PECTIC SUBSTANCES IN CEYLON TEA

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Introduction

An essential group of constituents of all plants is known by the general name of pectins or pectic substances. Commercially these substances are extracted on a large scale on account of their importance in the production of jams, jellies and canned fruit products. Even though pectic substances constitute from 4 to 8 per cent of the dry matter of tea leaf sufficient attention has not been paid to them, most of the investigations having been concentrated on other important constituents like polyphenols, caffeine, proteins, etc. However, occasional references have been made about the possible role of these substances in tea manufacture. Shaw (1) suggested that "the production of 'apple' smell in satisfactorily withered leaf may be ascribed to this transformation—(*i.e.* the demethylation of pectin during manufacture)—on analogy with the formation of the characteristic aromas produced during the ripening of fruits coincidental with pectic changes." Lamb (2) postulated that "characters such as stickiness of withered leaf and rolled leaf, often associated with good quality in Ceylon, maltiness of fired tea and the apple-like odour of well withered leaf may quite possibly be associated with pectin content," while Roberts (3) mentioned that "changes in the pectins are probable but these have been but little investigated."

Shaw suggested a technological differentiation between two kinds of pectic substances. They are the 'infusion' type (water soluble) and the 'texture' type (water insoluble); the former is present mostly in the tender portions of the flush while the latter occurs in older leaves and stalk. While the 'infusion' type imparts certain liquoring characteristics to tea, the 'texture' type helps in the keeping properties of made tea and also in protecting tea polyphenols by reacting first with heavy metals which would otherwise affect the polyphenols. Following this work in South India, Lamb carried out the analysis of Ceylon green leaf for pectic substances but he made no distinction between the various fractions. His results showed wide variations in pectic contents in samples of leaf of varying ages. It has recently been shown that the pectic content of tea leaf from shaded areas is slightly less than that found from unshaded areas (5).

Nomenclature of pectic substances

Pectic substances are complex colloidal carbohydrate derivatives containing a large proportion of anhydro-galacturonic acid residues which are probably linked in a chain structure. The carboxyl groups of these units may be partly esterified by methyl groups and partly or completely neutralized by one or more bases. According to the most recent nomenclature (4), the following terms are used for various fractions of pectic substances:—

Protopectin — Precursor of pectic substances, insoluble in water.

Pectinic acid — Colloidal galacturonic acids containing appreciable proportions of methyl ester groups, soluble in water. (Water soluble tea pectins fall into this group).

Pectin — Composition same as pectinic acids but capable of forming jelly with sugar and acid The commercial products of pectin belong to this group. The ideal pectin (completely esterified with methyl alcohol) contains 16.3 per cent methoxyl groups, but so far such a product has not been isolated from any vegetable source.

Pectic acid — Pectic substances free from methyl ester groups. This group is possibly present in the protopectin in combination with bivalent metals like calcium and magnesium. It combines readily with heavy metals to form a precipitate. In the determination of pectic substances in tea (for which 0.5% aqueous ammonium oxalate is used as solvent) this group along with the water soluble fraction is estimated as total pectin content.

Protopectin in tea

Protopectin is probably found in the middle lamella and primary cell membrane of the leaf. Being insoluble in water it is not likely to play an important role in the manufacture of tea. However, during withering part of it may be broken down to pectinic acid. An increase in the pectinic acid content is found to occur during withering—see table below:

St. Coombs Estate leaf

	Water soluble pectin (% dry matter)	Water insoluble pectin (% dry matter)
Green leaf ...	3.62	0.92
Withered leaf ...	4.24	1.60
Fermented leaf ...	3.84	1.60

Pectinic acid in tea

The pectinic acids from different plant materials are heterogeneous containing varying proportions of methoxyl groups. In Ceylon tea leaf, pectinic acid appears to have a fairly constant composition containing 4 to 5 per cent methyl alcohol. The composition of fractions of pectic substances from green leaf prepared by two different methods is shown below. In one experiment (I) the fractions were prepared directly from dried green leaf while in the other (II) the leaf pigments were removed completely with acetone prior to fractionation.

Fraction of Pectic substances:	*Experiment I*		*Experiment II*	
	(a)	(b)	(a)	(b)
(1) Soluble in water ...	51.6	3.84**	58.5	4.35*
(2) Soluble in 0.5% oxalic acid	45.5	3.39*	61.0	4.54*
(3) Soluble in 0.5% ammonium oxalate ...	54.8	4.08*	62.6	4.66*

A comparison between citrus pectin (B.D.H. 100 grade) and a highly purified preparation of tea pectinic acid from a single clone (clone No. 9) showed that the latter contained a higher percentage of methoxyl groups than the former.

(a) Methyl alcohol expressed as mgs. in 100 gms. dry leaf
(b) Methyl alcohol expressed as % pectinic acid
** Actual determination. *Calculated.

	Methyl alcohol (Moisture and ash-free basis)
B.D.H. Citrus pectin (100 grade) ...	4.28%
Tea pectinic acid from clone No. 9 ...	5.06%

Role of pectinic acids in tea manufacture

The biochemical changes that occur to the pectinic acids in tea during rolling and fermentation have been studied in detail in this laboratory and the results published from time to time (5–8). The presence and role of an esterase (enzyme) controlling these reactions was reported for the first time.

The general course of reaction of this enzyme-substrate system occurring during rolling and fermentation may be represented as follows:—

$$\text{Tea pectinic acid} \xrightarrow[\text{methyl-esterase}]{\text{Pectin-}} \text{Pectinic acid} + \text{Methyl alcohol}$$

The optimum conditions for this reaction are a pH of 6.8 and a temperature of 45°C. The reaction commences as soon as the leaf is rolled and continues during the period of fermentation. During firing, however, the enzyme is completely inactivated and the reaction is thus arrested. This reaction does not occur if the leaf is steamed prior to its rolling and fermentation. The enzymic nature of the production of methyl alcohol during fermentation has been clearly demonstrated.

	Methyl alcohol produced during fermentation (Results expressed as mgs. methyl alcohol from 100 gms. dry leaf).	
	Experiment 1	*Experiment* 2
Green leaf withered, minced and fermented for 2½ hours	39.4	26.8
Green leaf steamed, withered, minced and fermented for 2½ hours	0	0

Methyl alcohol production during fermentation has also been demonstrated in leaf subjected to different types of rolling treatment in the factory; quantities of methyl alcohol varying between 28 and 37 mgs. per 100 gms. dry leaf have been collected during the 4 hour period of fermentation. A combined collection of this methyl alcohol along with other volatile constituents from fermenting leaf (small scale collections made by sweeping the volatiles by a current of air, absorbing in water and extracting with ether) gave a minute quantity of a yellow crystalline material with a very strong and pleasant odour of tea. The quantity of the crystalline material collected was, however, insufficient for identification.

Part of the methyl alcohol formed during fermentation is possibly reacted upon by organic acids or other constituents to form non-volatile esters. Thus increase in this hydrolysable methyl alcohol was found to occur in the fermented leaf when compared with that present in withered leaf. In these experiments free volatile matter was removed by steam distillation before hydrolysis.

	Hydrolysable methyl alcohol expressed as mgs. in 100 gms. dry leaf	
	Experiment 1	*Experiment* 2
Withered leaf	146	215
Fermented leaf (4 hours) ...	179	242

During fermentation, the pectin-pectinmethyl esterase enzyme system does not exert its optimum degree of reaction since the temperature and pH of the

fermenting leaf are very much lower than the optimum conditions necessary for it. The temperature of the leaf coming out of the rollers is about 32°C while that of fermenting leaf is about 27°C. The acidity of the leaf also increases due to the formation of pectic acid, the pH changing from 5.66 in withered leaf to 5.10 in dhool fermented for 4½ hours.

One of the properties of pectic acid is its ability to form a gel under acidic conditions. Pectic acid formed during fermentation is therefore likely to form a gel under the acidic conditions of the fermenting leaf and remain as a thin layer over its surface. This would enable the leaf to retain its twisted appearance. The thin film of the gel may impede the easy diffusion of oxygen to the tea polyphenols and also act as an antioxidant or preservative to the made tea. The inhibition of the oxidation of tea polyphenols has been demonstrated by a number of experiments both with minced leaf (laboratory conditions) and factory rolled leaf. In these experiments minced leaf and rolled leaf were deprived of oxygen for varying periods so that only the reaction between tea pectin and pectin methyl esterase enzyme could take place. The material was then allowed to ferment under normal conditions in the presence of air. During the period of one hour's fermentation, treated samples showed a definite loss of capacity to absorb oxygen. Depending upon the conditions of experimentation, this loss was found to vary from 2 to 12% in the factory rolled leaf and 6 to 48% in the minced leaf. The lower figures obtained in the factory rolled leaf were due to pre-fermentation that occurred before the leaf could be brought to the laboratory and in minced leaf to oxygen (air) which was already held up in the leaf and reacted before it could be evacuated. The higher figures were obtained when the air was continuously swept off by a current of carbon dioxide during evacuation. In another experiment where green leaf was treated with a commercial grade of pectin (2.6%), the loss in oxygen absorption capacity was found to be nearly 40%.

Further proof of the action of pectic acid on the oxidation of tea polyphenols was adduced under practical conditions using the experimental machinery in the factory. Freshly harvested clonal leaf from St. Coombs covered with dew was dusted with different quantities of a commercial pectin, and manufactured under normal conditions. The made tea samples were sent to professional tasters in Colombo. The results are shown below:

Experimental details.	Tasters' report on liquors.
1st Manufacture: Fermentation for 4 hours.	
Control	... Great strength
Green leaf treated with 1.3% pectin	... Light and green
,, ,, 2.6% ,,	... Very thin and green
2nd Manufacture: Fermentation for 3 hours.	
Control	... Good colour and strength
Green leaf treated with 1.5% pectin	... Little greenish
,, ,, 3.0% ,,	... Very green
3rd Manufacture: Fermentation for 2 hours.	
Control	... Good colour and strength
Green leaf treated with 1.5% pectin	... Greenish
,, ,, 3.0% ,,	... Very green

The pectic acid formed was also found to protect the fermenting leaf from too rapid condensation of the oxidised polyphenols. The degree of condensation may be judged from the analysis of made tea for its ethyl acetate soluble fraction. The lower the percentage of this fraction the higher is the degree of condensation (9).

	% dry matter soluble in ethyl acetate	
	Experiment 1	*Experiment* 2
Control	11.26	7.75
Rolled or minced leaf held in absence of oxygen before fermentation ...	12.49	10.21

The property of gel formation was demonstrated in green leaf collected from the factory bulk as well as in a clone which was found to have a very high pectin-methyl esterase enzyme activity. When the leaf was rapidly comminuted with water and incubated at 50°C, the whole mass set into a firm gel in 4 hours. The surface of this mass assumed the characteristic coppery red colour during this period on account of its access to air. The rest of the mass remained perfectly green. Evidently the diffusion of oxygen to the lower layers of the mass was prevented by the gel.

It is probably fortunate that conditions in the leaf during fermentation are not optimum for the activity of the pectin-methyl esterase enzyme; otherwise fermentation might be inhibited before a satisfactory degree of oxidation of the catechins could be achieved. Undoubtedly there is a balance between the activity of the polyphenol oxidase and the rate of formation of pectic acid.

Variation in the pectinic acid content of clonal leaf

The study of the variations in the pectinic acid content of teas is more important than any other fraction on account of its role during fermentation. The usual method of its determination as calcium pectate (Carre and Haynes) (10) was found to be long and tedious. A rapid method was therefore evolved for a rough estimation of pectinic acid. In this method the leaf samples were freed from steam volatile matter, hydrolysed with mild alkali and the methyl alcohol formed was distilled. For oxidation and determination of the methyl alcohol in the distillate, the method of Walkley and Black (11) was adopted after a slight modification. Though the results were found to be quite reliable, the final values obtained were found to give 90% recovery on account of interference from volatile matter from constituents of leaf other than pectinic acid. For purposes of converting methyl alcohol into pectinic acid, it was assumed that tea pectinic acid contained 4.5 per cent methyl alcohol.

A summary of results of recent research on the variations in the pectinic acid content of clonal leaf from St. Coombs under different conditions is indicated below:

1. There is a progressive increase in the pectinic acid content of the flush fractions from bud to 3rd leaf while the stalk contains as much as the 2nd leaf. When the total pectic substances of the flush fractions are taken into account, the pectinic acid fraction appears to show similar increase except in stalk which contains the least percentage.

	Pectinic acid (% dry leaf)	Total pectic substances (9) (% dry leaf)	Pectinic acid as % total pectic substances
Bud ...	1.76	4.9	35.9
1st Leaf ...	2.31	6.1	37.9
2nd Leaf ...	2.38	4.7	50.6
3rd Leaf ...	2.64	5.0	52.8
Stalk ...	2.38	7.6	31.3

2. There is an increase in the pectinic acid content of flush as the age from pruning increases from 9 to 21 months, while it falls to a lower level in bushes 33 months old from pruning.

Age from pruning	Pectinic acid % dry leaf
9 months	1.44
21 months	1.49
33 months	1.02

3. A comparison between high and low quality clonal flush showed that the pectinic acid content of high quality clones was higher than that in poor quality clones.

Sampling period	Clones above Clone No.	average quality Pectinic acid % dry leaf	Clones below Clone No.	average quality Pectinic acid % dry leaf
November, 1957	1294	1.60	407	1.22
February, 1958	2023	1.49	1114	1.02

4. The pectinic acid content of flush appears to increase during dry weather, particularly when the variations in the day and night temperatures are extreme.

Quality of the clones	Difference between maximum and minimum temperature on the previous day to sampling	Pectinic acid % dry leaf
Above average	16°F	1.44
	8°F	0.47
Below average	16°F	1.38
	14°F	1.22

It has been shown that protopectin may be broken down to pectinic acid during withering. But further breakdown of pectinic acid is unlikely since measurable quantities of methyl alcohol were not found to occur during this stage.

Pectic acid in tea

It is not improbable that pectic acid formed during fermentation combines with heavy metals to form the respective insoluble pectates. The water soluble fraction in made tea would thus be less than that in withered leaf. Shaw's results on the pectin content of South Indian teas show a loss of about 17 per cent of the water soluble fraction (expressed as calcium pectate) during manufacture, evidently due to de-methylation of pectinic acid and subsequent combination of pectic acid with metallic ions. Experiments in this laboratory have shown that at least 16 per cent of the methyl alcohol of pectinic acid is hydrolysed and lost to the atmosphere during fermentation. Even though these two results are not directly comparable, they indicate the possibility of pectic acids being rendered insoluble during fermentation.

Summary

1. A partial breakdown of protopectin appears to take place during withering.

2. The methyl alcohol content of tea pectinic acid varies from 4–5 per cent.

3. Pectinic acids are broken down to pectic acid and methyl alcohol during fermentation. The pectic acid formed impedes the oxidation of tea polyphenols and possibly the condensation of oxidised polyphenols.

4. There appears to be some relationship between quality and the pectinic acid content of teas. The high quality teas produced during dry weather conditions may probably be related to the presence of a high percentage of this fraction.

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