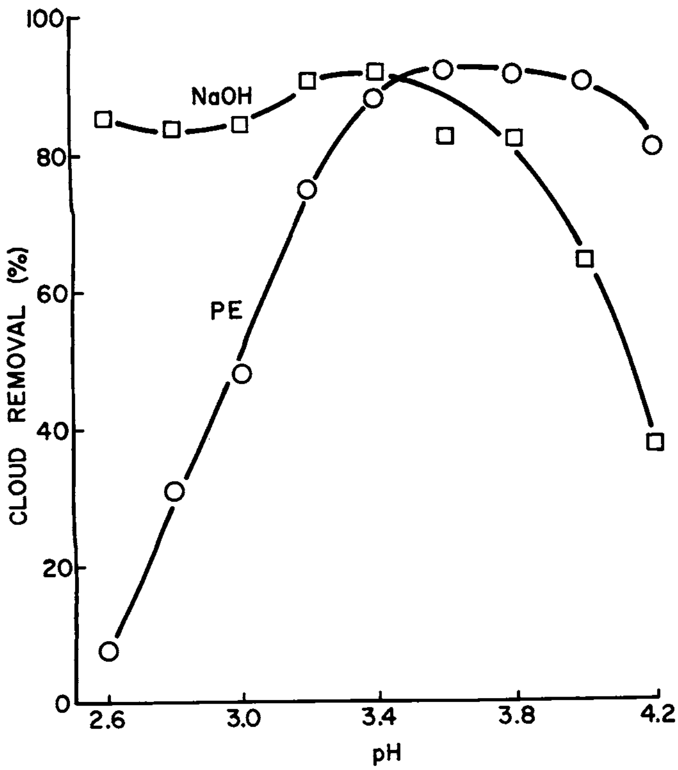

Fig. 3. Effect of pH on orange juice clarification with 200 ppm PE derived pectin (2.94% methoxyl) or NaOH derived pectin (2.42% methoxyl).

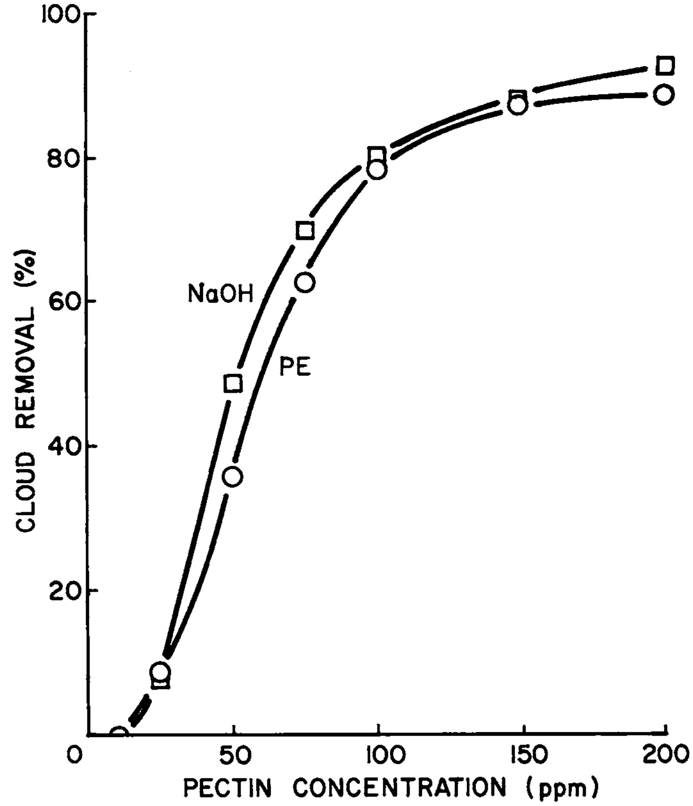

Fig. 4. Cloud loss in orange juice (pH 3.5) treated with varying concentrations of PE derived pectin (2.94% methoxyl) or NaOH derived pectin (2.42% methoxyl).

possibility may partly explain some of the problems encountered in the clarification of those citrus juices by aging or by addition of commercial enzymes.

Clarification with pectin No. 5 of the NaOH series was optimal at pH 3.2-3.4, and diminished slightly as pH was lowered to 2.6. Thus a low methoxyl pectin produced by NaOH demethylation would appear to be a satisfactory clarifier of low pH citrus juices. Some polygalacturonic acids, when dissolved by partial neutralization, are also suitable clarifiers of low pH juices (1). Low methoxyl pectins are advantageous because they are soluble in water without neutralization.

Intersection of the pH sensitivity curves in Fig. 3 indicated that the two pectins would both perform equally well at pH 3.5. When tested for minimum effective concentrations in orange juice at this pH, both pectins displayed similar clarifying activity (Fig. 4). Cloud loss was 80% with 100 ppm of either pectin.

In summary, two series of low methoxyl pectins were prepared by PE and NaOH demethylation of citrus pectin. Both series contained pectins which were effective clarifiers of fresh orange juice. Optimum methoxyl content for clarification at pH 3.7 was between 2.94 and 4.52% for the PE demethylated pectins, and near 2.42% for the NaOH demethylated pectins. The PE derived pectin containing 2.94% methoxyl clarified optimally at pH values between 3.4 and 4.0. The NaOH derived pectin containing 2.42% methoxyl was most active as a clarifier at pH values between 2.6 and 3.4. At pH 3.5, 100 ppm of either pectin reduced cloud by 80%.

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