PHYSICO-CHEMICAL CHARACTERISTICS OF ORANGE JUICE CLOUD

By SHIMON MIZRAHI and ZEKI BERK

Orange juice is a suspension of heterogeneous particles in a clear serum. The size of the particles varies between 0·05 μm and a few hundred μm. The particles with a size below 2 μm constitute the stable 'cloud'. This fraction consists of needle-like crystals of hesperidin, chromoplastids, amorphous (rag) particles and oil globules attached to some of these particles. The adsorption of oil globules on the rag particles enhances their stability in suspension, by decreasing their density. The hesperidin crystals are formed partly by crystallisation immediately after juice extraction. All the cloud particles exhibit a negative charge, which decreases with decreasing pH. However, it seems that hydration rather than electrical charge is responsible for the stabilisation of the cloud. Heat treatment of the juice causes an increase in the number of fine particles at the expense of coarser ones. In this process some extraction of pectin into the serum also takes place but this has little significance on the cloudiness and cloud stability of the juice.

Introduction

The fine particles of suspended material are responsible for the colour, appearance and much of the flavour of citrus juices. Without these particles known as the 'cloud', orange juice would be little more than a clear, almost colourless sour-sweet liquid of no particular value. Failure to keep the cloud particles in suspension is often the cause of quality loss in orange juice and beverages.

Under certain conditions, the cloud particles undergo flocculation and the suspension is physically decomposed. Cloud-break or clarification of citrus juices and concentrates has been extensively investigated. Work on this subject has been reviewed by Joslyn & Pilnik.[1]

Most investigators have studied cloud problems in connexion with the enzymic degradation of pectic substances. The physico-chemical characteristics of the cloud particles and the exact mechanisms responsible for the stability of the suspension have not been studied until quite recently. Scott *et al.*[2] reported on the chemical composition of the suspended material in orange juice. According to these authors, the fraction containing the finest particles (the cloud) has a composition quite different from that of the other fractions. They suggested that the cloud particles should be regarded as a distinct anatomical component of the fruit and not as small fragments of 'pulp'. A possible interaction between cloud particles and sugar has been postulated by Highby[3] and suggested as an explanation of the process of 'neck cloud loss' in citrus beverages.

The purpose of the work described here was to investigate some properties of the orange juice cloud, in connexion with the physical factors which may have implications for the stability of the suspension.

Experimental

Orange juice

The juice was prepared by hand reaming of Shamuti (Jaffa) oranges and strained through a screen with perforations 0·8 mm dia. The characteristics of the juice were as follows:

Soluble solids, % (Bx°)	11·0
Acidity, % anhydrous citric acid	0·93
pH	3·30
Soluble pectin, % anhydrous galacturonic acid	0·027

Pulp

Pulp is defined as the portion of the suspended particles precipitated by centrifugation at 360 × *g* for 10 min.[4] Pulp volume is the volume of the precipitate as read on the graduation of the centrifugation tube and expressed as percentage of the total juice volume.

Cloud

Cloud is defined as the portion of suspended particles retained in suspension after centrifugation at 360 × *g* for 10 min. The turbidity of this suspension as measured by means of a Klett-Summerson colorimeter equipped with Filter No. 66 is defined as cloud stability or retained cloud. Cloud stability is expressed in bentonite units.[5]

Serum

Serum is defined as the clear supernatant after precipitation of the cloud by centrifugation at 18,400 × *g* for 10 min.

Resuspended cloud

Resuspended cloud was prepared from the cloud particles precipitated as described above. The cloud was washed by repeated suspension in water and reprecipitation, and finally resuspended in the appropriate medium (water, buffer solution, serum etc.).

Electron micrographs

Electron micrographs were taken from carbon film preparation of twice-washed cloud, resuspended in water.

Zeta potential

Zeta potential was computed from the velocity of the particles of resuspended cloud, in an electrical field of known intensity. A Zetameter instrument was used for this purpose. The velocity used for the computations was the average of the velocity of ten particles.

Serum viscosity

Serum viscosity was determined by means of a No. 50 Ostwald-Canon-Fenske capillary viscosimeter, at 30·0°.

Enzymic demethoxylation of pectin

This was carried out by means of a purified pectin esterase (PE) preparation produced from fresh orange pulp.

Pectin content

Pectin content was determined according to the method of McComb & McCready.[6] The method is based on the precipitation of pectin substances by alcohol and subsequent treatment of the precipitate with sulphuric acid and carbazol. The colour developed was read in a Klett-Summerson colorimeter, using a No. 54 filter. The standard curve was established with anhydrous galacturonic acid, analytical grade.

Results and Discussion

Size and shape of the cloud particles

Fig. 1 is a photomicrograph and Fig. 2 is an electron micrograph of cloud particles. Photographs of coarser pulp particles are shown for comparison (Figs 3 & 4). Examination of these figures reveals the existence of four types of particles in the cloud:

Regular, intensely coloured, smooth-surface particles, approximately 1 μm dia. probably chromoplastids.

Irregular, light coloured, rough-surfaced, rag-like particles, 2–10 μm in size, probably fragments of pulp.

Spherical droplets of oil, found almost exclusively attached to the surface of rag-like particles, approximately 1 μm dia.

Needle-like particles 0·5 to 3 μm long, 0·05 to 0·2 μm thick. Some needles reach lengths up to 10 μm and thicknesses up to 0·5 μm. The following experiments were carried out in order to isolate these particles and determine their nature.

A clear serum was prepared from fresh juice by centrifugation of all suspended particles. This serum became turbid after 2 h and its turbidity continued to increase with time during 48 h. The turbidity was found to be retained almost completely after the usual centrifugation procedure used for the determination of cloud stability. Microscopic examination of the turbid serum showed that the suspended material consisted almost exclusively of needle-like particles similar to those observed in the juice cloud. The needles were isolated by centrifugation at 18,400 × *g*. They were found to be insoluble in water but they dissolved in dilute NaOH giving an orange-coloured solution. The needles showed a positive cyanidine reaction, typical of bioflavonoids.[7] The needles were also subjected to the borohydride reduction test typical to flavonones[8] and gave a positive reaction. According to Goren,[9] Jaffa orange juice is saturated with respect to hesperidin, a flavonone. Thus crystallisation of flavonones in orange juice may be expected. However, the quantity of crystals seem to indicate that freshly pressed juice is heavily supersaturated with the bioflavonoid. The turbidity of the serum after 48 h was approximately 2·0 bentonite units. Thus crystallisation of flavonones may be a significant factor in overall juice cloudiness. An increase in cloudiness of orange juice after juice extraction has been reported several times.[10–13] This effect may be due to the precipitation of bioflavonoids.

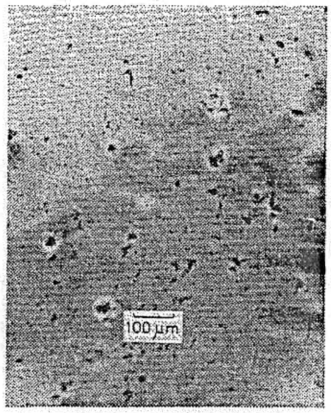


FIG. 1. *Photomicrograph of stable cloud*

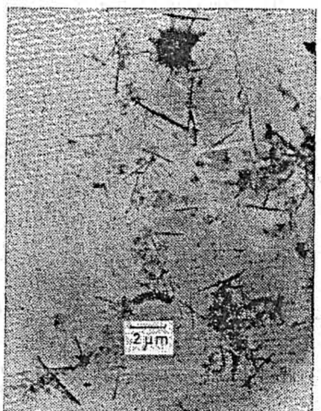


FIG. 2. *Electron micrograph of stable cloud*

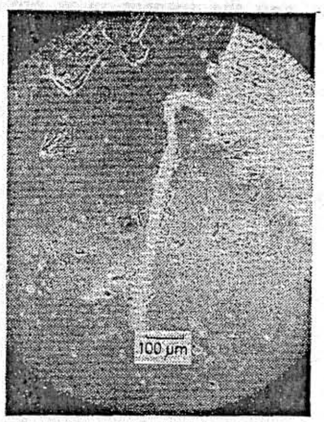
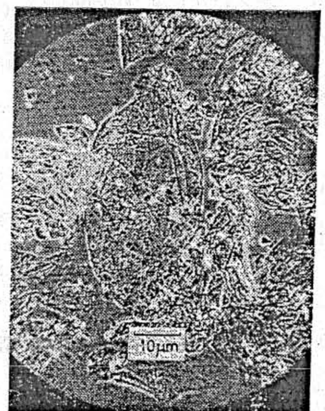


FIG. 3. *Photomicrograph of coarse pulp*



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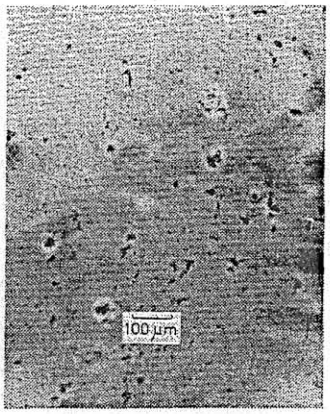

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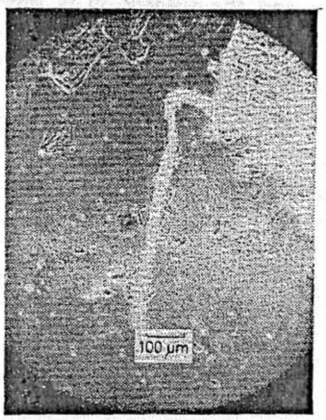

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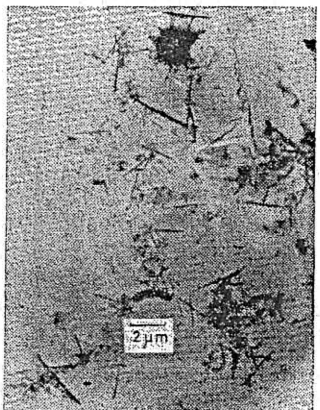

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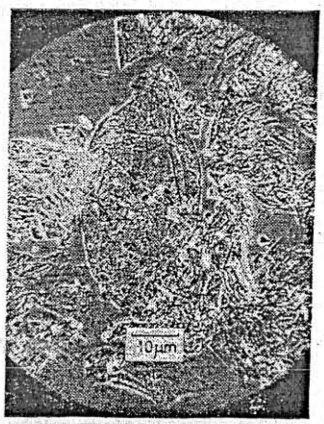

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Role of the oil in cloud stability

The droplets of oil attached to the surface of cloud particles could have a stabilising effect on the suspension by decreasing the average density of the particles and bringing it closer to that of the serum. In order to test this assumption, orange juice was shaken with added essential oil. The stability of the cloud was then determined in the usual way. The results are shown in Table I. Retention was found to increase with increasing amounts of added oil. Very large amounts of oil however, caused complete breakdown of the suspensions by floating most of the cloud material to the surface. This is indicative of the probable mechanism of stabilisation by the oil droplets suggested above.

Effect of heat treatment

The effect of heat treatment on the cloud stability, pulp volume, serum turbidity and serum viscosity is summarised in Table II. Heat treatment seems to enhance cloud stability. Since the experiment was carried out with fresh juice and cloud stability was measured shortly after pressing, this effect cannot be attributed to the inactivation of pectin esterase. According to Loeffler[12] heat treatment not only stabilises the cloud by enzyme inactivation but also enhances the turbidity of the stable cloud. Braverman[14] assumed that the turbidity is increased as a result of extraction of pectic substances into the serum. However, the results in Table II show that the increase in serum turbidity is too small to account for the increase in juice cloudiness. Extraction of pectic substances into the serum may have been the cause of the increase in serum viscosity as a result of that treatment. On the grounds of Stoke's Law the increase in serum viscosity could be responsible for the enhanced cloud stability. In order to establish the effect of serum viscosity alone, one portion of the juice was diluted with water to 7°Bx prior to heat treatment. The cloud stability of this juice was higher that that of the undiluted fresh juice while its serum viscosity was much lower. It can be concluded that the increase in serum viscosity alone cannot explain the stabilising effect of heat. On the other hand, heat treatment may have caused some disintegration of the larger cloud particles. Indeed, while the centrifuged pulp obtained from unheated juice was voluminous and fluffy, that precipitated from heated juice was finer and more compact.

Electrical change of the particles

Zeta potential values of the cloud particles of various pH are given in Table III.

The effect of calcium ion concentration on the stability of a cloud suspension prepared by suspending juice cloud particles in citrate buffer at pH 3·5 is shown in Table V.

Table VI summarises the combined effect of pH and calcium ion concentration on the stability of a cloud suspension which had been subjected to the action of pectin esterase.

All the particles are negatively charged. The charge decreases as the pH is lowered. The site of the negative charge could be carboxyl groups, e.g. from pectic substances.

In the cloud suspension at pH 3·5, the presence of calcium ions up to relatively high concentrations did not affect the cloud stability. Flocculation by calcium ions was observed only after dehydration by addition of ethyl alcohol.

After demethoxylation with PE, the cloud undergoes flocculation at pH 3·5 but not at pH 5·0. Calcium ions did cause clarification at pH 5·0 but not at pH 3·5. However, in freshly extracted juice, cloud retention was found to be practically unaffected by partial neutralisation to pH 5·4 (Table IV), despite the change in zeta potential which takes place as a result of this treatment.

It must be concluded that, with respect to cloud stabilising mechanisms, the stage of hydration of the particles is more important than their electrical charge.

TABLE I
Effect of added essential oil on cloud retention

Added oil, ml/100 ml juice	Retained cloud, Klett units	Pulp volume, %	Remarks
0	485	10	
0·2	550	9·4	
0·4	580	10	
0·6	600	8	
0·8	750	2	Pulp floats
20·0	—	2	Pulp floats

TABLE III
Zeta potential of cloud particles at various pH values

pH	Zeta potential, mV
5·1	−24·9
4·1	−21·7
3·4	− 7·1

TABLE IV
Effect of pH on cloud retention of freshly extracted juice

pH	Retained cloud, bentonite units	Pulp volume, %
Natural (3·5)	1·87	12·2
Partly neutralised (5·4)	1·80	10·2

TABLE II
Effect of heat treatment on cloud retention

Treatment	Retained cloud, bentonite units	Pulp volume, %	Serum turbidity, bentonite units	Relative viscosity of serum*
Unheated fresh juice	1·30	24	0·22	1·445
Heated 30sec at 60°c	2·50	20	0·30	1·515
,, ,, at 75°c	3·20	19	0·37	1·531
,, ,, at 90°c	4·12	15	0·37	1·531
Diluted to 7 Bx° and heated 30sec at 90°c	2·95	—		1·170

* Relative to the viscosity of serum after treatment with pectinase

Table V

Influence of calcium ions on the stability of cloud particles dispersed in citrate buffer, pH 3·5

Ca^{2+} concentration, ppm	Retained cloud, bentonite units*
0	0·50
50	0·47
100	0·50
200	0·47
500	0·65

* Initial turbidity of the suspension was 1·4 bentonite units

Table VI

Influence of calcium ions and pH on the retention of cloud particles subjected to PE action

pH	Ca^{2+} concentration	Retained cloud, bentonite units*
3·5	0	0·15
3·5	500	0·10
5·0	0	0·87
5·0	500	0·17

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