# Technical Papers

## Characterization of Pectin

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The molecule of the natural high polymer, pectin, is composed principally of anhydrogalacturonic acid residues, partially methyl esterified and linked together to form a long chain. Nongalacturonide materials, galactan and araban, may constitute one-third or more of the weight of the pectin. As has been shown by Schneider and Bock (*3*), the characteristic properties of pectin—gelation, film formation, and high viscosity in dilute solution—derive from the polygalacturonide chain; the nongalacturonide constituents or "ballast materials" act mainly as diluents.

In characterizing pectin by chemical means it has been the custom to give the ester content, computed as methoxyl and expressed as per cent by weight of the whole sample. Alternatively, the related quantity, neutralization equivalent, has been used for the same purpose. It is the object of this communication to demonstrate that because of variability in the proportion of nongalacturonide material neither the weight per cent of methoxyl nor the neutralization equivalent is adequate for the characterization of pectin, and to show that the properties of pectin are better described by the galacturonide content and the degree of esterification of the galacturonide chain.

Recently considerable interest has developed in low-ester pectin. De-esterification progressively increases the acidic nature of pectin and hence its reactivity with metallic ions. Beyond a certain stage in the de-esterification, the pectin is able to form stable gels with polyvalent cations such as calcium. These gels have promise of commercial importance.

It has not been recognized previously that the galacturonide content of a low-ester pectin may be greatly influenced by the method of de-esterification. Fig. 1a shows that in de-esterification by acid the methyl ester groups and the nongalacturonide materials are removed at approximately the same rate, while in enzyme de-esterification (Fig. 1b) the nongalacturonide content is reduced only slightly. The methoxyl content and neutralization equivalent of low-ester pectins produced by these two methods are not, then, in general comparable, because the pectins contain different amounts of diluent.

On prolonged acid treatment the nongalacturonide content of the pectin sample represented in Fig. 1a was reduced to 0.9 per cent. The corresponding average residue weight is 178 and thus approaches closely the theoretical limit of 176, the residue weight of

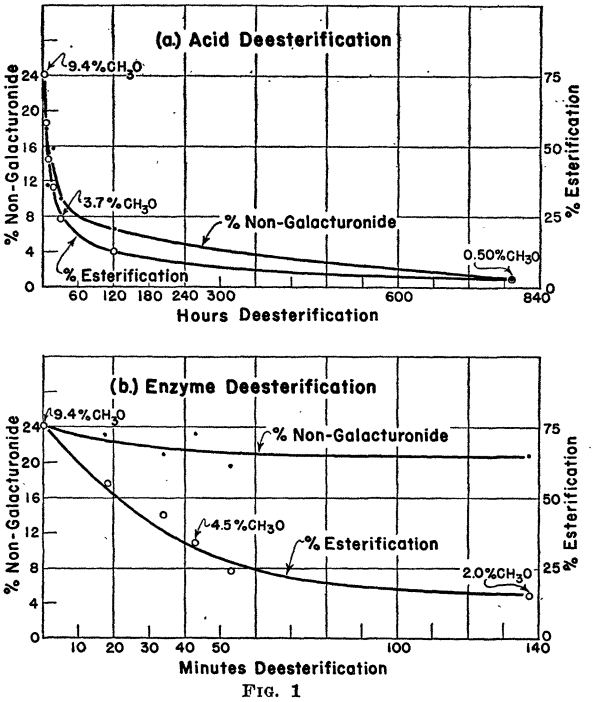


FIG. 1

anhydrogalacturonic acid. The fact that this material was of high molecular weight (at least 30,000 as indicated by viscosity and gel formation) means that probably none of the nongalacturonide constituents are situated in the pectic acid chain. This confirms the conclusion reached by Schneider and Bock (*3*) from entirely different experiments that the main pectin chain consists exclusively of anhydrogalacturonide units.

As stated above, a characteristic property of low-ester pectins is the formation of ionic-bonded calcium gels. The extent of de-esterification necessary for gel formation with calcium ions cannot be defined accurately by either the neutralization equivalent or the methoxyl value, since both quantities are affected by variation in the nongalacturonide materials present. This fact is illustrated by the data in Table 1. Sample A, a non-de-esterified pectin, did not form a stable calcium pectinate gel, although it had a lower methoxyl value than sample B-1, which did form a gel. This anomaly is explained by the difference in the per cent esterification of the two samples. It may

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be observed that calcium pectinate gels containing 35 per cent sugar are formed only when the degree of esterification of the polygalacturonide chain is less than 50 per cent.

Data for samples A and A-1 show that by removal of nongalacturonide material the methoxyl value and the neutralization equivalent may be changed greatly, while the degree of esterification is reduced only

TABLE 1

DATA CHARACTERIZING TWO SERIES OF ACID—DE-ESTERIFIED PECTINS

| Sample No. | Description | Ca-pectinate gel formation* | Per cent esterification | Per cent $CH_3O$ | Neut. equiv. | Per cent galacturonide |
|---|---|---|---|---|---|---|
| A | Commercial apple pectin | None | 68.0 | 6.57 | 990 | 57.8 |
| A-1 | Sample A purified† | None | 66.7 | 8.06 | 769 | 71.1 |
| A-2 | Sample A acid de-esterified | None | 50.6 | 6.61 | 476 | 78.3 |
| A-3 | Sample A acid de-esterified | Strong | 38.5 | 5.23 | 370 | 80.1 |
| B | Purified apple pectin† | None | 74.2 | 9.36 | 952 | 76.0 |
| B-1 | Sample B acid de-esterified | Weak | 45.2 | 6.78 | 391 | 85.7 |
| B-2 | Sample B acid de-esterified | Strong | 24.2 | 3.70 | 269 | 89.1 |

* Calcium pectinate gels, containing 35 per cent sugar, prepared by method of Hills, White, and Baker (Hills, C. H., White, J. W., Jr., and Baker, G. L. *Proc. Inst. Food Tech.*, 1942, 47–58).

† Purified by dissolving in water, adding 5 per cent conc. HCl by volume, immediately precipitating with ethanol, and washing with ethanol until free of chlorides.

slightly. Clearly, the increase in the jelly grade of pectins upon mild acid de-esterification observed by Baker and Goodwin (*1*) may be caused in part by the removal of inactive constituents and the consequent increase in galacturonide content.

The degree of esterification and the galacturonide content may be calculated readily from the methoxyl value and titration data. Each anhydrogalacturonide residue in the chain may be considered to contain either a free carboxyl or a methyl-esterified carboxyl group.[2] The number of moles of free carboxyl groups (N) per gram of pectin may be determined from the alkalinity of the ash and the amount of alkali required to titrate an aqueous solution of pectin to pH 7.5 (*4*). The number of moles of methyl-esterified carboxyl groups may be determined by methyl ester analysis (*2*) and the formula

$$Z = \frac{\text{wt. per cent } CH_3O}{3100}$$

From these two quantities one may compute the per cent galacturonide, the per cent nongalacturonide, the neutralization equivalent, the per cent esterification of the galacturonide chain, and the average residue weight by the relations

$$\text{Per cent galacturonide} = (176\,N + 190\,Z) \times 100$$

$$\text{Per cent nongalacturonide} = 100 - \text{per cent galacturonide}$$

$$\text{Neutralization equivalent} = \frac{1}{N}$$

$$\text{Per cent esterification} = \frac{Z}{Z+N} \times 100$$

$$\text{Average residue weight} = \frac{1}{Z+N}$$

In view of these considerations it is suggested that, instead of per cent methoxyl by weight, the per cent galacturonide and the per cent esterification of the galacturonide chain be used in the characterization of pectin.

[2] The close agreement between the decrease in methyl ester groups and the increase in carboxyl groups observed on de-esterification of pectin indicate that, for the purpose of calculation, it is valid to consider that all the ester groups are methyl. In any case, if the araban or galactan occurred as ester groups, the decrease in the total carboxyl content would be negligible because of their high molecular weight.

### References


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## Inhibition of Growth of *Mycobacterium Tuberculosis* by a Mold Product—the Effect on Pathogenic Human Tubercle Bacilli [1,2]


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A mold product capable of inhibiting the *in vitro* growth of a rapidly proliferating nonpathogenic strain of *M. tuberculosis* was described in a previous report (*1*). The present study is concerned with the *in vitro* activity of this mold product against pathogenic human tubercle bacilli.

For convenience the material has been named, temporarily, mycocidin and a working unit of activity has been established. The strength of a mycocidin preparation is determined as follows: A standard culture is prepared consisting of a 5-day-old pellicle of the nonpathogenic strain of *M. tuberculosis* var. *hominis* (American type culture collection No. 607) on Long's synthetic liquid medium. The standard inoculum, which measures approximately 2 × 2 mm., is taken from the periphery of this pellicle. With some experience nearly similar fragments are usually obtainable. Test tubes of 1 in. in diameter containing 5 ml. of Long's synthetic medium are set up in series and mycocidin is added in varying concentrations. The final volume is made to 6 ml. The inoculum is floated


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