"FROTHING AS A FOOD PROCESSING TECHNIQUE"

A Thesis

Presented to

the Faculty of Food Science and Biotechnology,

Massey University

In Partial Fulfilment

of the Requirements for the Degree of

Master of Food Technology in Food Processing

by

CHAIYUTE THUNPITHAYAKUL

1967

ACKNOWLEDGEMENTS

The author wishes to thank his supervisor, Mr H.A.L. Morris, for his continual enthusiasm in this project. Particularly appreciated were the many long discussions which always stimulated and encouraged.

Sincere thanks are also due to Mr G. Strachan (D.S.I.R., Auckland) for various samples, Dr W.R. Bannatyne and Dr. R.D. Reeves for their helpful advice, and Miss S.E. Quinn for her always willing technical assistance.

Special mention must also be made of Mr G.J.K. Packer for assistance with the photography, and Mrs S. Isarabhakdi for her able typing of this thesis.

CHAIYUTE THUNPITHAYAKUL

TABLE OF CONTENTS

I. SUMMARY

In this work, two major topics have been studied using frothing techniques.

(I) Studies on the possibility of using frothing techniques for recovery of proteins from solutions have been conducted.

Using sodium caseinate protein, frothing studies on various possible factors affecting the enrichment ratio e.g. P^H, concentration, pre-heat treatment, have been carried out. It was found that, to recover protein as soluble protein, is rather more theoretical than practical.

However, further studies on frothing insolubilization techniques (i.e. to insolubilize protein by frothing) have shown that, it was possible to recover up to 65% of egg white albumin from solutions. The key factor governing the recovery efficiency was the stability of the protein prior to frothing operation i.e. the less stable the protein, the greater the recovery. For egg white, the most important single factor in promoting the recovery was the effect of P^H near the isoelectric point.

When the same techniques were applied to cheese whey, no froth precipitation was experienced even after various efforts

to destabilize the whey proteins just preceding the frothing process. A postulate has thus been put forward to explain the results.

(II) Experiments have been carried out to investigate the possibility of using frothing techniques for removing some undesirable substances in citrus juices e.g. excessive essential oil in citrus juice, naringin in grapefruit juice. Studies based on model systems have shown that, while a large proportion of essential oil would come out with the froth, appreciable amount of naringin could be removed only when a suitable surfactant was used. When the frothing techniques were applied to natural orange and grapefruit juices, it was found that, by removing a significant quantity of oil, some flavonoids and possibly some limonin, the flavour of the juices could be improved as confirmed by taste panel results.

(III) In addition, on the basis of the experimental evidence, a tentative theory and proposed mechanisms have been put forward for the removal of undesirable substances in fluid food product using surfactant.

II. INTRODUCTION

Recognition of the phenomenon of surface adsorption dates back to J. Willard Gibbs (29) who first pointed out that the concentration of a solute at the surface of a liquid could be greater than in the bulk of the solution. The thermodynamic relationship expressing this behaviour is usually referred to as the Gibbs adsorption equation. For a dilute solution of concentration C this equation is

$$\Gamma = -\frac{C}{RT}\frac{d\sigma}{dc}$$

where Γ is the excess concentration of solute per square cm of surface, as compared with that in the bulk of the solution, $\frac{d\sigma}{dc}$ is the rate of increase of the surface tension of the solution with the concentration of the solute, R is the gas constant, and T is the absolute temperature. According to this equation then, if $\frac{d\sigma}{dc}$ is positive, Γ will have a negative value, the concentration of the solute will thus be lower in the surface than in the body of the solution. This behaviour known as "negative adsorption" has been observed with some electrolytes. On the other hand, any solute which causes the surface tension of the solvent to decrease i.e. $\frac{d\sigma}{dc}$ is negative, will have a higher

concentration in the surface than in the bulk of the solution, since Γ will be positive. In other words, a substance which decreases the tension at an interface will concentrate at that interface. This is known as "positive adsorption".

As the most common solvent in solutions is water, which has a high surface tension and most solutes reduce its value, hence the great majority of substances are positively adsorbed from aqueous solutions. This phenomenon of positive surface adsorption is the essential basis for foam separation techniques. As most surface active substances e.g. protein, organic salt and acid, alcohol, ester, etc. have the ability to foam. Thus when a solution containing surface active substances is foamed with a gas e.g. N_2, CO_2 or ordinary air, the foam will be richer in these solutes than the residual liquid. This foam can then be collected and condensed to produce a rich liquid product. Foaming, hence, permits the collection of interfacial material with ease in either batch or continuous systems. Successful foam separation is therefore dependent on the nature of the foam produced as well as on the adsorption characteristic of the system (59).

While ample information on theory and other relevant facts about surface adsorption and foam is readily

obtainable from most text books or literature dealing with surface activity (9, 10, 26, 30, 42, 52, 72), information about foam separation theory has been relatively scarce. Shedlovsky (63) has reviewed in detail various methods for fractionation of mixture by foam separation. More recently an excellent review on foam separation, giving comprehensive details of general theory of foam separation, apparatus, application, examples of application together with 135 references, was published by Rubin and Gaden in 1962 (59). They state that, foam separation can best be applied to complex, heat sensitive, and chemically, unstable materials which cannot be readily separated by the common unit operation, distillation and extraction for example. Probably the greatest advantage of the foam separation techniques is its effectiveness at low concentration. Foam separation methods may therefore be used in those regions where other separation methods commonly encounter economical or practical limitations.

As a matter of fact, foam separation techniques have found innumerable applications in the past. Reports of publication can be dated back since the beginning of this century. However, if one tries to divide these reports broadly into two categories i.e. literature dealing with

foam or foam separation in non-food field such as detergent, dye, organic acid and salt, ester, alcohol etc. and those which can be classified as food, one would find that enormous work has been done in the first category, which is beyond our interest in the present research project, hence, it is not intended to go through them here. On the contrary, reports pertaining to foaming of food either for separation or other purposes have been relatively few.

As early as 1934, Barmore (8) reported that egg white foam is more concentrated with a stabilizing agent such as acid and acid salts. However, this report and other similar ones on egg white foam (7, 33, 35) are mainly concerned with either stability or whipping characteristic of egg white foam.

Investigations of beer foam which is vital in brewing industry led to the discovery that protein is the main constituent of beer foam, and on foaming, protein tends to enrich in the foam phase (31, 51).

Also in a study of partially hydrolysed soybean protein foam, Perri and Hazel (55) found that the principal foam active ingredients were metaprotein and the protein fraction, and that the percentage of protein fractions were much higher in foam than in the residual liquid.

These two reports just mentioned, are again, the results of research aiming at improving foaming characteristic of foaming agents, rather than attempts to recover or remove any principal surface active ingredient by means of foaming.

Nevertheless, Ostwald and Siehr (53) described an apparatus and method for producing and separating foam from dilute solution, foam separation had first been successfully carried out with some inorganic compounds e.g. sodium oleate and aluminium stearate, and later they modified the original procedure to permit foam separation in a circulatory system. This new system was claimed to yield much greater concentrations of capillary-active substances. Experiments on potato and sugar beet juice resulted in complete separation of albumin after foaming for 18 minutes with CO_2.

Spengler and Dorbeldtz (65) attempted a full scale purification process of sugar juices by foaming separation techniques. They found protein enrichment in the foam was greater when CO_2 was used than with air. But there was not enough of a separation to make this a useful process to supplant the customary lime - CO_2 juice purification.

Arrazola (4),following a similar line, studied the foaming of fruit juices and saps by introducing of CO_2 as a possible industrial purification method. However, complete success has not been reported.

Frothing procedure has found useful applications in concentration and purification of enzymes (3, 6, 36, 43, 44, 54).

Foam separation has also been applied to fractionation of various proteins extracted from apples (21).

More recently Schnepf and Gaden (60) employed foaming methods to concentrate protein from very dilute aqueous solution of bovine serum albumin. They found that, all the results, at least, qualitatively, were in agreement with Gibbs adsorption equation, bovine serum albumin maximum enrichment ratio was found to increase with decreasing protein concentrations.

From the foregoing review of the literature, it can be seen that the foaming principle, is by no means an idea of recent origin, nevertheless, research work on application of foaming as a processing technique in food industry, apart from the few attempts cited above, has been relatively meagre. Therefore, it was the intention in this present research project to study the possibility of using foaming as a new processing technique, in addition to those conventional

processing unit operations already exist in the food industry or to be used when the conventional methods are not economically or practically feasible.

This study was divided into three broad sections. In the first section, the effort was devoted to study of frothing as a possible process to recover valuable substances from dilute food waste liquor with special reference to protein food.

The second section of experimental work was dedicated to the study of frothing as an unit operation to remove undesirable matters from liquid solutions, with special emphasis on the possible removal of undesirable flavour compounds from citrus juices.

In the third section of the work, based on experimental evidence, a tentative theory was evolved to account for the removal of undesirable substances in fluid food products using surfactants and froth treatment.

III. EXPERIMENTAL WORK

SECTION 1. RECOVERY OF VALUABLE SUBSTANCES FROM FOOD WASTE LIQUOR WITH SPECIAL REFERENCE TO PROTEIN FOOD

PART A.

INTRODUCTION

Of all the foam-active food substances, protein can justifiably be considered one of the most well-known. Many common examples can be illustrated. The ease with which milk froths, on stirring, pumping or during transportation is a technical nuisance in processing of milk or dairy products, and foam destroyers are frequently needed in dairies (17). On the other hand, foaming of beer and milk drinks like milk shakes enhance the palatability of the product. Perhaps, the ability of protein to foam well can best be illustrated by the widespread use of egg white, gelatin and partially hydrolysed soya bean as foaming agents in normal household cookery, confectionery and other food manufacturing purposes.

And throughout the brief survey of research work on foam separation, one can see that a great proportion of work have been concerned with biological substances of protein

nature, either to separate, purify or concentrate protein from dilute aqueous solutions.

However, hitherto, little quantitative work has been attempted to exploit the foaming technique as a means for recovery of protein in commercial practice.

Rubin and Gaden (59) pointed out that, foam separation techniques are particularly efficient with very dilute solutions. They can best be used where other methods are not economically feasible. Foam separation is indeed a very cheap process. In fact, a column fitted with a sintered plate, a foam collector and air are all that is needed in a simple design. It is this cheapness of foaming techniques that initiated the idea whether this method can be used to salvage protein from industrial waste liquors which are normally used as animal feeds or simply poured down the drain. Casein whey, soybean protein whey and cheese whey all fall in this category. This is considered a great waste, especially when millions of less fortunate people in the world to-day are on a protein-deficient diet.

Therefore, it is our hopeful intention to study the possibility of using frothing techniques to recover these

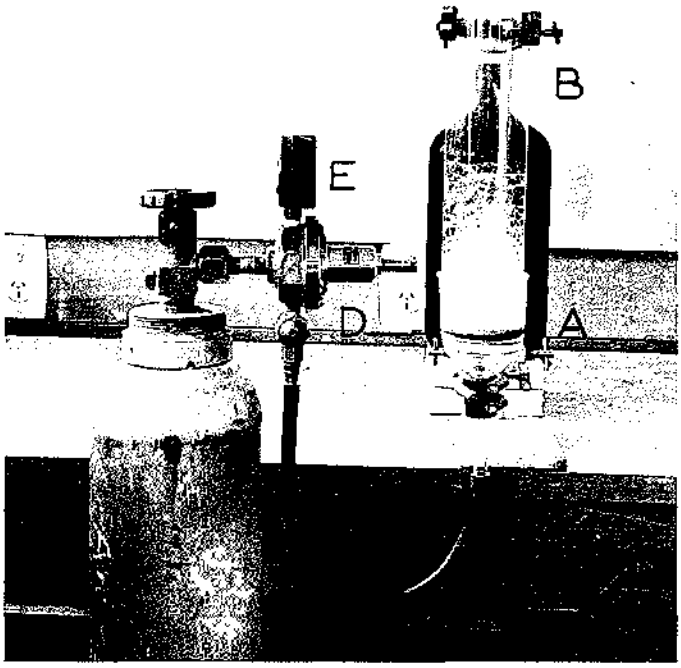


FIGURE 1. Apparatus for frothability tests of various protein solutions.

proteins, so that there may be more protein foods available to meet the demand of the needy world.

PART B. EXAMINATIONS OF FROTHABILITY IN VARIOUS PROTEIN SOLUTIONS

The aim of this experiment was to undertake a preliminary study on the frothability of various protein solutions so as to obtain an approximate idea of what sort of minimum concentration is required to produce durable foams. Three types of proteins available in powder form have been studied. They are sodium caseinate, albumin and lactalbumin.

EXPERIMENTAL

Apparatus

The apparatus adopted consisted of a sintered glass funnel (A) rigidly mounted on a stand as shown in Figure 1. The column (B) was made of rolled plastic with a diameter just suitably fitted on the base of the glass funnel. The glass funnel was then connected to the CO_2 cylinder tube by a rubber tube (C). The gas flow rate could be adjusted and maintained by means of a valve (D) and a pressure gauge (E).

Procedure and Methods

Stock protein solutions containing 0.1% of the above mentioned proteins were prepared initially with distilled water and diluted as needed.

100 ml of protein solution was introduced into the funnel (A) and frothed at approximately 15 psi.

The frothability was judged by the ability to yield foams.

RESULTS AND DISCUSSION

The results are illustrated in tabular form in Table 1.

Table 1: The Effect of Concentration on Frothability

Concentration % (w/v)	Remarks of Frothability		
	Sodium Caseinate	Albumin	Lactalbumin
0.05	Foamed well and gave stable foams.	The same as sodium caseinate.	The same as sodium caseinate.
0.025	Foamed reasonably well.	Foamed well at the initial stage but foaming ability decreased thereafter.	Foamed reasonably well.
0.006	Foaming ability decreased and foams became less stable.	Little foams at the beginning. Foams soon disappeared.	The same as sodium caseinate.
0.001	Visible foams only at the beginning.	The same as sodium caseinate.	The same as sodium caseinate.
0.0001	No foam.	No foam.	No foam.

It can be seen from the above descriptions in Table 1 that frothability of these proteins is equally good, as can be witnessed from their ability to yield froth even when the concentration was as low as 0.001%. However, the stability of these protein solutions as judged by visual

observations, decreased with lower concentrations. For example, at the concentration of 0.001%, although foams still could be seen at the beginning of the run, no stable foam persisted thereafter. The minimum concentration to obtain durable foams in this experiment appears to be at the region of 0.025%.

Perhaps the explanation for the decrease in stability of foam with lower concentrations can be drawn from Freundlich's work (27, 28). He states that, in order to obtain stable foams, in the first place, the surface tension of the liquid must be small, for otherwise its tendency to reduce the surface would be too powerful. A second condition for the production of stable foams is that the vapour pressure shall be small, for substances with high vapour pressure evaporate rapidly. These conditions are fulfilled by aqueous solutions of capillary-active substances, and especially by sols of many colloids, like soaps, saponins, tannins and proteins. Freundlich states that in protein solutions, there are "pellicles" or surface skins on the boundary layer, which tend to prevent evaporation. At least part of these pellicles are the layers of adsorbed surface-denatured protein. If the above statements hold, it is reasonable to deduce that, as the

concentration of protein solutions lowers increasingly, there will be less and less protein available to form surface skins on the boundary layer. The stability is accordingly decreased.

One interesting feature in connection with the frothing of albumin solution is noteworthy here. It was found that, albumin protein is very susceptible to surface denaturation. As the foaming proceeded, visible rings of denatured albumin precipitate could be seen around the wall of the frothing column. This phenomenon might help to clarify the peculiar observations in the frothing of albumin solutions at low concentrations such as 0.025% and 0.006%. The frothability of these solutions was found to decrease considerably as the experiment continued.

This may be explained by the fact that albumin protein was continuously precipitated out of the solution by surface denaturation process. As a result less and less albumin protein was available for foam formation.

The great susceptibility of protein to surface denaturation as exemplified by albumin gave rise to an idea that protein may possibly be recovered in this way i.e. using

frothing process to insolubilize protein in the solutions and collect the denatured protein as precipitated solid. This method will be investigated more fully in later section.

CONCLUSION

From this experiment, it is reasonable to conclude that protein solutions in general, will froth well at concentrations as low as 0.025%. This concentration is much lower than most of the industrial protein waste liquors which range from about 0.3% in soybean whey (64) to approximately 1% in cheese whey (71 (a)). Consequently, upon frothing of these protein waste liquors, durable foams should be readily obtained.

PART C. FUNDAMENTAL STUDIES ON CONCENTRATION OF A TYPICAL PROTEIN SOLUTION BY FROTHING TECHNIQUES

INTRODUCTION

It was the intention of this study to undertake some basic research on concentration of a typical protein from a dilute solution by frothing separation.

In order to obtain an efficient recovery of a protein from waste liquor, that protein must, on adsorption from the bulk liquid, be able to concentrate considerably at the gas-liquid interface. Therefore, if such a protein solution is frothed by means of a suitable gas disperser, foams rich in adsorbed protein are produced.

Many factors are known to affect the performance and efficiency of a foam separation system (59). It was therefore decided that, before going so far as to attempt recovery of real protein solutions, such as cheese whey, casein whey and the like, a rudimentary study on a typical protein solution should be undertaken. For this purpose, sodium caseinate protein was chosen on the grounds that it is comparatively more soluble than other available proteins and consequently a more uniform colloidal solution could be prepared.

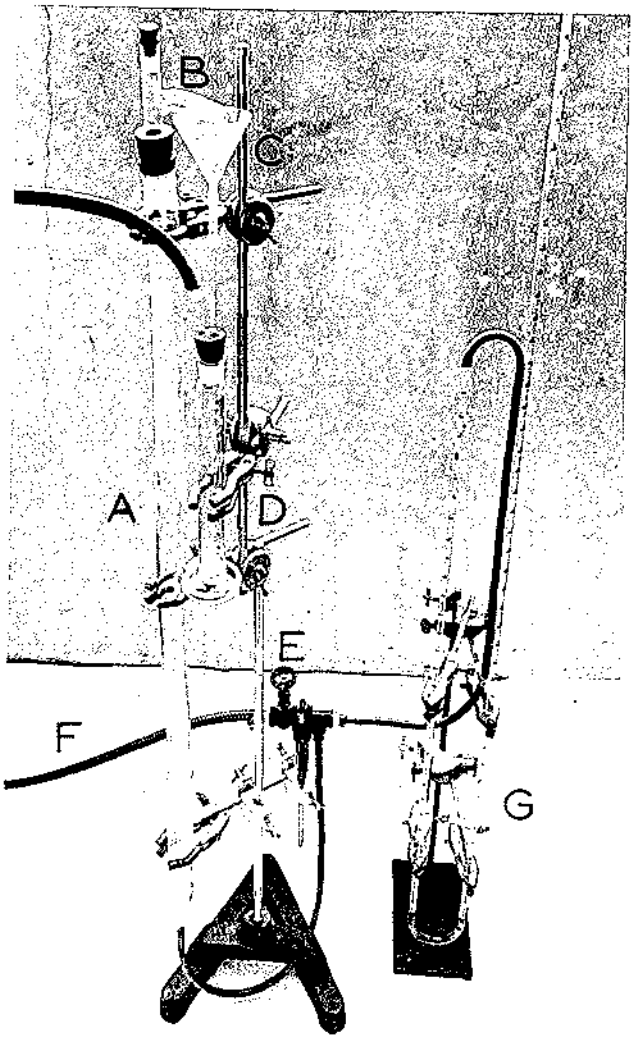


FIGURE 2 - Apparatus for frothing of sodium caseinate solutions.

As our prime effort was to concentrate protein by frothing for recovery purpose, an index useful of indicating the possible extent or degree of separation is necessary. The index adopted in this experiment is termed the "distribution factor" "enrichment ratio" or

$$\frac{\text{concentration of solution in the foam}}{\text{concentration of solution in the bulk liquid}} \text{ (designated by } \frac{C_F}{C_B}) \quad (59)$$

The effect of several factors on efficiency of foam separation has been studied. These are as follows:-

(1) Concentration

(2) p^H

(3) Pre-heat treatment

(4) External additives

EXPERIMENTAL

Apparatus

The apparatus used previously in Part A (see Fig. 1 p 12) was found to be unsatisfactory. Hence, a new type of apparatus was assembled. This is shown in Figure 2.

The frothing chamber (A) consisted of a glass column about 8" high and 1.25" in diameter with a sintered

glass sparger disc at the bottom. A rigid plastic tube of approximately 3 feet long and 1" in diameter was mounted on top of the column. The total column height was hence about 3' 8". This long column was to ensure that sufficient drainage will be obtained so that better concentration can be resulted in foams (1). Leaving the frothing chamber (A), foams then went through a side glass tube (B) before being sucked by vacuum into the receiving funnel (C). The final collapsed liquid was then collected in a graduated foam collector (D). Compressed air was found to be satisfactory and used in the present study. The flow rate of compressed air from the main line (F) was regulated by a valve (E) and could be kept at a constant flow by means of a water manometer (G).

Procedure and Methods

In the experiment, 150 ml of sample solution was fed into the frothing chamber (A). The gas valve was turned on and adjusted so that a slow-rising column of foams was obtained. A gas pressure of approximately 24" water was found to be satisfactory under the present conditions. When the froth reached the top and flowed into the receiving funnel, vacuum was applied. The vacuum sucked and broke the froth until the collapsed liquid reached the foam collector.

The experiment was allowed to proceed until 20 ml of foamate (i.e. liquid from collapsed foams) was collected.

Determination of Sodium Caseinate Concentration in the Solutions

The analytical method adopted was as follows:-

After each run, 10 ml samples of both the foamate and the remaining bulk liquid solution were pipetted into aluminium drying dishes and dried overnight at 100^{o}c in an electrically heated oven. The concentrations of the solutions were then calculated from the resulting total solid contents.

Determination of Stability

An arbitrary method was used here. In the determination, a standard glass funnel was filled up with froth and then inserted in a graduated 10 ml measuring cylinder. The stability was measured in terms of ml of drainage obtained in 5 minutes.

RESULTS AND DISCUSSION

(1) Effect of Concentration on Enrichment Ratio $\left(\frac{C_F}{C_B}\right)$

To determine the concentration effect, experiments were performed with a series of solutions. The concentrations in these solutions ranged from 0.1 to 1%. The P^{H}'s of all the

solutions were approximately 6.8, hence no adjustment was necessary.

Results of the experiments showing the effect of concentration on enrichment ratio together with the stability tests are presented in Table 2.

Table 2

The Effect of Concentration on Enrichment Ratio

Concentration % (w/v)	$\frac{C_F}{C_B}$	Stability ml/5 min.
0.1	2.61	2
0.3	1.59	1.3
0.5	1.38	1.3
0.7	1.29	1.4
1.0	1.12	1.6

It can be seen from the above table that, concentration has a great effect on the enrichment ratio. As the concentrations decreased from 1.0% to 0.1%, the enrichment ratio increased accordingly from merely 1.12

to 2.61. This indicates that foam separation of sodium caseinate is more efficient with low concentration solutions. This finding is very much in agreement with Gibbs' theory (29).

Rubin and Gaden (59), in their review on foam separation, have fully discussed the reasons why frothing is specially effective at low concentrations.

For an ideal solution of one solvent and one surface active solute, simplified Gibbs equation can be written as

$$\frac{\Gamma_A}{X_A} = -\left(\frac{1}{RT}\right)\left(\frac{d\gamma}{dX_A}\right) \qquad \text{——————(2)}$$

where Γ_A is the surface excess

X_A is the concentration in the bulk liquid

γ is the surface tension

R, T are constant and absolute temperature

$\frac{\Gamma_A}{X_A}$ is, therefore, the enrichment ratio. Equation (2) indicates that the distribution factor or enrichment ratio depends on the slope obtained by plotting surface tension versus concentration. For most surface active substances including protein, as concentration decreases the value $\left|\frac{d\gamma}{dX_A}\right|$ would decrease. Hence, $\frac{\Gamma_A}{X_A}$ or enrichment ratio on the left hand side of the equation (2) will increase with decreasing concentrations.

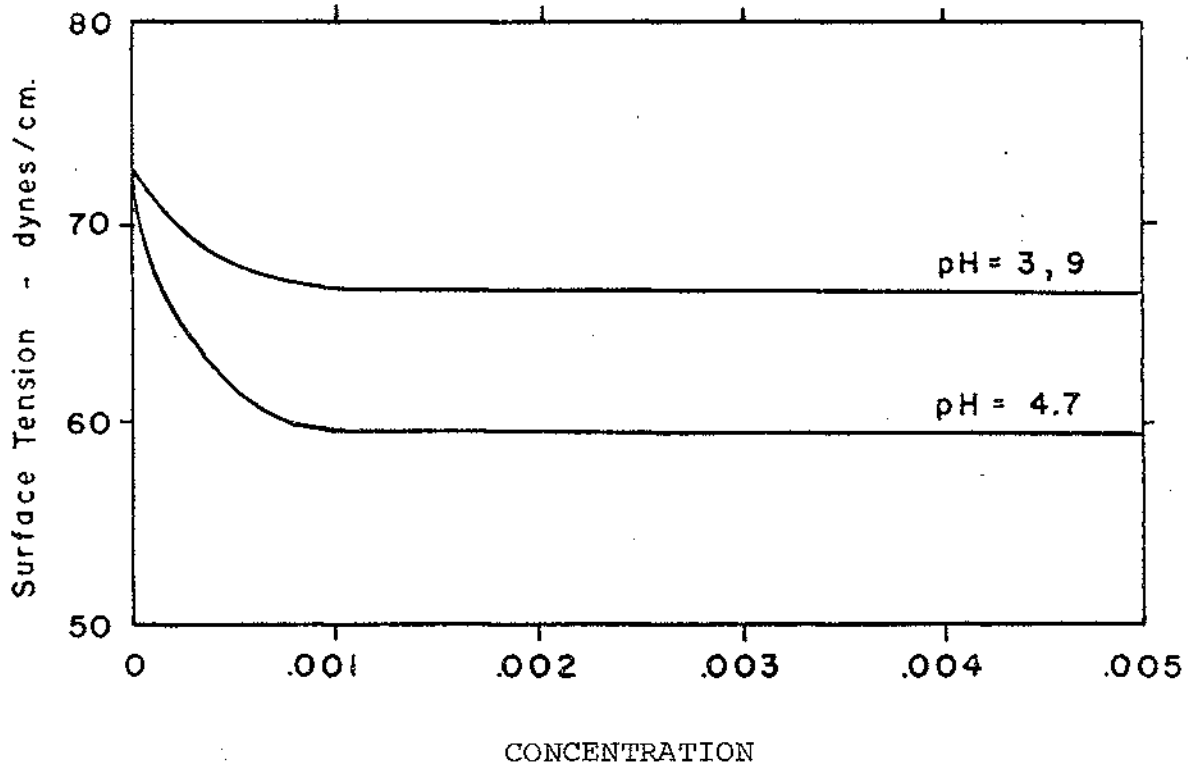


FIGURE 3 - Surface tension - concentration relations for bovine serum albumin.(60)

A typical example can be obtained from Schnepf and Gaden's work on bovine serum albumin (60) and shown in Figure 3.

Figure 3 shows that the slopes of surface tension versus concentration curves increase with decreasing concentration.

However, if we compare the enrichment ratios obtained from this experiment with those of Schnepf and Gaden, it can be readily seen that their enrichment ratios are much higher e.g. enrichment ratio of up to 20 was obtained. This could be attributed to the fact that, in this experiment, concentrations of the solutions were only as low as 0.1%, whereas in Schnepf and Gaden's experiment, most work were done at concentrations between 0.001 to 0.005%. According to Gibbs' theory just discussed above, greater enrichment ratio would be expected with these extremely low concentrations. However, as our main interest is to concentrate protein for recovery purposes, concentrations at such low region are therefore not practicable.

It was also noted from the above results in Table 2 that the stability of the froth appeared to increase from concentration 0.1% up to 0.5%, then fell off slightly from

0.7% to 1.0%. This is in conflict with the fact that, stability of protein normally increases with increasing concentration. The discrepancy of the last two concentrations could possibly be brought about by various causes. For example, slight variation in the volume of froth used in the determination of stability could account for small difference in drainage. Also, small change in gas pressure could result more entrainment liquid in the froth, thus giving wetter froths as possibly indicated by the greater drainage obtained.

(2) Effect of P^H On Enrichment Ratio

For this purpose, four different solutions of about 0.5% were adjusted to P^H 5.7, 6, 6.5 and 7.5 respectively by addition of either citric acid or $NaHCO_3$.

The results are shown in Table 3.

Table 3

The Effect of P^H on Enrichment Ratio

P^H	$\frac{C_F}{C_B}$	Stability ml/5 min.
5.7	1.63	1.7
6.0	1.41	2.5
6.5	1.38	3.3
7.5	1.30	3.7

The data in Table 3 show that, although the enrichment ratios were all fairly low (the maximum being 1.6), they followed a definite pattern. The highest enrichment was found near the isoelectric point which is somewhere near the region of 5.5. When the P^H's tended away from the isoelectric point, the enrichment ratio fell off correspondingly. These results correlate with predictions from Gibbs' adsorption equation (29) and the observations of other workers. Abribat (1) asserted that the most suitable condition for foam fractionation occurred at the isoelectric point of the protein. London, Cohen and Hudson (44) also noticed that the optimum P^H for separation is near the isoelectric point. Schnepf and Gaden (60) contended that, since a protein exhibits minimum solubility at its isoelectric P^H, it is at this point that the protein should have the greatest tendency to collect in regions of the solution where it is least surrounded by the solvent liquid (i.e. at gas-liquid and solid-liquid interface). From this, one would expect maximum enrichment to occur at the isoelectric point. Their work on frothing of bovine serum albumin also confirmed this.

The highest stability was similarly observed at near the isoelectric point. This may be due to the fact that, sodium caseinate protein becomes more and more

susceptible to denaturation as the P^H approached isoelectric point. Upon frothing, the already unstable sodium caseinate molecules were accentuated by surface spreading effect, so that partial denaturation of protein might occur. Therefore, it is reasonable to expect more rigid and stable froths as the isoelectric P^H is approached.

(3) Effect of pre-heat Treatment on Enrichment Ratio

Since the less soluble sodium caseinate near the isoelectric P^H gave rise to more concentration of protein in the froth fraction as discussed in the preceding experiment, it was thus reasoned that, pre-heating the sodium caseinate solution to reduce its solubility prior to frothing, may have a similar bearing in enrichment ratio. Therefore, in the present experiment, two samples of about 0.5% were pre-heated in a hot water bath. They were heated to $65^{\circ}c$ and $75^{\circ}c$ and kept at these temperatures for 15 minutes. The heated samples were then cooled to approximately $23^{\circ}c$ and adjusted to P^H 6 with citric acid.

The experimental results were tabulated and presented in Table 4.

Table 4: The Effect of Pre-heat Treatment on Enrichment Ratio

Pre-heat Treatment ^{o}c/15 min	$\frac{C_F}{C_B}$	Stability ml/5 min
Control	1.36	2.2
$65^{o}c$	1.49	1.8
$85^{o}c$	1.43	1.8

From the figures presented in Table 4, it appears that pre-heat treatment did increase the stability of the froth and at the same time increased the enrichment ratio as expected. However, the increase was still very small, being about 8% in the sample treated at $65^{o}c$ for 15 minutes. Enrichment ratio of a sample pre-heat treated at $85^{o}c$/15 min. seemed to be lower than the sample heat treated at $65^{o}c$/15 min. This may be explained on the assumption that the solution in the $85^{o}c$/15 min. sample had been over-heated despite the fact that no precipitation was noticeable. The property of sodium caseinate protein may have been impaired to some extent. Therefore, the ability of slightly damaged sodium caseinate to be adsorbed from the bulk of the liquid into the air-liquid interface was slightly decreased. However, its enrichment ratio was still higher than the control. From the evidence in this experiment we can thus conclude that pre-heating has

some influence over the solubility, i.e. reducing the solubility of protein so that higher enrichment could be obtained. And it seems that there may be an optimum temperature/time relationship which will give the best enrichment at a particular P^H.

(4) Effect of External Additives on Enrichment Ratio

To examine whether external agent has any influence over enrichment ratio, three additives were chosen. They are, sodium carboxy methyl cellulose (Edifas B), gelatin agar and $CaCl_2$. A small quantity of each additive was added into a 0.5% sodium caesinate solution and mixed thoroughly prior to frothing. The P^H of the solutions were then adjusted to about 6.5 with citric acid or $NaHCO_3$. The tabulated results can be illustrated in Table 5.

Table 5

The Effect of External Additive on Enrichment Ratio

Kind of Additive	$\frac{C_F}{C_B}$	Stability ml/5 min.
Control	1.3	4
Sodium carboxy methyl cellulose	1.22	3
Gelatin agar	1.19	2.2
$CaCl_2$	1.50	2.7

(a) Effect of Sodium Carboxy Methyl Cellulose (Edifas B) and Gelatin Agar - these two additives were considered because it was noticed from the previous experiments that higher enrichment ratios frequently associate with more stable froths. It was therefore wondered whether the enrichment index might be increased if more stable foams were purposely produced. For this purpose, the above foam stabilizers were chosen and investigated. None the less, as the data in Table 5 show, while the stability of froth did increase from 4 ml/5 min. in the control sample to 3 ml/5 min. and 2.2 ml/5 min. in the samples containing Edifas B and gelatin agar respectively, the enrichment ratios did not increase correspondingly. Instead, they were found to be less than the control sample. The most probable explanation to account for this phenomenon is that, the foam stabilizer did not increase the adsorption of sodium caseinate in the froth but only increased the stability of foams because of their viscous property. This increased viscosity in foams will consequently retard the rate of drainage (1). As a result, less concentrated protein foams were obtained.

(b) Effect of $CaCl_2$ - Some polyvalent metal ions are known to have an effect in destabilizing soluble protein. For example, Ca^{++} was found to destabilize caseinate protein in milk (39). This experiment was conducted to study this effect on sodium caseinate protein.

The results show that Ca^{++} increased the stability of froth to some extent as shown by the increase of stability from 4 ml/5 min. to 2.7 ml/5 min. Ca^{++} was also found to increase the enrichment ratio somewhat. However like other factors investigated earlier, the effect of Ca^{++} is still not substantial enough to significantly increase the enrichment index, as shown by the data that the concentration was only 1.5 fold of that in the remaining bulk liquid.

SUMMARY

The main findings from the previous experiments on sodium caseinate solution can be summarised as follows:-

(1) The enrichment ratio increased with decreasing concentration. Foam separation was shown to be most effective with very dilute solutions.

(2) P^H was found to have a great influence upon enrichment ratio which tended to become higher as P^H approached isoelectric point. The best enrichment was observed at P^H 5.7.

(3) Pre-heat treatment prior to frothing increased the enrichment ratio slightly.

(4) Ca^{++} was found to exert positive effect on enrichment ratio, while foams stabilizers like Edifas B and gelatin agar had a negative effect.

DISCUSSION AND CONCLUSION

In the light of the above experimental results, it is apparent that variables such as concentration, P^H, pre-heat treatment, Ca^{++} were found to affect enrichment index to some extent. However, concentration effect seems to be the most significant. The enrichment ratio always found to increase with more dilute protein solution as can be shown by the fact that maximum concentration ratio of 2.6 was obtained with the lowest concentration (0.1%) in this experiment. On the basis of this evidence and reports from other workers (1, 23, 24, 60), it is reasonable to

speculate that, if sodium caseinate protein solutions are diluted even further below 0.1%, higher enrichment ratios could be attained. This effective concentration in very dilute solution may suggest the use of frothing techniques for preliminary concentration of dilute protein waste liquor. However, as mentioned earlier (see Section I, Part B., p 18), most industrial protein wastes are much higher than 0.1%. Therefore, the application of frothing process for preliminary concentration of waste protein liquid is not practical.

The poor adsorption pattern obtained throughout the experiments could possibly be ascribed to the innate characteristic of sodium caseinate solution. For example, the equilibrium enrichment ratio obtainable in the system may be the prime important factor. It was pointed out by Rubin and Gaden (59) that, in contrast to the separation of metal ions, in which equilibrium enrichment ratio can be as high as 100 or more, for most systems containing surface-active substances, the equilibrium enrichment ratio is usually of the order of magnitude of 1-5. This might not be true with very dilute protein solutions in which enrichment ratios could be higher than 5 (60), but it could very well be the case in solutions above a certain concentration such as those concentrations range in this experiment.

Although much further work is needed on frothing of other protein solutions, before a definite conclusion can be drawn, it seems, from the work on sodium caseinate, that concentration of soluble protein solution by frothing separation is rather theoretical than practical.

However as will be seen in the following section, recovery of proteins would seem a practical possibility provided they can be destabilized to a certain optimum extent and permitting precipitation in the froth phase. Egg albumin, for example, can be readily recovered from a colloidal solution under an optimum destabilized condition.

PART D. STUDIES ON RECOVERY OF EGG WHITE PROTEIN BY FROTHING INSOLUBILIZATION PROCESS

INTRODUCTION

Concentration of protein solution as soluble protein has been shown not very practical (see Section I, Part C., p 19). Some effort was therefore, directed to looking for other ways whereby protein may be successfully separated out of the solution. On doing so, it was recalled that during the frothing of albumin solution (see Section I, Part B., p 17), noticeable solid particles were visible on the wall of the frothing column. Actually, these solid particles were denatured egg white protein as a result of egg white protein being adsorbed into the air-liquid interface and stretched enormously as the foams formed. The precipitated protein was then carried up by the ever-rising froth column and finally deposited on the wall.

This phenomenon is by no means a new observation. Even as early as in 1894, Ramsden (56) showed that simple shaking of certain protein solutions brought about a separation of the protein as fibrous or membrano-fibrous solids. In this way it was possible to remove all the protein from egg white solution.

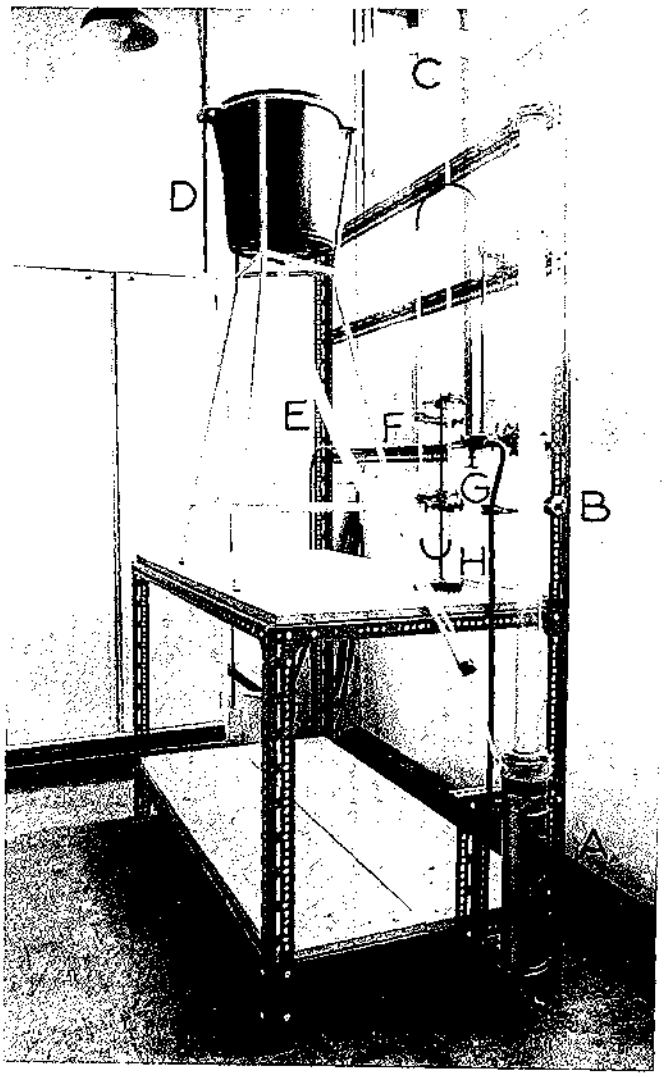


FIGURE 4 - Apparatus for the recovery of egg white from solutions.

On the basis of this fact, therefore, if a receiving system is provided somewhere in the frothing apparatus to collect the insoluble egg white protein, a proportion of protein originally present in the solution may be recovered. This concept led us to investigate the possible recovery of protein by a frothing insolubilization process i.e. using a frothing process to insolubilize the proteins and then recover them in a precipitated form. Since egg white has been shown to be easily denatured by frothing, it was chosen for further investigation in this experiment. The aims of the present project are to find out how efficient can egg white protein be recovered in this way, and what are the likely factors which would accelerate insolubilization upon frothing. It was hoped that, the knowledge gained from studies based on egg white may shed some light upon the plausible recovery of other industrial waste proteins.

EXPERIMENTAL

Apparatus

The apparatus was assembled and built from material available locally. It can be seen in Figure 4. The main frothing chamber (A) consisted of a steel cylindrical

column about 1' 6" in length and 4" in diameter with a metal gas sparger at the bottom. On top of this was a long draining column (B) made of cylindrical glass about 5' in height. When foams reached the top of the draining column, they passed up a curved plastic tube (C) into a receiving bucket (D) from which stable foams or denatured egg white protein were collected. The liquid drained from collapsed froths flowed through a fine metal mesh sitting on the bucket outlet and recycled back into the frothing chamber by a connecting plastic tube (E). In this way, egg white protein solution could be continuously foamed and drained until precipitation by surface denaturation took place. Compressed air for foaming was fed into the frothing chamber from a main compressed air line (F). The gas pressure was regulated by a valve (G) and a constant gas pressure could be maintained by means of a water manometer (H).

Procedure and Methods

Preparation of Egg White Solution - The egg white used in this experiment was either in the form of pan-dried egg powder or liquid egg white from fresh eggs.

Preparation of Egg White Solution from Pan-dried Egg White - A weighed quantity of power was thoroughly mixed with

an appropriate amount of distilled water. When all the suspending powder was well dissolved, the solution was then ready for use.

Preparation of Egg White Solution from Fresh Egg White - Protein in normal egg white is known to be about 10% (19). Based on this figure, a known weight of egg white was thoroughly mixed and stirred with an appropriate volume of distilled water. The resultant solution was then filtered to get rid of the insoluble floc resulting from some minor protein components which are insoluble in distilled H_2O e.g. ovoglobulin (75). The final solution was clear in appearance and ready for use.

Determination of Concentration of Egg White Solution - 10 ml sample was pipetted into an aluminium drying dish and dried overnight in a 100^{o}c oven. The concentration of the solution was then calculated from the total solid content.

In the experiment, compressed air of low pressure was first fed into the egg white solution. This is to prevent liquid seeping through the gas sparger by gravitational force and thus causing a fluctuation in gas pressure. One

litre of egg white solution was then introduced via the opening of the draining glass column (B). Thereafter, the gas pressure was increased gradually until a steadily rising column of froth was obtained. After a few preliminary tries, a gas pressure equivalent to approximately 25" water on the manometer was found to be optimum for this purpose. Hence, all the following experiments were conducted at about 25" water. The frothing operation was allowed to run for 2½ hours. The drained coagulated protein was then collected, placed in small trays and dried overnight in a 100°c oven. The total weight of recovered egg white protein on bone dry basis was subsequently determined and the percentage recovery efficiency (P.R.E.) was expressed as:

$$\text{P.R.E.} = \frac{\text{total weight (b.d.) of egg white recovered}}{\text{total weight (b.d.) of egg white originally present in the solution}}$$

RESULTS AND DISCUSSION

(1) Preliminary Frothing of Egg White Protein Solution

A preliminary run was performed with 0.5% solution prepared from pan-dried egg white. It was shown that upon frothing, very stable foams were produced, as can be seen from Figure 5 that a continuous foam column could be maintained

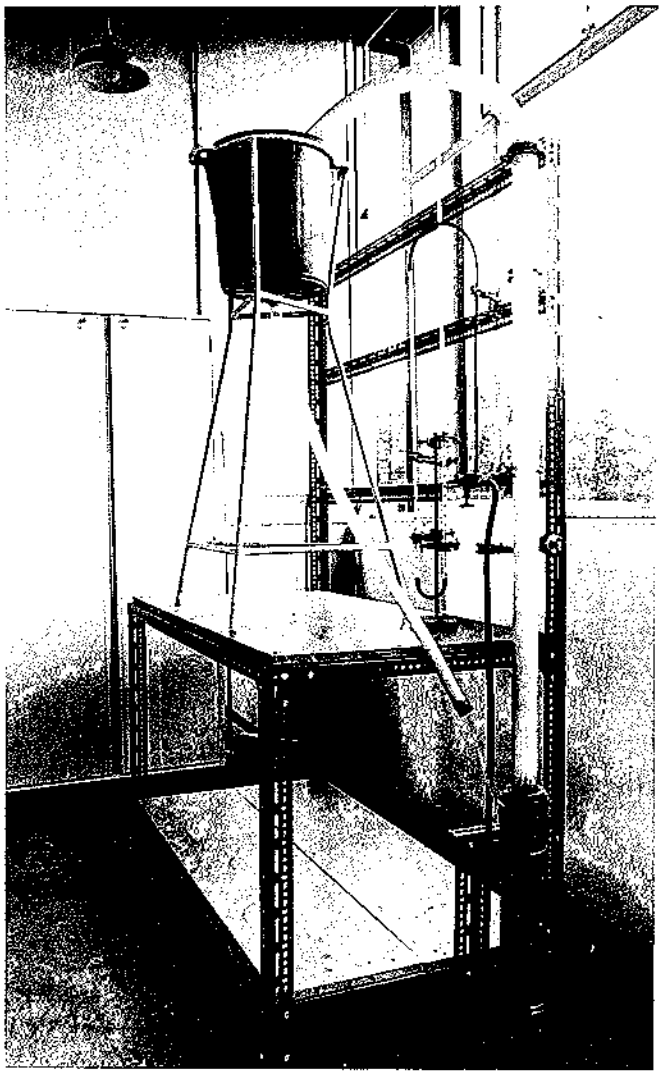


FIGURE 5 - Showing the continuous foam column.

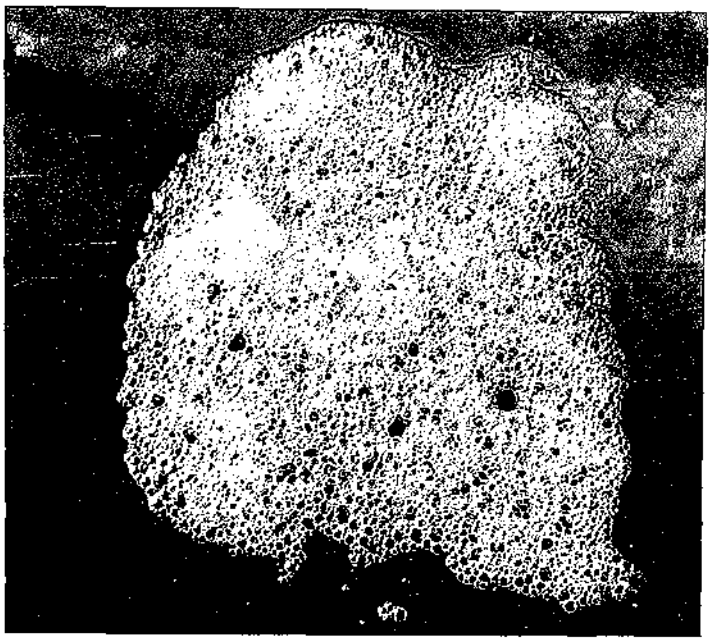


FIGURE 6 - A close-up sample of uncoagulated egg white foam.

FIGURE 7 - Showing the coagulated egg white foam in the receiving bucket.

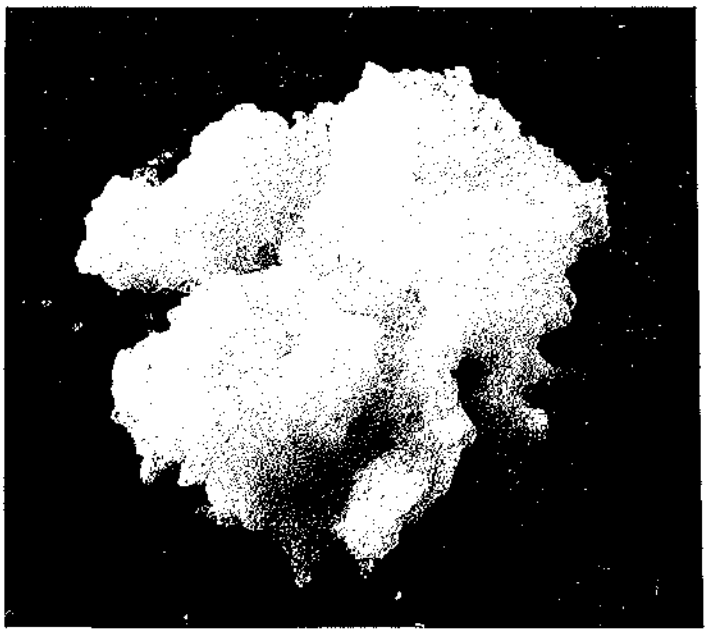


FIGURE 8 - A sample of coagulated egg white foam.

starting from the main frothing chamber until it actually reached the receiver. One interesting feature about the behaviour of the froth is worth mentioning here. At the beginning of the run, although froths emerging from the delivery tube (C) were quite stable, no visible coagulation of froth was taken place. These froths eventually collapsed and returned to the frothing chamber. A sample of froth taken at the initial stage is shown in Figure 6. Nevertheless, as the operation proceeded, probably due to the increasing degree of denaturation, the outcoming froths became more and more rigid until flocculation occurred. The resulting coagulated froths would then stay in the receiving bucket (D) (see Figure 7) and a close-up sample of the coagulated egg white foams can be seen in Figure 8. The accumulation of coagulated froths went on fairly consistently except that towards the end of the experiment, comparatively larger and less stable foams were obtained probably, due to the gradual depletion of egg white protein in the bulk solution.

Total solid tests shows that, when sufficient drainage time was allowed after frothing, the concentration of recovered egg white froth could be up to 6 - 7%.

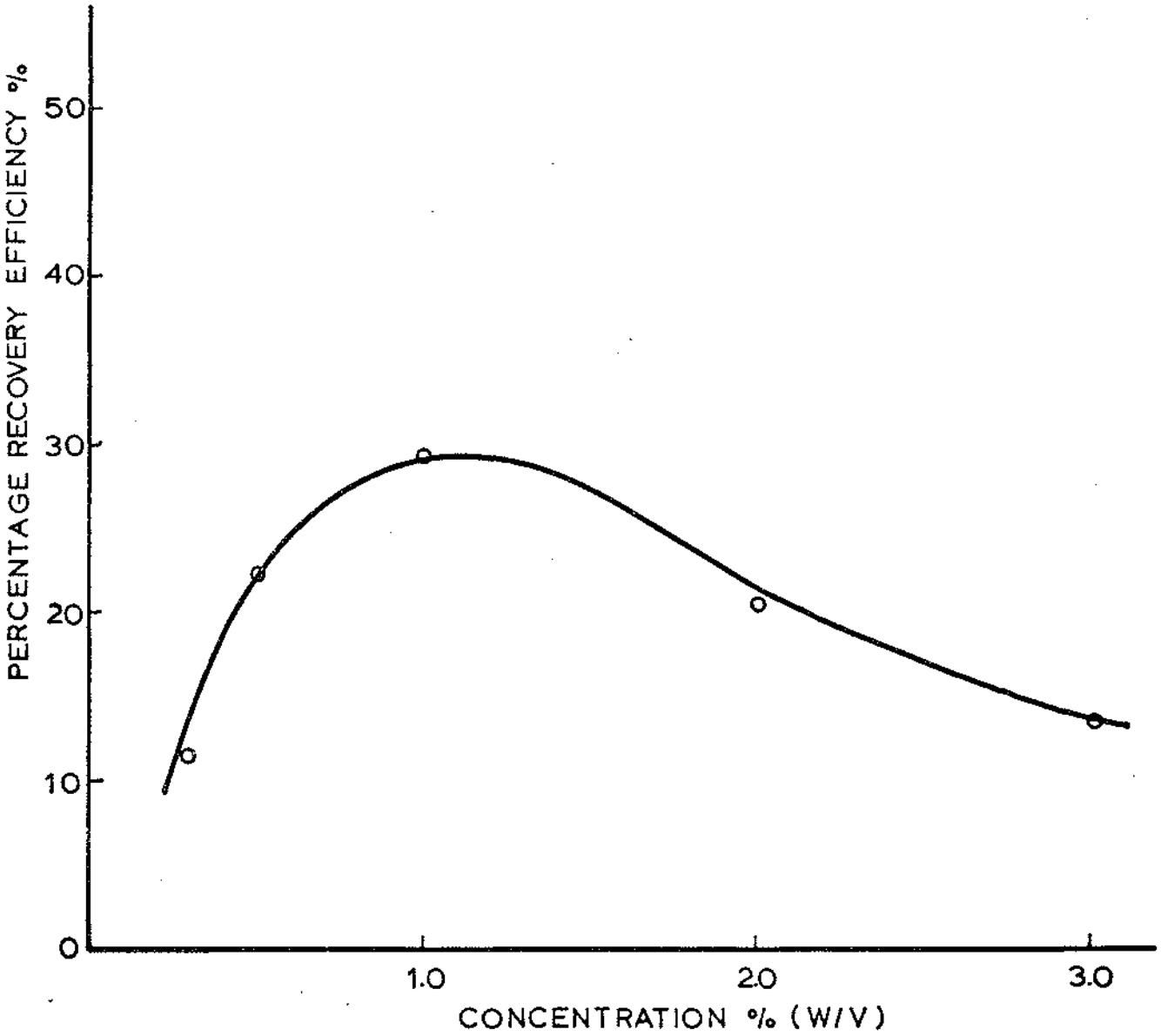


FIGURE 9 Effect of concentration on Percentage Recovery Efficiency of egg white solution.

(2) Effect of Variables on Percentage Recovery Efficiency (P.R.E.)

(a) Effect of Concentration of Solution on Percentage Recovery Efficiency - Experiments were made with solutions of different concentrations viz 0.3, 0.5, 1, 2 and 3% prepared from pan-dried egg white. P^H's of these solutions after preparation were found to be fairly constant at approximately 7.8. Therefore,no adjustment of P^H was necessary.

The relationship between the concentration of the solution and Percentage Recovery Efficiency in graphical form is shown in Figure 9.

It can be seen from Figure 9 that P.R.E. increased from merely 11.6% at 0.3% to a maximum of 29.5% at 1% and then fell off with solutions of higher concentrations.

These results, ostensibly do not follow Gibbs' adsorption equation (29) which predicts that greater separation efficiency will be obtained with more and more dilute solutions as confirmed by earlier experiments on sodium caseinate (see Section I, Part C, p 23). However, attention must be drawn to the fact that there is a striking difference between the present system and that of sodium caseinate. In sodium caseinate solution, the enrichment ratio will be primarily

dependent upon the ability of sodium caseinate protein to decrease the surface tension of the solution or in other words, dependent on the amount of sodium caseinate molecules present in the air-liquid interface of the froth, if everything else is constant. Nevertheless, the recovery of egg white in this experiment is probably dependent on the degree of susceptibility to surface denaturation of egg white protein. For example, if egg white in one solution is more prone to surface coagulation than the other, greater recovery may be expected in the former solution in a given time.

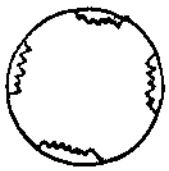
According to the graph in Figure 9, the optimum concentration for maximum surface coagulation to take place is 1%. This phenomenon is however, contradictory to the work of Wu and Ling (74). They studied surface denaturation of egg white albumin by shaking solutions of egg white albumin in glass bottles. They reported that the rate of surface coagulation was independent of time and concentration, but strongly influenced by the rate of shaking and by the size of the bottles. Since in this experiment, apart from concentration, every other variable such as air pressure, pore size of gas sparger, volume of sample used are approximately constant. Therefore, no satisfactory theory could be put forward to explain why the recovery is maximum at 1%, if

Wu and Ling's findings are valid. On the other hand, the experimental procedures in this system and those of Wu and Ling are not quite the same. One salient difference is that, in the present system, there is a recycling process whereby collapsed liquid from uncoagulated froths could be returned to the frothing chamber and refrothed until surface coagulation eventually takes place. As a result, results obtained from these two systems are probably not comparable. Examining further the results of this particular study, attempt has been made to explain why at concentration of 1% is optimum for maximum recovery efficiency of the protein. It is thought that the key factor in efficiency of recovery with a standard process time will involve making use of the fullest surface area available for denaturation. At 1% concentration, it is postulated that the total surface is filled with protein molecules and so throughout a process time a maximum rate of denaturation is obtained. In solutions more dilute than 1%, less efficent use of the total surface available, hence the rate of denaturation achieved is less, and after a given process time, a small percentage protein will be recovered. In solutions more concentrated than 1%, more protein is available than the surface can accommodate and this along the line of the above approach, leads to a reduced efficiency and a smaller percentage recovery after a given time of frothing.

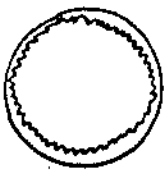
represents air-liquid surface available in the froth.

represents stretched protein molecules spread on the surface.

represents unstretched protein molecules.



(a) In dilute egg white solutions such as at concentrations of 0.3 and 0.5%, a relatively small number of protein molecules are available for surface spreading relative to the surface available, hence only a small amount of protein is denatured in a standard process time.



(b) At an optimum concentration e.g. 1%, it is thought that the air-liquid surfaces are fully occupied by protein. Thus protein denaturation is at its maximum rate and hence a maximum recovery is achieved in a standard process time.

(c) At higher concentrations e.g. 2, 3%, there are more protein molecules than air-liquid surface can accommodate as illustrat by undenatured protein molecules underneath the surface layer. Therefore, total proteins stretched and coagulated are similar to those at 1% solution, but the total recovery in a standard process time will be less than that at 1% concentration.

FIGURE 10. Diagrams showing the possible effect of concentration on Percentage Recovery Efficiency of egg white solution.

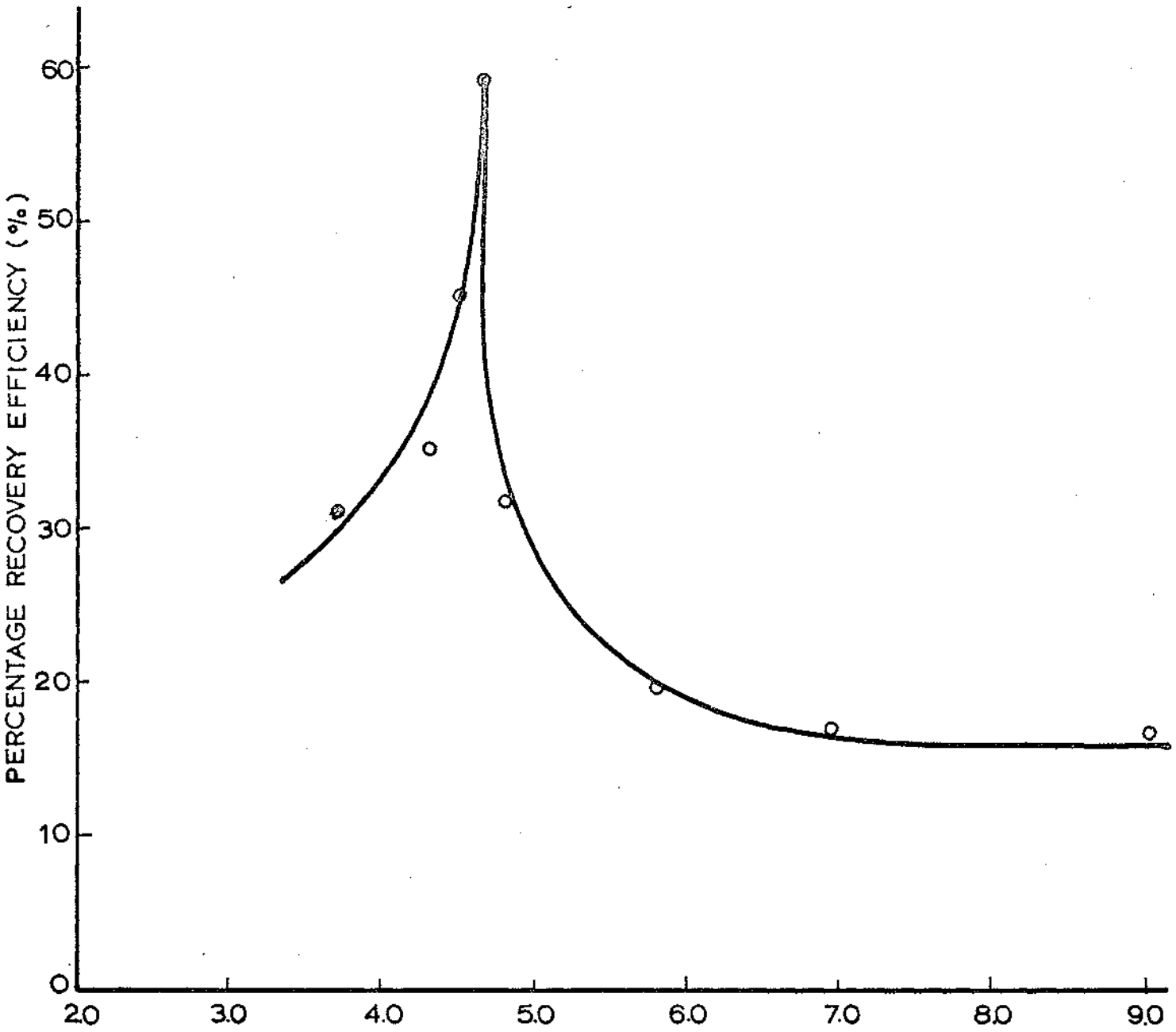


FIGURE 11 Effect of pH on Percentage Recovery Efficiency of egg white solution.

It can be seen, therefore that concentration is primarily a rate influencing factor and a 1% concentration is the optimum for the particular process time used in this work (2½ hours). On this basis therefore, larger process times with both concentrations of less than 1% or more than 1%, would be expected to achieved ultimate percentage recoveries similar to the optimum.

Diagramatic representation of the mechanisms involved in the rate efficiency of protein recovery through denaturation is shown in Figure 10.

(b) Effect of P^H on Percentage Recovery Efficiency - In this experiment, samples of about 1% egg white solutions prepared from fresh egg white were adjusted to an initial P^H ranging from 3.7 to 9.0 by addition of citric acid or $NaHCO_3$.

Results were plotted as P.R.E. versus P^H as illustrated in Figure 11.

From the results shown in Figure 11, it is obvious that P^H has a pronounced effect on P.R.E. of egg white protein. The recovery ranged from as low as 17% at P^H 9 to a maximum of 59% at P^H 4.65. Apparently, P^H 4.65 is near the

isoelectric point of ovalbumin, a water soluble protein which constitutes 75% of the total egg white protein (58). Longsworth et al (45) have also shown that the isoelectric point of ovalbumin lies in the range of 4.6 to 4.8, and appears to vary with the ionic strength of the buffer and the valency of the cations present. It is therefore fair to say that the actual isoelectric point of ovalbumin in this experiment is in the vicinity of 4.6.

It can be seen from the shape of the curve in Figure 11 that when P^H came towards the isoelectric point from both sides, the P.R.E. increased. The increase was at first fairly small as indicated by a slight increase when the P^H decreased from 9 to 5.8. However, as the P^H approached the isoelectric point, P.R.E. started to rise sharply and finally reached a peak at P^H 4.65.

The above results are by no means unexpected and could possibly be explained in terms of the stability of protein. It is well recognised that stability of protein is very P^H-dependent and is least at isoelectric P^H. Therefore, the stability of egg white protein was already different in these various solutions due to the differences in their initial P^H's and therefore, the nearer the P^H of the solution was to

the isoelectric point, the less stable it would be. Prior to frothing, all the components of egg white protein apparently were still in soluble form as no noticeable precipitate was noted in all samples apart from trace of precipitate at P^H 4.65. However, when frothing was initiated, surface effects came into play, further reducing the stability until surface coagulation took place. Undoubtedly, those solutions with P^H near the isoelectric P^H will tend to denature more easily, and consequently higher P.R.E. was obtained near the isoelectric point.

(c) Effect of Pre-heat Treatment on Percentage Recovery Efficiency - Just as P^H is renowned for its influence on stability of protein, heat treatment has long been regarded as a principal method by which precipitation or denaturation of most proteins can be effected. The main purpose of this experiment is to see if destabilization of egg white protein by heat treatment just preceding frothing operation would be as effective as P^H in increasing the P.R.E. To test out this idea, two series of experiments were carried out with approximately 1% egg white solution prepared from fresh egg white. In the first series, 3 samples were heated to $55^{\circ}c$, $60^{\circ}c$ and $70^{\circ}c$ and held at these temperatures for 5 minutes respectively, in a hot water bath. The heated samples were then cooled

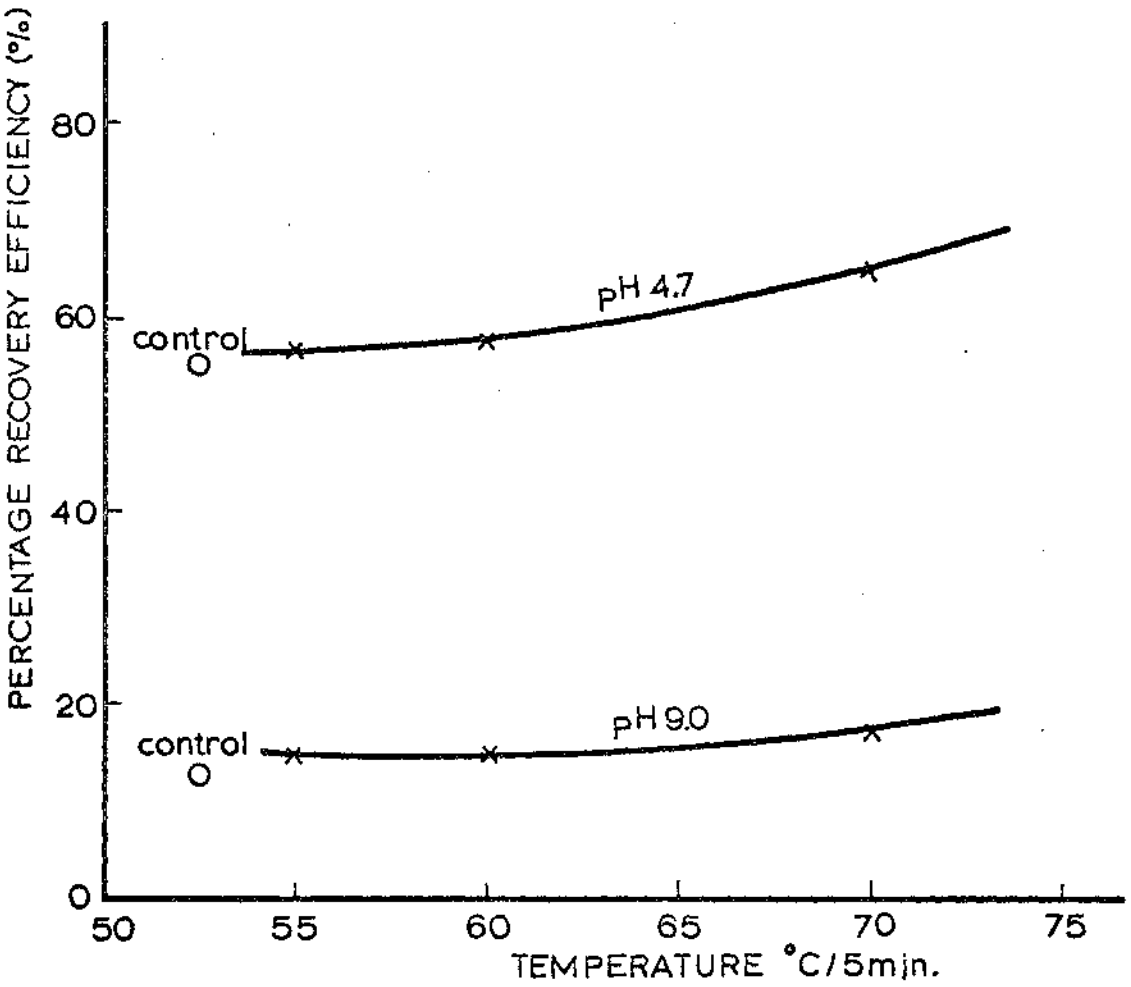


FIGURE12. Effect of Preheat treatment on Percentage Recovery Efficiency of egg white solution.

immediately in cold water to about 23°c. After heat treatment, pH's of the solutions were found to be unchanged and remained at about pH 9. Therefore no pH adjustment was needed.

The same procedure was repeated in the second series except that after heating and cooling, the samples were adjusted to pH 4.7 by citric acid.

In addition, an experiment using a control sample without heat treatment was carried out in each series.

Although undiluted egg white is known to coagulate around 60°c (46), egg white protein in the above heat treated samples seemed to be still very stable. Apart from a trace quantity of precipitate in 70°c/5 min. heat treated samples at pH 4.7, no coagulation was noticeable in all the other solutions.

Results were plotted as P.R.E. versus °c temperature/ 5 mins. as shown in Figure 12. The P.R.E. of the control solutions at pH 4.7 and 9.0 were found to be 56% and 13% respectively. To facilitate comparison, they were shown alongside the curves in Figure 12.

Figure 12 shows that the pre-heat treatment has some effect on P.R.E. of egg white protein. P.R.E. of samples with heat treatment were all higher than the control samples

at both P^H's. However, from these two curves presented, it appears that the effect of pre-heat treatment is comparatively small when compared to that of P^H. Perhaps, this statement needs further elaboration. If one considers the lower curve, it can be seen that the P.R.E. increased from 13% in the control to a maximum of 17.8% in the sample pre-heated at 70°c for 5 minutes, the most drastic heat treatment attempted in this experiment. These figures clearly indicate that the increase of P.R.E. is fairly small. Furthermore, the increase must be due to the effect of pre-heat treatment alone. Why? This is because P^H's of all the egg white solutions used in this case were about 9, a P^H which is quite a distance away from the isoelectric point of ovalbumin (approx. 4.6). Therefore, stability of egg white protein could hardly be affected by such a high P^H. Any destabilization of protein and hence subsequent increase in P.R.E. from the control solution must be due to the effect of pre-heat treatment.

The above arguments could be further substantiated by the upper curve in which the operating P^H was close to the isoelectric point of ovalbumin. It can be seen that while the P.R.E. only increased from 57% at 55°c/5 minutes to

about 65% at 70°c/5 mins., the control samples had jumped from nearly 13% at P^H 9 to 56% at P^H 4.7, an enormous increase clearly attributable to the change of P^H. The P^H ranges from P^H 9.0 where egg white protein is in its most soluble form to P^H 4.7 where egg white protein is in its least soluble form.

The above evidence is ample to give a conclusive finding that, although pre-heat treatment increase P.R.E. slightly, P^H is still a predominant factor governing the extent of possible recovery of egg white protein from the solution.

SUMMARY

The following points can be summarised from all the above experiments:-

(1) Concentration has been found to exert considerable influence on P.R.E. The optimum concentration found so far has been 1%.

(2) P^H has the most significant effect on P.R.E., the nearer the isoelectric point, the greater is the recovery. The maximum recovery was found to be 59% at P^H 4.65.

(3) Pre-heat treatment by itself has little effect on P.R.E. But pre-heat treatment carried out in conjunction with adjustment of P^H to near isoelectric point of egg white protein could yield P.R.E. as high as 65%.

CONCLUSION

The recovery of protein from solution by frothing insolubilization techniques has been proved feasible for egg white protein. With appropriate pre-frothing manipulations, recovery could be as high as 65%.

The key factor determining the possible recovery was the stability of the soluble protein just prior to the frothing operation. Any process which would promote destabilization of the protein would consequently increase the recovery efficiency.

PART E. ATTEMPT TO RECOVER INDUSTRIAL WASTE LIQUOR BY FROTHING INSOLUBILIZATION PROCESS WITH SPECIAL REGARD TO CHEESE WHEY

INTRODUCTION

In New Zealand, chiefly due to economic reasons, production of protein from wheys is usually not a commercial practice. Consequently, dairy wheys such as cheese and rennet casein whey, are normally used for pig-feeding, pasture irrigation, or if circumstances allow, simply disposed of as liquid waste. Frothing techniques are simple and impose little cost problems, thus, if proved successful, they may justifiably be used to save these valuable proteins for better usage e.g. as supplimentary diet in protein-deficient countries. Since, frothing insolubilization has shown promising results for egg white protein, it was therefore the main objective of this study to see whether this method can be similarly exploited for recovery of cheese whey protein.

EXPERIMENTAL

Apparatus - was the same as described in Figure 4 (see Section I, Part D, p 40).

Procedure and Methods

Experiments were primarily carried out with Gouda cheese whey available from nearby Dairy Research Institute factory. The whey had previously been separated in the factory to a very low level of fat (approx. 0.08%) so that it would not reduce the frothability of the whey proteins (25).

One-litre sample was then introduced in the frothing chamber and frothed with an approximately constant pressure at 26" water on the manometer.

The experiments were carried out under two main headings: (1) Frothing of whey as is

(2) Frothing of whey with pre-frothing treatment

(a) P^H adjustment

(b) Pre-heat treatment

(c) Miscellaneous

(1) Frothing of Whey as is:- In this experiment, Gouda cheese whey with an original P^H 6.5 was frothed and found to give reasonably stable froth. The leading froth column rose slowly without any interruption. When froths reached the receiving bucket, they tended to decay fairly quickly and the collapsed liquid returned to the frothing chamber and

subjected to further frothing. However, no precipitation or coagulated froths were noted even when the frothing operation was allowed to continue for an hour.

(2) Frothing of Whey with Pre-frothing Treatment

(a) P^H adjustment

The isoelectric points of the major whey proteins i.e. lactalbumin and lactoglobulin are in the region of 4.1 - 4.8 and 5.18 respectively (39). Therefore,Gouda cheese whey samples were adjusted to P^H 4, 4.5, 5, 5.5 and 6.5 (the original P^H) respectively by addition of citric acid.

When these P^H - adjusted wheys were being foamed, it was observed that there was no appreciable difference in the frothability, neither was there any precipitation or coagulation of froths visible in the receiving bucket at all P^H's after prolonged period of frothing.

(b) Heat Treatment plus P^H Adjustment

It is known that if milk is heated, lactalbumin and lactoglobulin are changed so that they precipitate by acidification at about P^H 4.6 (39). Thus, it was thought that may-be whey protein in cheese whey can be destabilized in a similar way. Therefore, three samples of Gouda cheese

whey were at first adjusted to P^H 4.7 and then heated to 60°c, 65°c and 70°c - and maintained at that temperature for 5 minutes respectively. The samples were then removed from the water bath and cooled in water to about 23°c. This combination of P^H adjustment and heat treatment aims not to actually precipitate out the whey proteins but just to destabilise the dispersing proteins considerably so that on subsequent frothing process they may become less stable and precipitate out.

Trace amounts of precipitation was observed in all the samples before they were frothed. This suggests that a small percentage of whey proteins had already been precipitated by the heat treatment.

However, although frothability of all three samples were as good as the control, once again no coagulated froths or precipitate were obtained after a period of one hour.

(c) Miscellaneous

In addition to those previous experiments in pre-frothing treatment, many other attempts have been made to destabilize whey proteins.

(i) Two lots of Gouda cheese whey were heat treated at 50, 55, 65 and 70°c for 5 minutes one at P^H 5 and the other at P^H 5.5 respectively.

(ii) Experiments were also carried out with other types of cheese whey viz. Mozzarella and Cheddar cheese whey, using same pre-frothing manipulations as described in (a) and (b).

No evidence of froth precipitation were noticed in all the above treated samples.

DISCUSSION OF RESULTS

From all the experiments, it is readily seen that, the behaviour of whey proteins is different from that of egg white. While egg white readily gives coagulated froth, whey proteins, on the other hand, do not show any sign of doing so in spite of all the efforts to destabilize the whey proteins prior to foaming. The failure of the major whey proteins i.e. lactalbumin to precipitate or coagulate was rather surprising, since it is in contrast to the findings of an earlier worker. Ramsden (56), in an experiment involving foaming of lactalbumin and various other proteins by shaking, found that insoluble precipitates of these proteins are formed. Clearly, frothing either by blowing air through the protein solution as in this experiment or simply by shaking as in Ramsden's is analogous to one another on theoretical considerations. Therefore, the contradictory results were

possibly not due to the difference in experimental procedure, but some other genuine causes.

It should be noted that in Ramsden's experiment, the only protein in the solution was lactalbumin itself, but in cheese whey, apart from two major proteins, lactalbumin and lactoglobulin, there are other minor proteins, a proportion of lactose and various minerals. Thus on the basis of the experimental evidence and the compositional difference in the solution just pointed out, it could be said that, lactalbumin by itself is coagulable by frothing, but in the presence of other proteins and other components present in whey, it has no tendency to do so. This has led to a postulate that, there may exist between lactalbumin and other proteins, possibly lactoglobulin, a mutual stabilizing effect which would enhance the resistance of whey proteins against flocculation on frothing.

In the case of whey as is, whey proteins, evidently, were undenatured and retained their good stability. Upon frothing, it was certain that, whey proteins were continuously being adsorbed and spread on the air-liquid interface. However, it is apparent that, whey proteins in their native state were just too stable for coagulation to take place.

Nevertheless in the case of whey proteins which had been subject to pre-heat treatment such as those pre-heated at pH 4.6, denaturation must have occurred to some extent, as indexed by the trace precipitate in the solutions after heat treatment, and yet, at no stage was there any sign of froth coagulation upon frothing.

Perhaps, it is worth pointing out herein that denaturation and coagulation of protein are two distinctive phenomena. Denaturation generally, denotes the unfolding of polypeptide chains of protein from its coiled native structure to the much more randomly arranged form. Coagulation or flocculation, on the other hand, refers to the aggregation of denatured protein molecules, bringing about precipitation of proteins. While in some instances, protein may undergo denaturation and flocculation almost at the same time e.g. egg white albumin, in other instance only denaturation takes place. Likewise, the same reasoning could be applicable in this experiment. Although, some denaturation of lactalbumin or lactoglobulin had occurred, it appears that the mutual stabilizing effect was not yet impaired and continued to exert its influence on these whey proteins. As a result, even a prolonged frothing was not capable of disrupting this stabilizing force and bringing about flocculation.

However, it is a well recognised fact that, if cheese whey is subject to a continuous heating process, serum proteins would eventually precipitate at an appropriate time-temperature relationship. This means that this stabilizing force between lactalbumin and lactoglobulin may be destroyed under suitable treatment conditions. Therefore, if one is able to destabilize whey proteins to a point just preceding the flocculation point by some suitable chemical on physical means, one may be able to bring about flocculation on frothing process. Perhaps, all the various efforts attempted so far have not yet reached this critical point or it could well be that this pre-flocculation point is so narrow that it is not possible to attain before the actual coagulation takes place.

CONCLUSION

From the experiments attempted thus far, it has not been able to bring about precipitation of cheese whey proteins in spite of various effort to destabilize the whey proteins prior to frothing.

SECTION II. REMOVAL OF UNDESIRABLE SUBSTANCES BY FROTHING TECHNIQUES WITH SPECIAL EMPHASIS ON UNDESIRABLE FLAVOUR COMPONENTS IN CITRUS JUICES

PART A.

INTRODUCTION

It is apparent, from the literature, very little use has been made of the frothing techniques as a means to remove undesirable substances from fluid food products.

Thus far, few recorded attempts have mainly been made in the sugar industry.

Meyer (48) and later Spengler and Dorbeldtz (65) attempted to purify the raw sugar juice by foaming techniques. However, complete success has not been reported.

In this section, the intention was to study the possibility of utilizing a frothing process as an unit operation to remove unwanted substances from citrus juices. It was hoped that, by doing so, the overall flavour quality of the juice could be improved.

In the present project, three undesirable components of the citrus juice have been considered, they are:

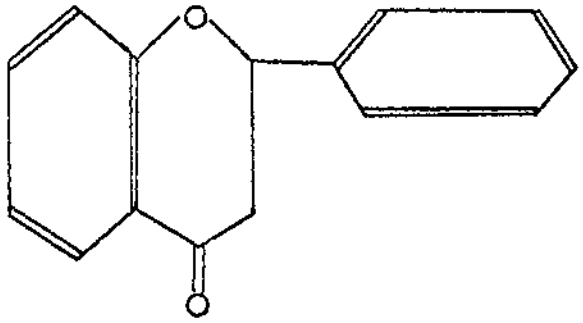


FIGURE 13. Flavone.

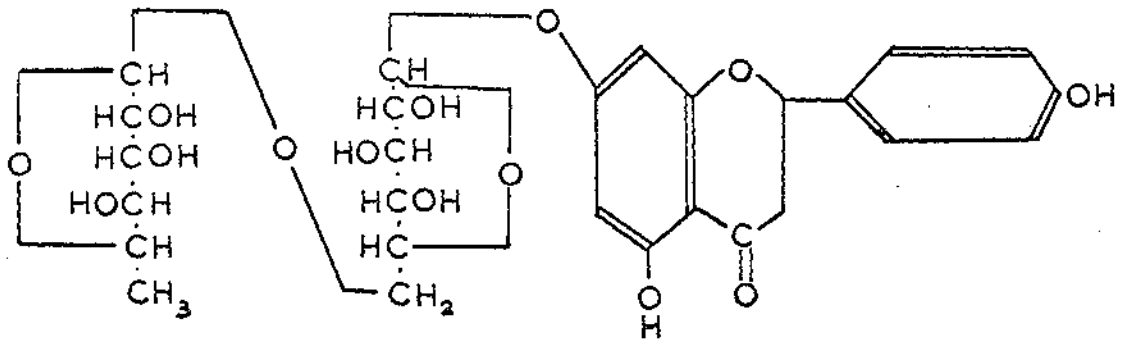


FIGURE 14. Structure of naringin.

(1) Naringin

(2) Limonin

(3) Excessive essential oil

(1) Naringin

Naringin is an organic compound belongs to a class of compounds called flavonoids. Chemically, flavonoids are organic compounds possessing the carbon framework of a flavone (see Figure 13) or more broadly are $C_6 - C_3 - C_6$ compounds. Flavonoids occur in the bark, twigs, leaves, flowers and fruits of a large number of higher plants, and are frequently found combined with carbohydrate molecules as glycosides. Flavonoid compounds may compose as much as ten per cent of the dry weight of citrus fruits. The flavanone rhamnoglucosides hesperidin and naringin and their corresponding aglycones hesperetin and naringenin are the flavonoids associated with commercial varieties of citrus fruits (69).

However, our main concern in the present work is naringin.

Naringin, the 7 - rhamnosido - β - glucoside of 4', 5, 7 - trihydroxy flavanone, has the structural formula (5, 76) as shown in Figure 14. Naringin is insoluble in ether, chloroform and benzene; yet is soluble to a varying

degree in water, alcohol, acetone, glacial acetic acid and pyridine. Calcium hydroxide, as well as other alkalis, greatly increases the solubility of naringin in water and is the basis for a patent on the recovery of naringin from grapefruit peel (38). Other physical and chemical properties can be referred to Kesterson and Hendrickson (41).

Naringin is a principal flavonoid in grape fruit. Its presence in the fruit is generally considered undesirable because of its intense bitterness. In fact the bitterness is so intense that one part in 50,000 is detectable by taste (41). The flavour and general acceptability of grapefruit juice are thus strongly affected by the amounts of this bitter principle and juices high in naringin concentration are excessively and disagreeably bitter. The New Zealand grapefruit is well known for its bitterness and juice prepared from it is generally much more bitter and astringent than overseas products. From some studies undertaken by the D.S.I.R. it seems probable that this bitterness is contributed solely by higher naringin contents although this alone may not entirely account for the poor acceptability of local products in juice form - this is discussed further later in this work. The need for a practical method of reducing such excessive bitterness is clearly desirable.

Hitherto, a number of different methods have been proposed for removing naringin from grapefruit juice or grapefruit products.

Burdick and Maurer (14) have patented a method for removing naringin from solutions using active carbon. They reported that under the optimum conditions of ^{pH} and temperature, active carbon will almost completely remove naringin from solution, but desirable flavour constituents are also removed. For this reason, this method is not very applicable for practical purposes.

Acid hydrolysis of the glycoside yields rhamnose, glucose and the non-bitter aglycone, naringenin, but such a procedure would be too drastic for practical application (67).

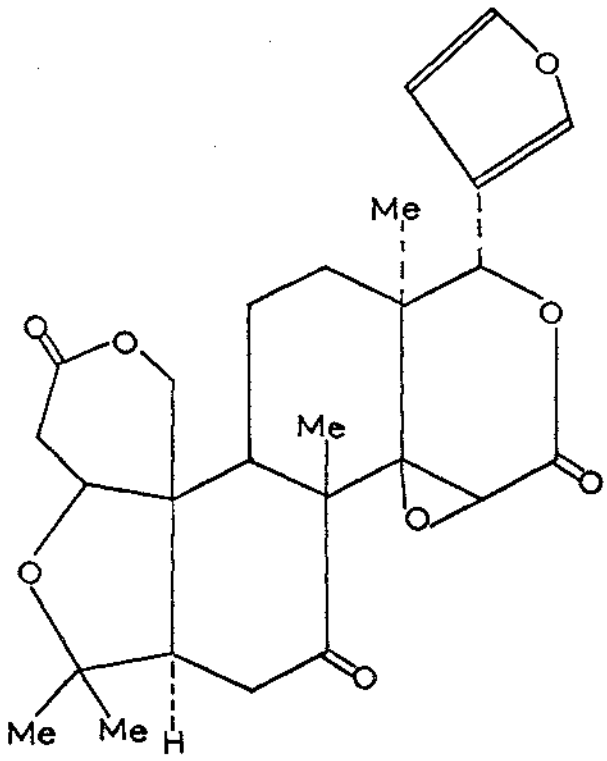
As yet, the method that has claimed some success is the enzymatic hydrolysis of naringin.

Hall (32) reported in 1938 the isolation of an enzyme from celery seeds which hydrolysed naringin in vitro, at ^{pH} 7 and 37^{o}c to naringenin and intact disaccharide. He also reported the occurrence of similar or identical enzyme in the leaves of the tree Citrus Decumana and in smaller amounts in the region between the flavedo and albedo of grapefruit.

Ting (68) found, upon treating grapefruit juice with relatively high concentration of Pectinol (a commercial pectic enzyme preparation of fungal origin) to facilitate filtering, that the total flavonoid glycoside content of the juice was greatly reduced. Naringin in aqueous solution was also found to be completely hydrolysed at P^H 4 by similar concentration of Pectinol, yielding, as the only products, glucose, rhamnose, and naringenin (67).

Thomas, Smythe and Labbee (1958) (67) in an effort to discover some more active enzymes, have investigated the possibility of obtaining active "naringinase" from micro-organisms. Using a large number of micro-organism they found that a number of the organisms were able to produce such enzymes, and one particular enzyme which they termed "Naringinase C" was found to contain a high order of activity. Naringinase C was found to rapidly hydrolyse naringin in vitro in the P^H range 3.5 to 5.0 and at temperatures of from 20-50^{o}c. It was also found to rapidly debitter natural grapefruit juices.

From the above brief survey, it seems that enzymatic hydrolysis is by far the most promising method for debittering grapefruit juice. However, to put an enzymatic method into commercial practice, several difficulties may be involved.



Structure of limonin (22a)

firstly, a suitable enzyme like Naringinase C has to be produced in sufficient quantity. Secondly, during the application, much trouble must be taken to ensure that juices are at the right P^H and right temperature for that particular enzyme to function. Furthermore, a considerable period of incubation time may be required.

(2) Limonin

Limonin is another bitter principle present in citrus juice. It has been isolated as a white crystalline substance having a melting point of 555° to $557^{\circ}F$ and with the empirical formula $C_{26}H_{30}O_8$. Its full structure can be seen on the opposite page.

Limonin is soluble in alcohol, acetone, and benzene, but relatively insoluble in petroleum ether and extremely insoluble in water. It forms tasteless salts with alkaline earth metal but below P^H 6 or 7, the salts revert to the bitter lactone form of the compound.

It has a pronounced bitter taste, and as little as 1 part of limonin in 100,000 parts of water will give a very bitter taste (37, 15).

Normally the limonin bitter principles occur chiefly in orange, mainly in the seeds, albedo and segment wall, and may pass into processed orange juices in amount sufficient to cause bitterness unacceptable to consumers. The bitterness is normally not apparent in freshly extracted juices but develops at a rate that depends upon storage and processing conditions. Navel orange varieties are especially affected and are therefore usually avoided for processing (16).

Numerous attempts have been made to prevent the development of, or to remove bitterness of limonin particularly in Navel orange juice (16) in order to make processing feasible. In one of the first methods the combination of a special type of burr was used for reaming the juice to prevent excessive extraction of rag and pulp, in conjunction with a process of increasing the hydrogen-ion concentration of the juice to P^H 3.8 - 4.0 by the addition of carbonate salts to minimize formation of the bitter lactone (15, 70). The difficulty in this method lies in the fact that it is not easy to prevent complete bitterness formation without increasing the P^H beyond the point where juice flavour is affected. Another attempt involved the use of pectic enzymes. The objective was to use their action on natural pectic substances in the juice and thus

coagulate the dispersed colloids that precipitate and, incidentally, to remove the limonin in suspension (47).

In a third method (47) activated carbons completely removed the bitter principle or principles and their precursors. However, the method presents technical difficulties, the chief of which are the tendency of carbon "fines" to be suspended in the juice and a partial loss of vitamin C.

None of these methods are considered successful or feasible for commercial application at the present time. (69)

(3) Excessive Essential Oil

The eseential oil of citrus juice consists mainly of the volatile constituents that can be removed from the juice by steam distillation methods. They will include large amounts of water, and volatile oils that are steam distilled along with the water, and other constituents that boil at temperatures lower than water or are removed as azeotropic mixture with water. Generally, the essential oil in citrus juice includes the following five general classes of compounds: carbonyls, alcohols, esters, terpene hydrocarbons and volatile organic acids (73). It is now generally recognized that the aroma of citrus juice is associated with the essential oil. For example, the flavour of a concentrated orange juice that

has been restored to its original consistency by the addition of pure water tastes insipid and is lacking in orange character (57). Grapefruit juice from which a portion of the water and volatile oil have been removed tastes only sweet and sour (49). Therefore, from this evidence, the presence of essential oil in citrus juice can hardly be considered undesirable. However, it has been shown that as well as contributing to the flavour of citrus juice, it is also responsible for some certain off-flavours in the processed juice (11, 12, 34). Hence, excessive amount of essential oil is objectionable in processed juice. Consequently, to control the amount of peel oil in the juice, deoiling sometimes becomes necessary. At present the principal method by which this may be accomplished is by vacuum distillation (71).

From the above brief consideration of the substances which have been classified as possibly undesirable in the present project, it is apparent that, thus far, there have not been any successful methods for removal of naringin and limonin in commercial practice. As regards the essential oil, the method used for removal of the excessive oil is fairly satisfactory. However, to do so, extra unit operation sometimes may be required in the processing line. Since frothing techniques are simple and economic, therefore it was

the primary intention of this work to investigate the possibility of using this method to remove these undesirable substances so that their overall flavour may be improved.

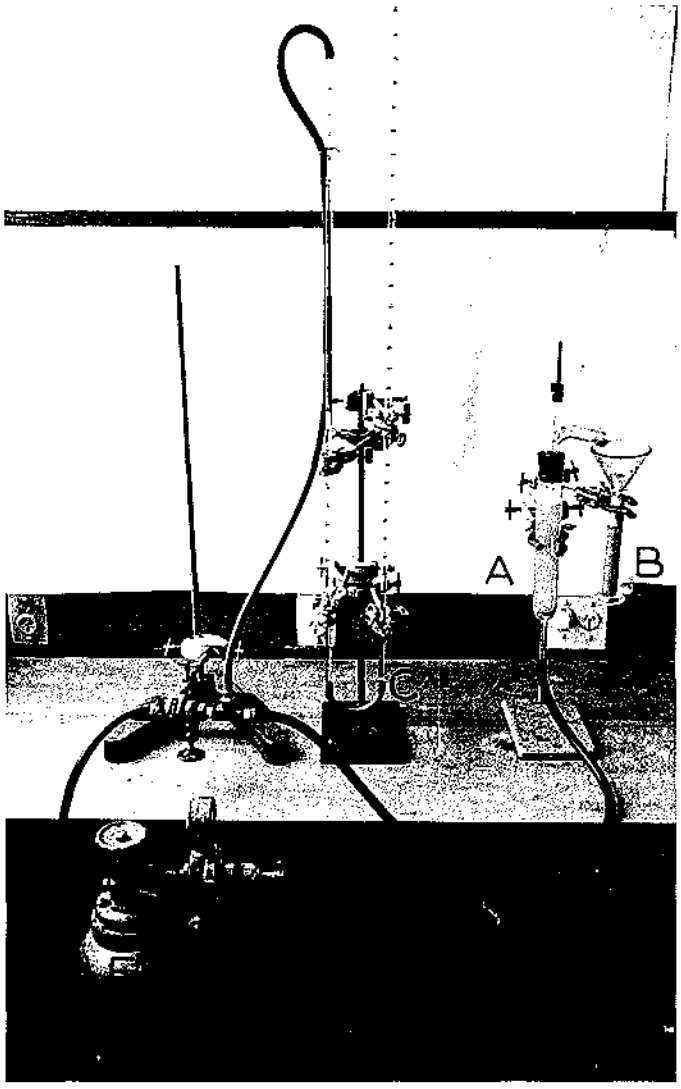


FIGURE 15 - Apparatus for frothing of citrus juices.

PART B. STUDIES ON THE EFFECT OF FROTHING ON THE UNDESIRABLE SUBSTANCES OF CITRUS JUICES BASED ON MODEL SYSTEMS

INTRODUCTION

According to the literature, thus far, there has not been any published work reporting attempts to improve citrus juice by frothing. This paucity of information necessitated a basic study on the principal compounds which have been classified as "undesirable substances" in the preceding section, i.e. naringin, excessive essential oil and limonin. It was considered that, in order to obtain some knowledge as to how these compound behave upon frothing, it is best to study them individually in model systems. However, because the analytical method of limonin in citrus juices is complicated and lengthy (16) study of limonin as a result has not been included in this project. The following experiments are carried out under two main headings:-

(I) Frothing studies on naringin based on model systems

(II) Frothing studies on essential oil based on model systems

(I) Frothing Studies on Naringin Based on Model Systems

EXPERIMENTAL

Apparatus

The apparatus used in this experiment and all the others in this part of the work is shown in Figure 15. The main

frothing chamber (A) consisted of a glass column fitted with a side glass tube as shown. The gas was introduced through a sintered glass sparger on the bottom of the main frothing chamber. Foams produced in this chamber, rose slowly, passed up the side glass tube, and flowed into the measuring cylinder (B) via a receiving glass funnel. Gas for frothing either CO_2 or N_2 was supplied from a gas cylinder (E). The gas pressure could be adjusted and maintained at a particular level by a valve (D) and a water manometer (C).

General Procedure and Methods

The naringin used in this study was pure naringin available in a form of dry powder.

Preparation of Naringin Solution

The method of preparing naringin solution is to add a small quantity of distilled water to a weighed sample of naringin (in milligram). The suspension was then heated to about $70^{\circ}c$, cooled and made up to 100 ml in a volumetric flask. The concentrations of the naringin solutions were then expressed in terms of mg/100 ml.

Determination of Naringin Concentration

The method used for estimation of naringin was based upon the colorimetric method developed by W.B. Davis (22).

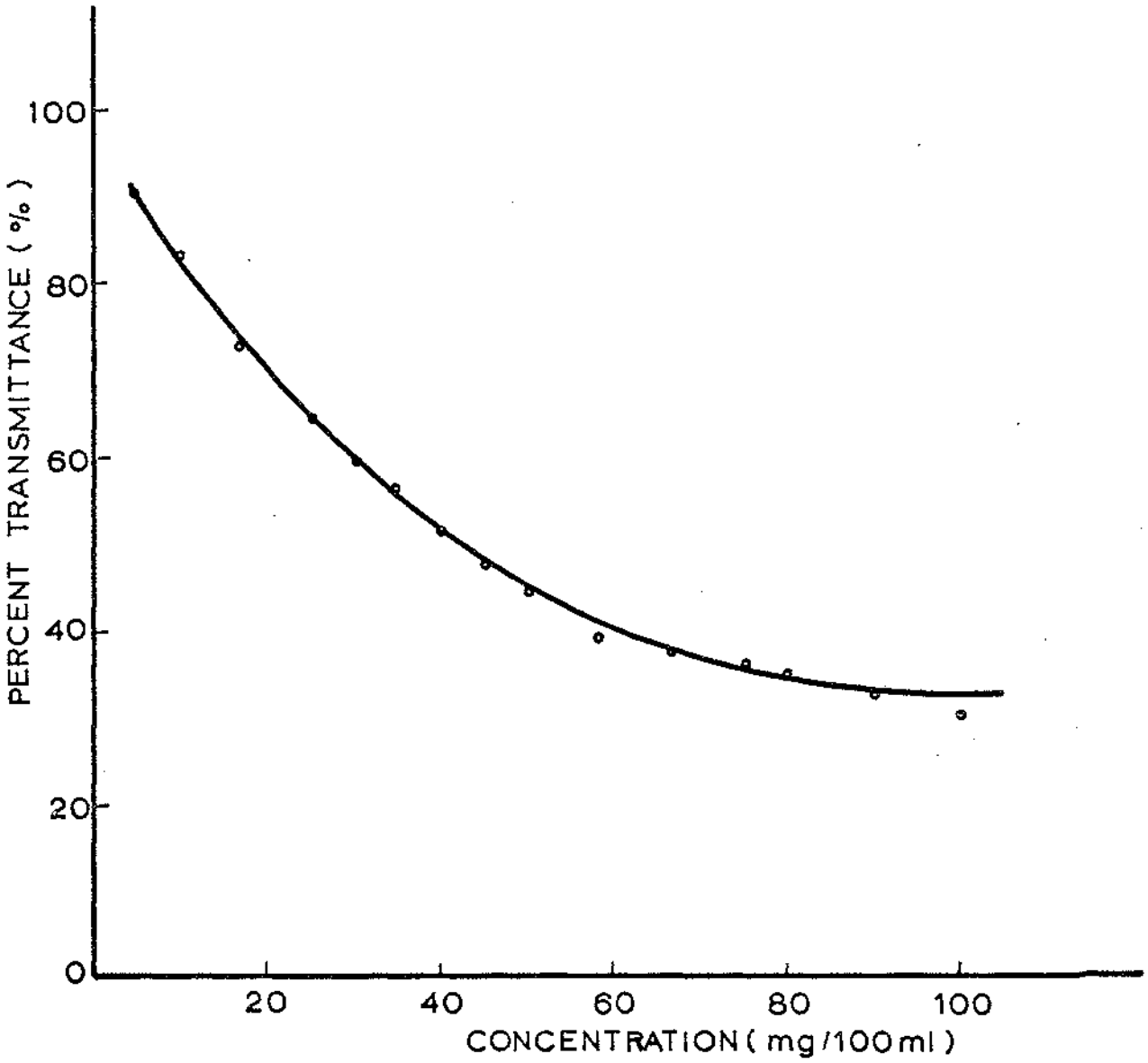


FIGURE 16 Naringin concentration versus Percent transmittance.

The colorimetric measurement was made with a Hilger Biochem Absorptiometer, using a blue filter at 430 mμ.

Preparation of Standard Curve for Naringin Concentration

The standard curve was prepared from colorimetric measurements carried out with a series of naringin solutions of known concentrations. The results were then plotted as % transmittance versus concentration (mg/100ml) as shown in Figure 16.

(a) Preliminary Study on Surface Activity of Naringin

Since it is well-recognised that surface adsorption is a prerequisite of frothing phenomena, it was thought that by judging the ability of naringin to foam, one could obtain some idea as to how likely effective naringin will be removed by frothing. Thus it was decided to examine the frothability characteristics by simple observation methods.

Experimental Method - Experiments were performed with 100 ml naringin solutions of different concentrations viz 24, 37 and 60 mg/100 ml respectively. CO_2 gas was used and a gas pressure of 16" water was maintained throughout the experiments.

Discussion of Results - It was found that naringin is surface-active as indicated by its ability to give a fair amount of foams in all three samples. However, probably due to the low concentrations, froths were unstable and the foam heights were only about ½" - ¾" for all the three solutions.

The implication from these preliminary tests is that, since naringin is surface active, it may be adsorbed at the liquid-air interface in froths and hence it may be possible to remove it by frothing.

(b) Frothing of Naringin Solution as is

Experimental Method - naringin solution with a concentration of 37 mg/100 ml was employed. The volume of solution used was increased to 150 ml so that the distance between the level of the liquid surface to the foam outlet was approximately 1". The CO_2 pressure found most suitable was 18" water which produced a froth column of approximately 1" without too much entrainment from the bulk solution. Naringin estimation were made with both the froth fraction and the mother liquid after each fraction of froth was taken out.

Results and Discussion - The results were tabulated in Table 6.

Table 6: Frothing of Naringin Solution as is

Order of froth fraction taken out	Volume of fraction taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100ml		
		F*	L*	C*	F	L	C
1st	1	50	53	53	41.5	38	38
2nd	1	49.5	53		42	38	
3rd	1	50	53		41.5	38	

F* = froth fraction

L* = bulk solution

C* = control solution before frothing

It can be seen from Table 6 that, naringin was positively adsorbed on frothing. However, the naringin concentration in the froth fractions were only slightly more than the bulk solution, a difference of about 3 - 4 mg/100 ml. This low increase in the froth fractions indicates that the ability of naringin to be adsorbed from the bulk solution is not good. Another possible cause for low concentration of naringin in foams could be ascribed to the poor drainage of foams. Since naringin foams were not stable, they collapsed quickly soon after they were formed. As a result, it was not possible to obtain a good drainage.

(c) Frothing of Naringin Solution with Addition of Ethanol

Solubility of naringin in ethanol is known to be much greater than in that of water at ordinary room temperature (41). Since ethanol is itself a surfactant, therefore, it was thought that if frothing of naringin solution is performed in the presence of ethanol, maybe more naringin could be removed together with ethanol froth. To test this, the following experiment was carried out.

Experimental Method - 10 ml of absolute ethanol was mixed well with 140 ml 45mg/100ml naringin solution before frothing. The mixture was then frothed with CO_2 gas. Foaming ability was found to be better than that of naringin solution alone. The gas pressure was adjusted to about 16" water such that froth was removed consistently with some drainage.

Results and Discussion - The results are presented in Table 7.

Table 7: Frothing of Naringin Solution with Addition of Ethanol

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100ml		
		F	L	C	F	L	C
1st	1	51	49.5	49.5	40	42.5	42.5
2nd	1.5	50.5	50		41	42	
3rd	3	49.5	50		42.5	42	

The figures in Table 7 clearly point out that there is only a small concentration difference between the froth and the bulk solution. In fact, there was a negative adsorption of naringin in the first two fractions. This implies that the surface activity of ethanol was greater than that of naringin and hence when CO_2 was introduced, ethanol seemed to be preferentially adsorbed and formed a foam first, leaving the less surface active naringin molecules in the bulk solution. This could explain why the concentrations of naringin in the first two froth fractions were less than that of the bulk liquid.

It was only in the third fraction that the positive adsorption pattern of naringin was restored, possibly due to

the gradual decrease of ethanol concentration so that naringin molecules may have had a chance to be adsorbed and concentrated at the air-liquid interface. Thus from the experimental evidence, it can be concluded that ethanol does not help to promote the naringin concentration in froths, instead, it will compete with naringin for the air-liquid interface available resulting less adsorption of naringin.

(d) Frothing of Naringin Solutions with Addition of Foaming Agent

Since frothing naringin solution alone gave fairly unstable froths and very poor adsorption, the use of surfactant as frothing agent was therefore considered. It was hoped that some surfactant may have strong affinity for naringin molecules, hence upon foaming, may be able to bring naringin out of the solution effectively. The following experiments were therefore carried out.

(i) Frothing of Naringin Solution with Addition of Sulphonated Oil Detergent

Experimental Method - A few drops of dilute sulphonated oil detergent was mixed well with 100 ml of approximately 24 mg/100 ml naringin solution and frothed at 13" water using CO_2.

Table 8: Frothing of Naringin Solution with Addition of Sulphonated Oil Detergent

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100 ml		
		F	L	C	F	L	C
1st	1	70	68.5	69	18.5	20.5	20
2nd	1	65	69.5		24	20	
3rd	1	65	69.5		24	19.5	

Results and Discussion - Table 8 demonstrates the results obtained in this experiment. It can be seen that, in the initial period of frothing operation, preferential adsorption of sulphonated oil surfactant was observed, as shown by the lower concentration of naringin in the first froth fraction. Thereafter, positive adsorption of naringin took place. However, the increase in froth concentration was still no better than when naringin solution was foamed alone (Refer to Table 6). This infers that the affinity between the naringin molecules and the frothing agent was not a strong one. As a result, no great increase of naringin concentration was resulted in the froth phase.

(ii) Frothing of Naringin Solution with moderately lipophilic surfactant (Span 40)

Experimental Method - A few drops of dilute Span 40 solution was added to 100 ml of approximately 24 mg/100 ml naringin solution. The mixture was then frothed at about 16" water using CO_2. At this pressure foams obtained rose very slowly. Consequently good drainage of foams was obtained.

The results are presented in Table 9.

Table 9: Frothing of Naringin Solution with Span 40

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100 ml		
		F	L	C	F	L	C
1st	1	67	67.5	68	22	21.5	21
2nd	2	67	68		22	21	
3rd	2	66	68		23	21	

(iii) Frothing of Naringin Solution with Moderately hydrophilic Surfactant (Tween 60)

Experimental Method - the same procedure as described in (ii) was applied to another 100 ml naringin solution of approximately 24 mg/100 ml except that the surfactant used was Tween 60.

The results are tabulated and presented in Table 10.

Table 10: Frothing of Naringin Solution with Tween 60

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100 ml		
		F	L	C	F	L	C
1st	1	53	64.5	64	38	24.5	25
2nd	2	53	65		38	24	
3rd	3	44	65.5		50	23.5	
4th	6	62	66		27	23	

(iv) Frothing of Naringin Solution with Strongly Hydrophilic surfactant (Tween 20)

Experimental Method - The same as (iii) except Tween 20 was used. Results obtained are presented in Table 11.

Table 11: Frothing of Naringin Solution with Tween 20

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100 ml		
		F	L	C	F	L	C
1	1	63.5	65	65	25.5	24	24
2	1	58	65		31.5	24	
3	1	58	65		31.5	24	
4	1	62	65.5		27	23.5	

Discussion of Results in (ii), (iii) and (iv) - From the figures given in Table 9, it is obvious that Span 40 did not have much influence on the naringin concentration in froths. The naringin concentration in the froth fractions were only 1 or 2 mg more concentrated than that in the bulk solution. On the other hand, naringin solution with Tween 60

added gave significant increase (see Table 10). The concentration in the froth fractions could be increased from about 25 mg/100 ml to 50 mg/100 ml in some fraction. Tween 20 also gave considerable increase but on the whole, it was less effective than Tween 60 (see Table 11). From these evidence, it appears that different type of surfactant would have different influence on naringin concentration in the froth phase. If this is so, what is the most possible explanation then?

It must be pointed out first of all that, the surfactants chosen in these experiments are only selections out of a tremendous range of surfactants commercially produced. A typical classification of surfactants on the basis of lipophilic-hydrophilic character is given by the Atlas Chemical Co. whose products were used in these experiments. In this system each surfactant is assigned a numerical value on the HLB system (66). The HLB (Hydrophile - lipophile Balance) of a surfactant is an expression of its Hydrophile-Lipophile Balance i.e. the balance of the size and strength of the hydrophilic (water-loving or polar) and the lipophilic (oil-loving or non-polar) groups of the surfactants. Span 40 (Sorbitan mono palmitate) has a HLB rating of 6.5 or 32.5% hydrophilic. Tween 60 (Polyoxyethylene sorbitan mono stearate) on the other hand has a rating of 14.9 or 74.5 hydrophilic.

Tween 20 (Polyoxyethylene sorbitan mono laurate) is by far the most hydrophilic among the three surfactants used, it has a HLB rating of 16.7 or 83.5% hydrophilic.

Evidently, these three surfactants are quite different in their hydrophilic properties. It would appear, therefore, that the proportion of hydrophilic group in the surfactant may have a bearing on froth concentration. For example, Span 40, a lipophilic surfactant, showed no tendency to bring naringin out of the solution on frothing. On the other hand, Tween 60, a hydrophilic surfactant, showed great affinity for naringin molecules and tended to pull naringin along with them upon frothing. However, Tween 20, an even more hydrophilic surfactant than Tween 60 was found to be not as effective as Tween 60. The immediate deduction from these evidence is that, naringin will come out readily with the froth only when a hydrophilic surfactant is used. In other words, the conditions in the froth must be hydrophilic. However, excessive hydrophilic property seems somewhat undesirable, since it tends to reduce the froth concentration. It is possible that, there exist between naringin molecules and hydrophilic portions of surfactant, a loose association. The tendency of the combined naringin-surfactant to come out of the solution appears to depend very much on the delicate balance of hydrophilic and lipophilic balance in the surfactant, which will be discussed more fully in Section III, p 142.

(e) CO_2 VS N_2 in Frothing of Naringin Solution

All the previous experiments have been carried out using CO_2. It was the intention of this experiment to see if N_2 gas could be used as effective as CO_2.

Experimental Method - To 100 ml naringin solution (24 mg/100 ml), 0.4 ml of dilute Tween 60 solution was added. The initial P^H of the mixture was measured and found to be 6.45. Then the solution was first frothed with N_2 at a gas pressure of about 16" water. After several froth fractions had been collected, the air flow was turned off, P^H of the bulk solution checked, and the remaining solution was frothed with CO_2 at the same gas pressure. At the end of the experiment, the final P^H of the bulk solution was once again taken.

Results - The results are tabulated and presented in Table 12 and Table 13.

Table 12: Frothing of Naringin Solution Using N_2

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100 ml		
		F	L	C	F	L	C
1st	1	51	67	67	40	22	22
2nd	2	65	67		24	22	
3rd	3	63	67.5		26	21.5	
4th	6	65	68		24	21	
5th	3	64	68[1]		25	21	

Table 13: Frothing of Naringin Solution Using CO_2

Order of froth fraction taken out	Volume of froth taken out (ml)	% Transmittance			Naringin concentration from standard curve mg/100 ml		
		F	L	C	F	L	C
1st	1	67	68[1]	68	22	21	21
2nd	1	55	68.5		36	20.5	
3rd	1	55	68.5		36	20.5	
4th	1	54	68.5		37	20.5	
5th	3	55	69[2]		36	20	

1 pH = 6.4

2 pH = 4.1

Discussion of Results - In the experiment using N_2 gas, it can be seen from Table 12 that, the concentration of naringin in the froth fraction was significantly higher than that of the bulk solution only in the first fraction as shown by the increase of naringin content from merely 22 in the bulk solution to about 40 in the froth fraction. All the succeeding froth fractions however, showed very little increase as far as naringin concentration in froths is concerned (see Table 12).

When CO_2 was introduced into the remaining solution, at first there was only a negligible naringin concentration difference between the first froth fraction and the bulk solution. However, as the frothing process proceeded, the froth naringin concentrations increased and were found to be consistently much higher than those of the corresponding bulk solutions as shown in Table 13.

The above results clearly indicate that CO_2 is more efficient than N_2 for removing naringin from the solution. This efficiency difference is possibly due to the difference of ^{P}H in the operating solutions. Because when foaming was performed with N_2, ^{P}H of the solution apparently was not affected by the presence of N_2 gas and found to remain at about 6.4 throughout the experiment. Nevertheless, when frothing was

produced by CO_2, it was found that incorporation of CO_2 rapidly decreased the P^H of the solution. P^H of the final solution was found to be 4.1. It appears that P^H variation may have significant effect on the solubility of naringin. For example, in the recovery of naringin (38), to solubilize naringin, the solution was adjusted to high P^H's of 8.4 - 9.1 with alkaline, and the extract was then adjusted to P^H between 4 - 5 for crystallization of naringin. Therefore, it is possible that at relatively high P^H 's such as 6.4 naringin might be still very soluble and hence tended to remain in the bulk solution rather than seeking air-liquid interface. However, at a relatively low P^H such as 4.1 when CO_2 was used, the solubility of naringin may have decreased considerably, thus it had more tendency to be adsorbed. As a result, naringin concentration in the froth phase was higher when CO_2 was used.

Conclusion - Because of its effect on P^H of the solution, CO_2 appears to be better than N_2 as dispersing gas for frothing of naringin solutions. However, in actual practice, P^H of most grapefruit juices are already between 3 - 4, therefore, either N_2 or CO_2 may be used according to convenience and cost.

Summary - The following points can be summarised from all the experiments.

(1) Naringin is surface active and thus tends to be adsorbed at the air-liquid interface. However, the adsorption of naringin from the bulk solution is generally poor.

(2) Some hydrophilic surfactants were found to be effective for removal of naringin from the solution. By far, the most suitable hydrophilic surfactant being Tween 60.

(3) Because of its effect on P^H of the solution, CO_2 appears to be better than N_2 as dispersing gas for removing naringin from pure naringin solution.

(II) Frothing Studies on Essential Oil Based on Model Systems

Here again, like in the case of Naringin, information about how the essential oil content will be affected by frothing is lacking. No research is reported in the literature concerning the frothing behaviour of essential oil in citrus juice and the question one might ask is would the essential oil concentrate in the froth phase? To answer such a question, an elementary study on effect of frothing on essential oil in an artificial system was considered essential. Therefore, the following experiments were carried out to fulfil this purpose.

(1) Development of a Method for Rapid Essential Oil Estimation in the Model System

In order to follow the change of essential oil upon frothing, it was a prerequisite to have a routine method of determining the essential oil content in the solution. Consequently, some effort was spent looking for a suitable method for the present purpose. The development has been developed in the following steps.

(a) First of all, all the conventional methods involving steam distillation and measurement of the distilled oil by volume e.g. method devised by Scott (61), Clevenger (18) and those recommended by Curl (20), have been considered. However,

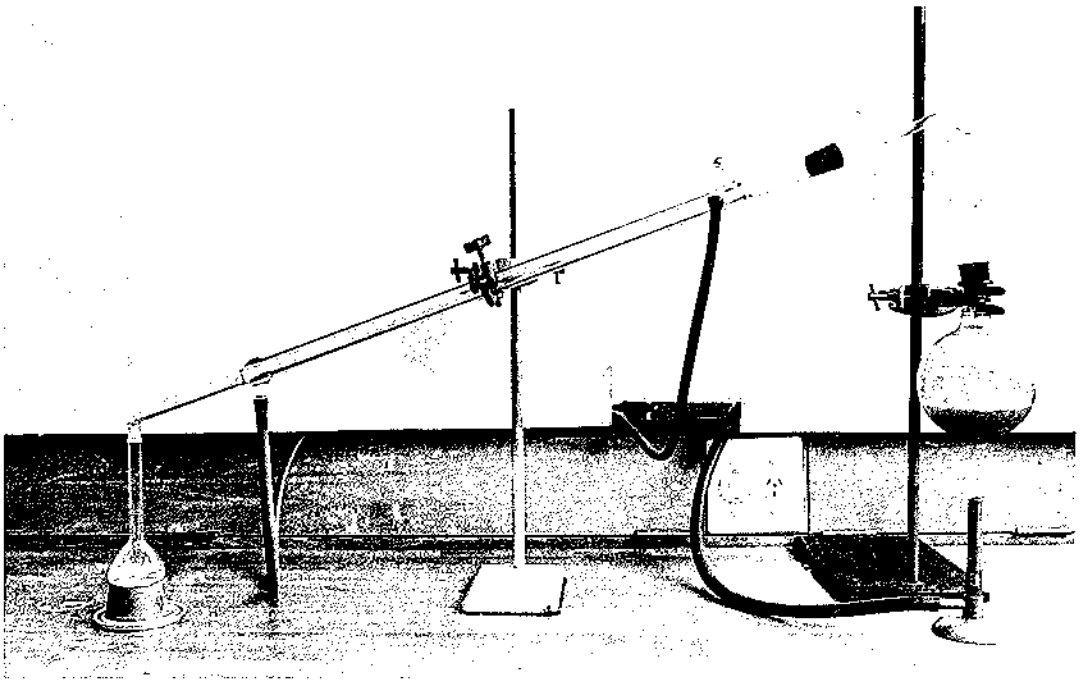


FIGURE 17 - Showing the distillation apparatus.

they were found to be unsuitable for the present project, as all these methods require a very large sample size. For example, a sample of one litre is normally used in these methods.

(b) Hence, it was decided to try some other methods which would permit use of smaller size of sample. As a result, the method for rapid estimation of essential oil in grapefruit juices published by Burdick and Allen (13) was subsequently considered. In this method 25 ml acetone was added to 100 ml juice, and 50 ml was distilled. When an aliquot of the clear distillate was diluted a turbidity, proportional to the oil content, developed and was measured colorimetrically. This method was considered very rapid and simple, furthermore, only 100 ml of juice was sufficient for one determination. Therefore, at the initial stage, it was decided to try out this method.

A distilling equipment was assembled and can be seen in Figure 17.

It consisted of a round bottom 500 ml flask, a Kjeldahl safety head, and a Liebig condenser, having its delivery end fitted into the opening of a 50 ml volumetric flask which served as the distillate collector. Heating was applied with a Bunsen burner. Turbidimetric measurements were made with Hilger Biochem Absorptiometer, using the blue filter (430 mμ).

Preliminary trials were made with canned orange juice available under the brand "Raro".

In the experiment, 4 separate 150 ml Raro juice were frothed at about 18" water using CO_2. The foaming ability of the juice was good and hence needed no addition of any surfactant. Approximately 25 ml of froth was collected in each run. The determinations of essential oil based on Burdick and Allen's method were then carried out with the combined 100 ml froth, the original juice and the combined froth treated remaining juice.

The same procedure was repeated for additional experiments.

Results - The data obtained from three runs are illustrated in Table 14.

Table 14

Run No.	Optical Density			Approximate Recoverable oil content (%)		
	O*	F*	T*	O	F	T
1	0.60	0.56	0.20	0.054	0.051	0.180
2	0.66	0.70	0.22	0.060	0.063	0.020
3	0.63	0.53	0.15	0.057	0.048	0.014

O* = original juice

F* = froth treated juice

T* = juice obtained from froth

It must be pointed out herein that in this present work, the primary intention was to obtain a comparative rather than an actual essential oil content in various fractions. Therefore, for the sake of convenience and in order to get a better understanding in the discussion below, the proportional factor 0.0906 of Burdick and Allen was adopted. And the subsequently calculated % recoverable oil were tabulated along side the optical density reading as shown in Table 14.

Discussion of Results

The data from Table 14 show some inconsistent and conflicting results. In all the three runs, the % recoverable oil in the froth treated samples was far less than the original juices. This suggests that oil tends to concentrate in the froth phase upon frothing and when froth is continuously taken out, the remaining juice is gradually deprived of its essential oil. This assumption is true, as will be proved in later experiments (see Section II, Part B, II (2), p 113 and Section II, Part C, II(i),p134). Therefore, one would logically expect high concentrations of essential oil in the froth fractions. However, using Burdick and Allen's turbidimetric method, it was not found so, instead in run No.1 and No.3, the % recoverable oil were even less than the original juice. It was only in run No.2 that the froth

fraction was slightly higher than the original juice. This evidence tends to indicate that this turbidimetric method is not very reliable, - since, it would not account for the loss of oil content in the froth treated juices. Nevertheless, if one has a close look at the % recoverable oil of original orange juice in all three runs, one would see that they are fairly consistent and the slight difference is within reasonable limits. This finding implies that the turbidimetric method may still be applicable to original and froth treated samples but not to the froth fractions. At this stage of the experimental work, no satisfactory explanation could be put forward to account for the experimental results obtained in this experiment. Nevertheless, in our later work on grapefruit juice, the probable cause of the irregular results with froth samples was postulated and confirmed (Refer to Section II, Part C, II(i), p134).

(c) Development of a simple method using colour index for approximate oil content.

It has been noted with interest during the preliminary frothings of orange juice that, the colour of the orange juice in froth fractions were always more intense than both the original and froth treated samples.

It is generally known that, most of the colouring pigments in citrus juice are mainly carotenoid substances e.g. carotenes, lycopene and numerous others (69). Nearly all of these carotenoid compounds are insoluble in water and only soluble in fat or oil. For example, even in their natural occurence, they are normally found to be present in colloidal suspension in the cell lipoids or in admixture with solid or semi-solid fat (40). This salient fact about the close association of carotenoids with oil and the afore-mentioned experimental observation have led to the thought that the increased colour intensity in the froths may be mainly due to the increase of carotenoid-carrying essential oil. If it is so, then the colour intensity of carotenoid in the juice may be useful as an approximate index for the essential oil concentration.

To test the validity of this theory, a model emulsion system made up of essential oil, carotene and distilled water was adopted.

The essential oil used was vacuum distilled Valencia orange oil and the carotenoid pigment used consisted of α and β carotene mixture in a form of a fine powder.

The following tests were carried out to prove that the colour intensity is an index of oil content.

(i) To prove that Colour of Carotene does not Develop in the Absence of Oil

To a 250 ml flask containing 150 ml of distilled water a trace of carotene was added. The solution was then shaken with 0.2 ml 5% Tween 20 (an emulsifier recommended by Atlas Co.) (66).

Only a faint pink colour was observed, probably due to the suspended tiny carotene particles. However, when the solution was filtered using a Whatman No.1 filter paper, no colour was visible. This is because most suspending carotene particles had already been filtered out. This evidence shows that in a predominantly aqueous system essential oil may be a vital solvent for carotene without which no carotene colour could be developed in the solution.

(ii) To Prove that the Carotene Colour Only Develops in the Presence of Essential Oil

0.05 ml essential oil was pipetted into another flask containing 100 ml distilled water. Approximately, the same amount of carotene was added. The mixture was shaken with 0.2 ml of 5% Tween 20 emulsifier. The resultant emulsion was orange in colour. When this coloured emulsion was filtered, the orange colour remained since filtering only removed the excessive undissolved carotene.

This experiment clearly proves that the colour of carotenes only develops in the presence of essential oil in an aqueous system (such as citrus juice).

(iii) To Prove that the Intensity of the Carotene Colour is a Function of Oil Content

(a) To two samples of 10 ml Raro orange juice were added equal amounts of carotene. Then to one sample was added 0.05 ml oil while the other was untreated. After thorough shaking and filtering, increase of orange colour was observed only in the one with oil added.

(b) In the experiment, 0.01, 0.03 and 0.05 ml essential oil were pipetted into three different flasks respectively. Thereafter, a small quantity of carotene powder was added into each flask and mixed well with the essential oil so that most of the carotene powder was nearly dissolved in the oil drops. The oil-carotene mixture was then shaken with 100 ml distilled water and 0.5 ml 5% Tween 20 for a considerable time until the emulsion was saturated with carotene and judged by the presence of undissolved carotene particles on the surface of the emulsion. The resultant emulsions were then filtered. It was found that the colour intensity of the emulsions distinctly increased with an increase in oil content. To measure the colour intensity objectively, 5 ml sample from each emulsion was

dissolved in 5 ml of absolute ethanol. The colour intensity of the resultant clear solution was then determined with the Hilger Biochem Absorptiometer using 430 mμ blue filter. The readings were made using a blank solution made up of 5 ml distilled water and 5 ml ethanol.

The % Transmittance readings of the three samples are presented in Table 15.

Table 15: Showing the Relation between the Colour Intensity of Carotene and Oil Content

Concentration of oil % (V/v)	% Transmittance
0.01	90
0.03	88
0.05	84

Therefore, from the observation in (a) and the data in (b) it is evident that the capacity of a given essential oil content to dissolve carotene is fairly fixed. Only an increase in the oil content, can it absorb and dissolve more carotene as shown by the figures in Table 15.

In other words, colour intensity of a carotene saturated solution is a function of oil content.

Conclusion

From the evidence obtained from (a), (b) and (c) it can be concluded that in this model system of saturated carotene/essential oil emulsion, the increase in colour intensity means increase in essential oil content.

(2) Basic Studies on the Effect of Frothing on the Essential Oil Content in Carotene/Oil System

The major aim of this experiment was to find out whether essential oil will be enriched in the froth phase. If so, how effective can it be removed from the mother solution by frothing techniques?

Experimental Method

Preparation of Standard Curve - A series of artificial carotene/essential oil emulsions containing different amounts of essential oil viz 0.01, 0.03, 0.05, 0.07, 0.1, 0.2 and 0.3 ml respectively were prepared. The procedure of preparation was the same as that described in the preceding experiment (see (iii) b, p111) except that 1 ml of 5% Tween 20 was used in order to assure a stable emulsion. 5 ml sample

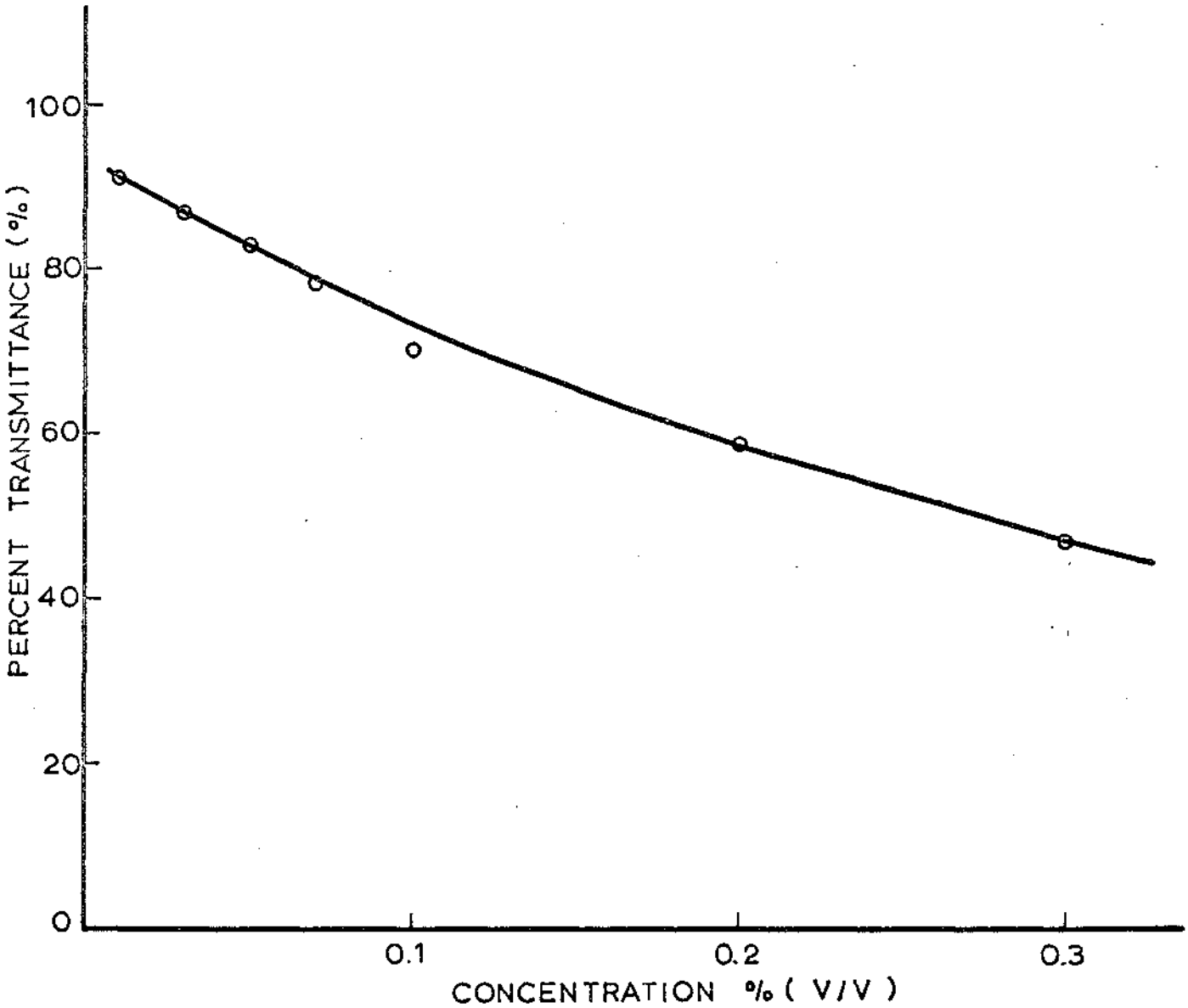


FIGURE 18. Concentration of essential oil versus Percent transmittance.

from each concentration was then dissolved in 5 ml absolute ethanol and the colour intensity of the resultant clear solution was read with the Hilger Biochem Absorptiometer using 430 mμ blue filter. The readings obtained were then plotted against the essential oil concentrations as shown in Figure 18.

It was found that the reproducibility in the low concentration region was within ±1% Transmittance, but in higher concentration range such as concentrations from 0.1% upwards, the variation could be ±2% Transmittance. However since in the present work, only comparative estimates of reasonable accuracy were required, the standard curve obtained in Figure 18 was thus considered satisfactory for this particular study.

In the experiment, 90 ml solution of 0.01, 0.03, 0.05% carotene/oil emulsion were frothed at approximately 15" water. In each run, 6 ml froth was collected. The % transmittance readings of both the froth and treated samples were subsequently determined.

Results - The results are shown in tabular form in Table 16.

Table 16: Effect of Frothing on the Essential Oil Content in Carotene/Oil System

Concentration of the carotene/oil emulsion % ($^{V}/_{V}$)	% Transmittance			Concentration obtained from the standard curve		
	C*	T*	F*	C	T	F
0.01	91	94	69	0.01	0.004	0.110
0.03	87	89	59	0.03	0.020	0.200
0.05	83	85	49.5	0.05	0.045	0.285

C* = control solution before frothing

T* = froth treated bulk solution

F* = froth fraction

Discussion of Results - It can be seen from the data given in Table 16 that froth fractions were always very much more intense in carotene colour than the bulk solutions. This indicates that the oil content in the froth phase was much more concentrated than the bulk solution. For example, based on the standard curve (see Figure 18). The froth concentrations obtained from 0.01, 0.03 and 0.05% solution were found to be approximately 11, 6 and 6 times their corresponding original solution. Hence, it is indisputable that frothing

techniques are very effective for removing essential oil from the mother solution. The percentage removal of essential oil from the 0.01, 0.03 and 0.05% solutions was found to be about 73, 46 and 37% respectively, after only 6 ml froth had been taken out.

The amounts of volatile oil present in commercially extracted grapefruit and orange juice have been reported to vary from 0.008 to 0.014 per cent and 0.016 to 0.075 per cent respectively (50, 57, 61). Judging from the results in this experiment in which the oil content was fairly closed to the above-mentioned commercial juices, it is reasonable to believe that if so desired, the excessive oil content resulting from any extraction process could be easily reduced to a desired level simply by taking out an appropriate portion of froth.

The reason why oil tends to come out of the solution dramatically could possibly be explained on the basis of solubility. In the original carotene/oil emulsion, one can imagine that essential oil globules are dispersed as colloidal particles in the bulk liquid. Since the oil globules are very insoluble in water, a great portion of them will seek air-liquid interface, it is thus natural that more oil particles would stay away from the bulk water phase and concentrate greatly

at the air-liquid interface. As a result, concentration of oil in froths will be much higher than the bulk liquid as already shown in this experiment.

Conclusion - frothing has been found to be very effective for removal of essential oil in aqueous dispersions.

PART C. STUDIES ON FROTHING OF CITRUS JUICES

From the studies based on model systems in previous work, it has been shown that frothing can be used efficiently to remove essential oil. Naringin, on the other hand, could be removed only under suitable conditions.

The aim of this project was to study whether frothing could be employed to remove some undesirable substances e.g. excessive oil and naringin in grapefruit juice, excessive oil in orange juice so that the overall flavour of the citrus juices could be improved.

Citrus juices of interest to us in this work are sweet orange and grapefruit juice.

Therefore, the following experiments were carried out under 2 different headings i.e.

(I) Frothing Process to Improve Sweet Orange Juice

(II) Frothing Process to Improve Grapefruit Juice

(I) Frothing Process to Improve Sweet Orange Juice

All the oranges used in this experiment were Jamaican Sweet orange.

Experimental Method

Preparation of Orange Juice - The oranges were cut into two halves, the extraction of juice was then carried out with a hand juicer. The resultant orange juice was filtered through Muslin cloth to remove gross suspending matter.

In the experiment, 150 ml orange juice was frothed in the frothing column using N_2 gas. The frothability of the orange juice was found to be reasonably good hence no addition of surfactant was necessary. A gas pressure of 20" water was found to be optimum to keep a continuous and quite stable froth column. During the frothing operation, massive lumps of oil carotene mixture could be seen coming out with the froths. As the operation continued, the orange colour of the original juice gradually diminished, while the colour intensity of froth fraction increased. To ensure an adequate removal of oil so that a definite flavour difference could be detected, the process was allowed to continue until 30 ml of juice was frothed off. Analytical tests were then carried out with the original juice, the froth fraction and the froth treated juice.

Determination of Colour Intensity - In the determination of colour intensity of the juice, 5 ml juice sample was thoroughly mixed with 10 ml absolute ethanol.

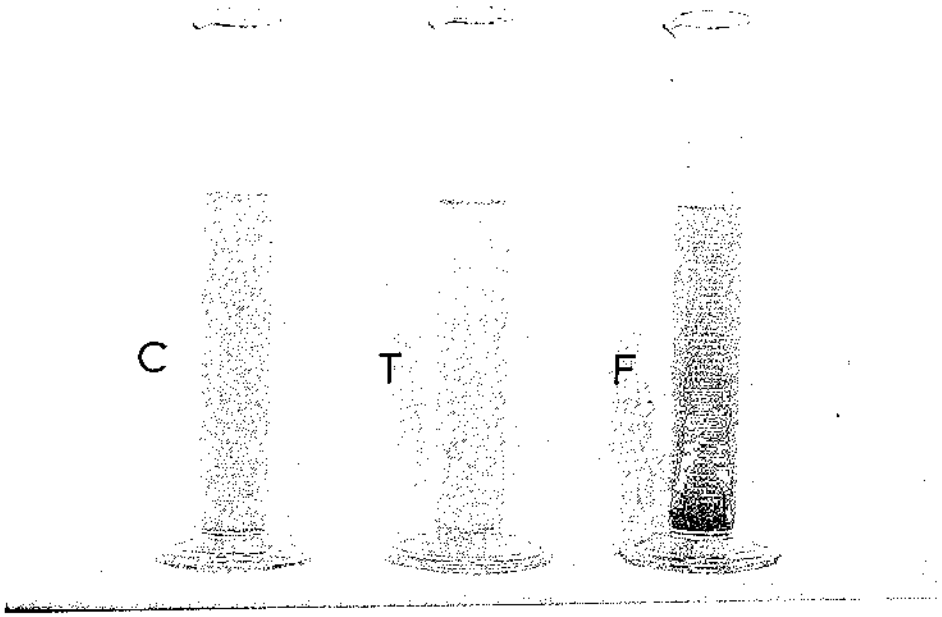


FIGURE 19 - Showing the colour of the different orange juice fractions after the frothing operation.

The resultant clear solution was filtered with filter paper to screen out suspended matter. The % Transmittance reading was determined with the Hilger Biochem Absorptiometer using a 430 mμ blue filter. The blank solution adopted consisted of 5 ml distilled water and 10 ml absolute ethanol.

Determination of Flavonoids - The determination of the flavonoids content was based upon the colorimetric method of W.B. Davis (22).

Results and Discussion

(a) Effect of Frothing on Essential Oil - It was noted, at the end of the run, that nearly all the orange colour of carotenoid compounds had disappeared from the remaining bulk juice. As a matter of fact, the remaining treated sample was only faintly yellow. On the other hand, the froth fraction was intensely orange. A photo showing the colour of these various fractions of juices can be seen in Figure 19.

Typical results of colorimetric measurements are presented in Table 17.

Table 17: Colorimetric Readings of the Different Juice Fractions

% Transmittance		
T	C	F
93	79	52

Figure 19 and the data in Table 17 clearly show that the colour intensity of the treated juice was much less than both the original juice and the juice obtained from the froths. Since it has already been proved previously that colour intensity is an approximate indication of oil content, the essential oil content of the treated juice after frothing must be very small. In other words, a large proportion of essential oil originally present in the juice must have been removed with the outgoing froth. This proves that frothing techniques may be used effectively to get rid of the excessive essential oil in orange juices.

It has been generally accepted that, excessive peel oil is undesirable because it may be responsible for the occurence of off-flavour in processed orange juice. However, from the evidence in this experiment, it appears that excessive oil content in fresh juice may also contribute some undesirable flavour to the orange juice. For example, the oil content

of the original orange juice must be quite high, as the extraction of juice was done by pressing the half-orange with a hand juicer, as a result, a considerable amount of peel oil must have been incorporated into the juice. When this original juice was subjected to tasting, it was found that the flavour was so strong that it had a very unpleasant hot and bitter after-taste. However, when flavour test was performed with the treated sample, it was found that the taste was much milder and yet retained its pleasant orange flavour. In order to prove that the flavour difference between the original juice and the froth treated juice was statistically significant, a subjective flavour taste panel was carried out. Paired-Comparison test has been adopted. In the testing procedure five judges were trained to distinguish different concentrations and types of orange flavour in citrus juices. Each judge was then presented with 6 paired samples coded with random numbers. Each pair contained one sample from the original juice and one sample from the froth treated juice. These judges were then instructed to identify the sample with the stronger orange flavour (i.e. excessive oil-less acceptable).

A clear-cut result was obtained from this flavour test, 28 samples out of the 30 samples being correctly identified.

The difference in flavour would be significant statistically (at 5 per cent level), if 20 out of 30 correct identifications are made (2). Hence, the difference in flavour of the orange juices was highly significant.

Therefore, it confirms that frothing may be successfully employed for removal of excessive oil present in citrus juice and thus improve its flavour.

(b) Effect of Frothing on Flavonoids Content - The results of the flavonoid tests made with various fractions of juices are presented in Table 18.

Table 18: Effect of Frothing on Flavonoids Content

% Transmittance		
T	C	F
73	71	70

According to the data shown in Table 18 the intensity of yellow colour in the froth fraction was slightly higher than that of treated juice. In other words, the concentration of the flavonoid compounds (mainly hesperidin) in the froth fraction

was slightly higher than the treated juice. The most probable explanation for the poor adsorption of hesperidin upon frothing could be that, because orange juice is made up of multitudinous chemical compounds among which many constituents are more surface-active than hesperidin e.g. amino acids, proteins, enzymes. Therefore, upon frothing, it is natural that, the more surface-active constituents would be preferentially adsorbed. Consequently, the chance of hesperidin being adsorbed is less, since it has to compete with so many other surface-active compounds. As a result, the concentration of the hesperidin in the froth fraction was only slightly higher than the bulk juice.

(c) Effect of Frothing on Limonin - As pointed out earlier, the analytical determination of limonin is complicated and lengthy and has not been attempted in this study. However, it is known that limonin is extremely insoluble in water but soluble in non-aqueous solvents such as alcohol, acetone, and benzene (69). Thus, if the limonin present in the juice is in a soluble form, it is most likely to be solubilized by some natural solvent system in the juice, for instance, dissolved in the oil phase. If this is the case, it is fair to say that a large proportion of the limonin would be removed with the oil-rich froth fractions.

Non the less, it is more probable that the limonin present in the juice is in a form of a suspension as can be

illustrated by the fact that, in the past, many attempts have been made to remove the limonin in suspension from the juice (see Section II, Part A, Introduction, p 78). Frothing has been found to be very effective for removing the suspended matter from solutions. For example, the froth treated juices were nearly always free from any suspended substances. Thus, on the basis of this experimental evidence, there is every reason to believe that, if present, the majority of limonin suspending in the juice would be removed during the frothing process.

Therefore, it appears that a frothing technique could be valuable when utilized as a possible unit operation for removing the limonin from citrus juices, e.g. for removal of excessive limonin in Navel orange juices to make their processing feasible in commercial practice. However, further detailed work is needed to confirm the full potential of the froth "refining" technique proposed.

Summary

The following main points may be summarised from the results of these experiments.

(1) A large proportion of essential oil in the orange juice can be removed by frothing.

(2) Some flavonoid compounds were shown to be removed upon frothing.

(3) Considerable amount of limonin may also be removed.

Conclusion

From all the results, it appears that frothing can be used to improve the overall flavour of orange juice.

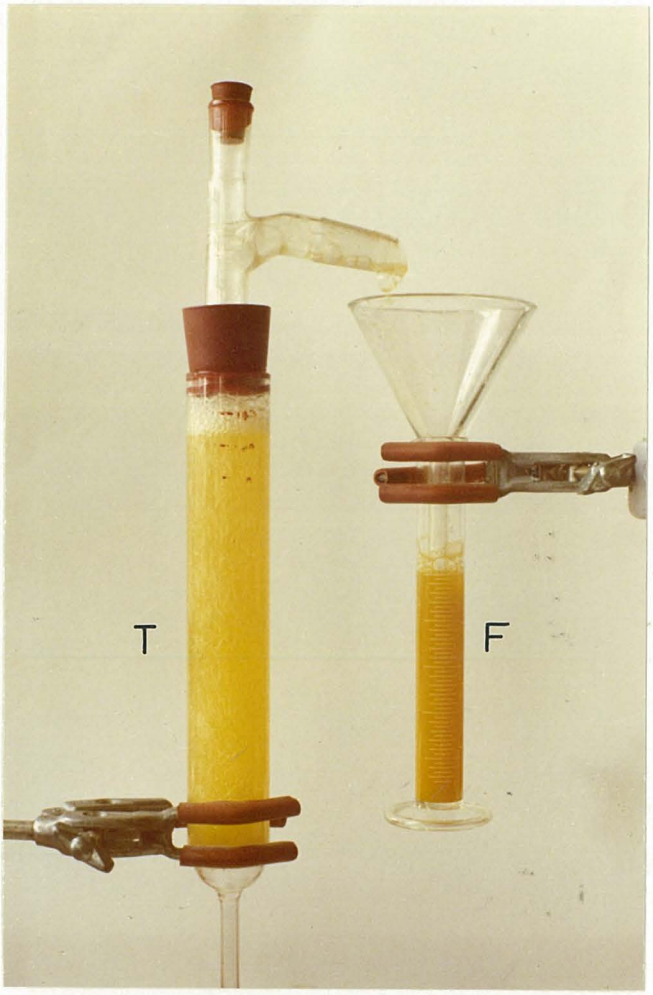
(II) Frothing Process to Improve Grapefruit Juice

New Zealand grapefruit is generally more bitter than overseas varieties probably due to the higher naringin and oil content. Therefore, our primary intention in this study was to investigate the possibility of using the foam separation methods to improve the flavour of grapefruit juice.

(i) Study of Frothing on Grapefruit Juice without Addition of Surfactant

Experimental Method

The preparation of juice and experimental procedure were the same as those used in frothing of orange juice except that only 25 ml froth was collected in each run.



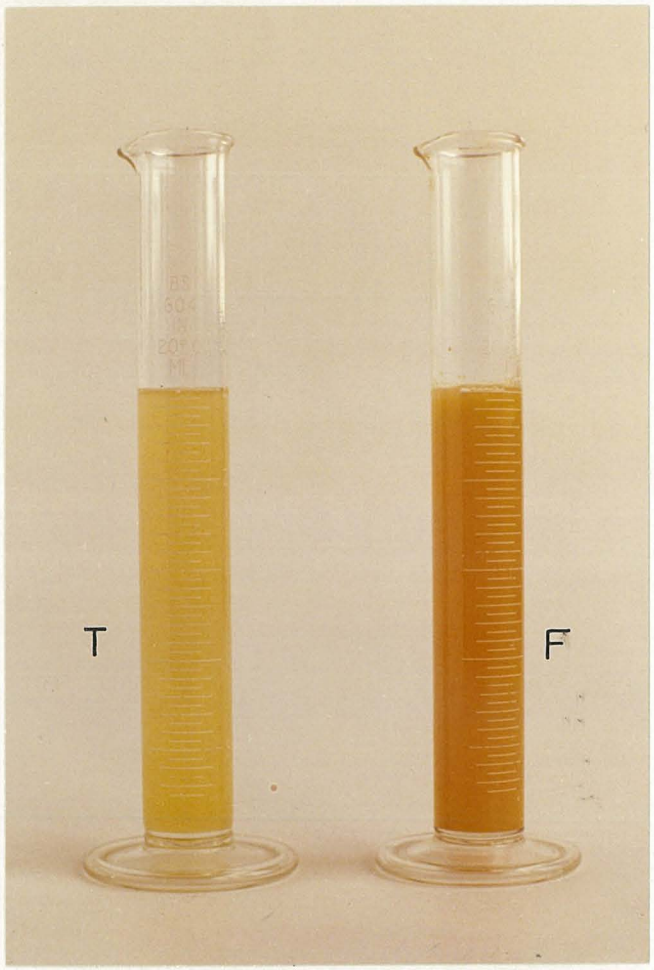
(a)

FIGURE 20 - Showing the colour of the different grapefruit juice fractions after the frothing operation.

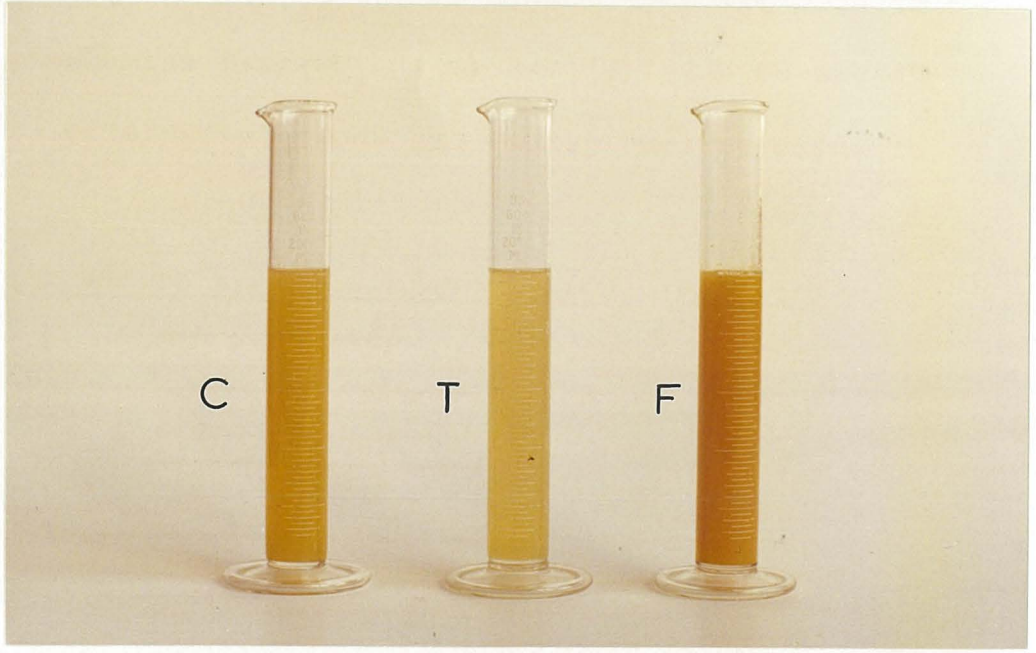
C = original grapefruit juice

T = grapefruit juice after frothing

F = grapefruit juice obtained from the froth.



(b)



(c)

FIGURE 20 (see p 129)

Determination of Colour Intensity of Juice

The test involved mixing 5 ml of juice sample with equal volume of absolute ethanol. The colorimetric test was then carried out with the filtered juice using 430 mμ blue filter on the Hilger Biochem Absorptiometer.

Determination of Oil Concentration in Juice

The test was based on Bromate Titration Method by Scott and Veldhuis (62).

Results and Discussion

(a) Effect of Frothing on Essential Oil - Similar to frothing of orange juice, there was a dramatic colour intensity difference between the froth fraction and the froth treated juice as can be best illustrated by reference to Figure 20 (a), (b) and (c). Typical colorimetric readings of these various juices are shown in Table 19.

Table 19: Colorimetric Readings of the Different Juice Fractions

% Transmittance		
T	C	F
90	86	80

The colour differences as shown by Figure 20 and Table 19 tend to suggest that the oil concentration in the froth phase was more concentrated than the original juice and very much more so when compared with the froth treated juice. This statement seemed to be supported by preliminary taste testing. It was found that, the flavour in the original juice, although not very strong, was always much more intense than the froth treated samples and the froth fraction was always the most intense.

However, to further confirm this quantitatively, determinations of essential oil content in these various juices were carried out using Scott and Veldhuis's Bromate Titration Method (62). Briefly, the analytical procedure involved distillating 25 ml juice with 25 ml 2-propanol and the distillate is titrated with bromate solution. In acid solution, the bromate releases bromine which reacts quantitatively with d - limonene, (the principal terpene hydrocarbon which constitutes about 90 - 95% of the essential oil).

The distillating apparatus was the same as that shown in Figure 17 (p 104).

At the initial stage, several tests of various juice fractions (i.e. froth, control and treated juice) were carried out using 25 ml juice and 25 ml solvent. However, the results obtained were inconsistent and sometimes contradictory. For example two typical results are given in Table 20.

Table 20: The Concentration of Recoverable Oil in various Juice Fractions

Run No.	Recoverable oil % by volume		
	T	C	F
1	0.00308	.00836	.01364
2	0.00308	.00870	.00740

It could be seen from Table 20 that, the concentrations of recoverable oil in the control and froth treated juice in both runs were approximately the same. However, the froth fractions were far from consistent, in run No.2 the froth concentration was even lower than the control. This evidence infers that irregularity only occurred in froth samples. Some effort was thus directed to looking for the cause. By

observation, it was noticed that, the juice obtained from froths always contained a considerable amount of yellowish flocular particles which possibly were demulsified oil in admixture with carotenoid pigments. Therefore, when only 25 ml of 2-propanol was used, these discrete oil particles may not be solubilized properly by the solvent before they had a chance to be dispersed and carried over into the distillate. This postulate has been proved in the subsequent tests in which twice as much solvent (i.e. 50 ml) was employed and thoroughly stirred with 25 ml juice prior to distillation. By doing so, consistent and reproducible results were obtained. Typical results are shown in Table 21.

Table 21: Concentration of Recoverable Oil in various Juice Fractions

Run No.	Recoverable oil % by volume		
	T	C	F
1	0.00264	0.0088	0.0264
2	0.00286	0.0088	0.02552

From the data in Table 21 the recoverable oil concentrations of froth fractions in both runs were approximately 3 times and 10 times of those of the control and froth treated juice respectively. This quantitative

evidence amply illustrates that frothing is a very effective means for removal of oil from grapefruit juices.

The oil contents of the grapefruit juices used here were rather low, being only about 0.009% (by volume), probably due to the variety of this particular kind of grapefruit.

None the less the majority of New Zealand grapefruit are known to contain exceptionally high oil content. Therefore, on the basis of this experiment, it appears that this frothing technique may be a plausible method for reducing such excessive oil.

Moreover, the discovery of the possible source of difficulties on determination of froth fraction in this experiment would also serve to explain the inconsistent results obtained from froth samples by Turbidimetric Method of Burdick and Allen (13) in our earlier work on canned orange juice. (Refer back to Section II, Part B, II(1), p 105)

(b) Effect of frothing on Naringin - Davis's method was used in determination of the naringin concentration. Typical results are shown in Table 22.

Table 22: Effect of Frothing on Naringin in Grapefruit Juice

% Transmittance		Naringin concentration from the standard curve mg/100 ml	
T	F	T	F
53	51.5	38	40

On the basis of the figures shown in Table 22, it is apparent that naringin concentration in the froth sample was only about 1 - 2 mg higher than that of the treated juice. The same explanation already discussed in the study of orange juice (see p126) could be used to account for the poor adsorption of naringin. Some preliminary flavour tests were carried out with the original juice and the froth treated juice. From these preliminary tests, it was found that, although according to the data given in Table 22, only a small quantity of naringin had been removed with the froth fraction, the froth treated sample seemed to taste less bitter than the original grapefruit juice. This suggests that the overall bitterness in grapefruit juice may not be due to the naringin content alone. Essential oil and possibly other compounds may

play some part in enhancing the bitter taste. This statement may be confirmed by the comparison flavour tests carried out between the juice obtained from the froth fraction and the treated juice. In this test, a distinctly more bitter and hot taste was always experienced with the froth fraction. Therefore, it is reasonable to believe that the milder taste in treated juice may be due to the combined removal of a large proportion of essential oil, some naringin and possibly other constituents present in the grapefruit juice.

However, whether the difference in bitter taste is significant is hard to determine. Since New Zealand grapefruit juice is extremely bitter, in testing, the bitter taste tends to remain on the tongue even a considerable time has lapsed. This would tend to upset the taster's subsequent attempts. Hence, unless a judge can detect the difference in the first attempt, further tests would be even more inaccurate and unreliable. Thus, to confirm whether the flavour of grapefruit juice was significantly improved by frothing, a special panel must be selected and thoroughly trained until they are able to detect detectable difference between different samples. Such a panel was not available in this experiment. As a result, a proper subjective flavour test has not been carried out. Further work would be required towards this direction.

Conclusion

Frothing appears to remove a considerable amount of essential oil and some naringin from the grapefruit juice. Preliminary flavour tests show that the froth treated juice was always less bitter than the original grapefruit juice.

(ii) Study of Frothing on Grapefruit Juice with Addition of Surfactant

In earlier study on pure naringin solution (see p 94) Tween 60 was found to be rather effective in concentrating naringin in the froth. This study was to find out whether Tween 60 will be effective as a naringin-carrier in natural grapefruit juice.

Experimental Method

0.4 ml of 5% Tween 60 solution was thoroughly mixed with 120 ml of grapefruit juice. The mixture was then subject to frothing at approximately 14" water using N_2 gas. Then several froth fractions (ff) of 3 ml each were successively collected. The results of naringin determinations made with the froth samples and the remaining treated juice are tabulated and presented in Table 23.

Table 23: Showing the Naringin Concentrations in the Froth Fractions and the Treated Juice

Samples	% Transmittance	Naringin content from the standard curve mg/100 ml
1st ff	53	38
2nd ff	54	37
3rd ff	51	40
4th ff	52	39
5th ff	54	41
6th ff	50.5	36
7th ff	51	40
treated juice	56	34

Discussion of Results

As seen from the data in Table 23, naringin concentrations in the froth fractions were all higher than the final bulk juice, the maximum difference thus far being about 7 mg/100 ml. This indicates that adsorption had taken place to some extent during the frothing process. However, in comparison with the results obtained from the study on pure naringin solution (see p 94) in which the concentrations

in the froth samples could be up to 25 mg/100 ml higher than the bulk juice, it is apparent that Tween 60 surfactant is much less effective in the grapefruit juice. This is not unexpected. On the basis of our earlier postulate (see p 97) which put forward that, there may be some loose association between naringin and Tween 60 molecules, thus, when the surfactant was being adsorbed, they would tend to carry naringin molecules along, resulting higher naringin concentration in froths. However, in grapefruit juice, apart from Tween 60 surfactant and naringin, there are so many other chemical constituents (69) it is possible that, some of these components may some way or another interfere with the association between naringin and Tween 60, hence reduce the chance of naringin to attach itself to the Tween 60 molecules. As a result, the naringin concentration in the froth was not as high as it should be. Nevertheless, the degree of naringin adsorption in the presence of Tween 60 was considerably better than when it was absent (see p 135). This evidence serves to point out that, if one finds it necessary to remove more naringin from the grapefruit juice, it could possibly be done by using an appropriate surfactant. As Tween 60 is only one of a large number of hydrophilic surfactants available on the market, it could well be that there are some other hydrophilic surface active substances which are more suitable both in terms

of taste (i.e. it must be tasteless) and effectiveness than Tween 60. To achieve this, further work is therefore required.

Conclusion

Adsorption of naringin in grapefruit juice is better in the presence of Tween 60. However, because of the interference of other components present in the juice, the adsorption of naringin is not as effective as that obtained in the pure naringin solutions.

(iii) A Study of Frothing of New Zealand Canned Grapefruit Juice

It was found that, the frothability of canned juice was very poor, probably due to the destruction of natural foaming agents such as enzymes, proteins and amino acids, during the pasturisation process. As a result all work was undertaken using fresh juices. It is recognised, however, in commercial practice, there could be a need for applying debittering and deoiling to pasturised or heat treated juices. In this instance, therefore, addition of surfactant may be necessary to make up for the reduced foamability. From preliminary observations, this is not believed to pose any major problems.

SECTION III. A PROPOSED THEORY AND MECHANISMS FOR REMOVAL OF UNDESIRABLE SUBSTANCES IN FLUID FOOD PRODUCTS USING SURFACTANT

Generally, undesirable substances present in fluid food product can be classified into two broad categories i.e. they are either hydrophilic (water-loving or polar) or lipophilic (oil-loving or non-polar).

By the same token, surfactants can be classified as hydrophilic or lipophilic depending on whether they have more hydrophilic or lipophilic groups in the molecule.

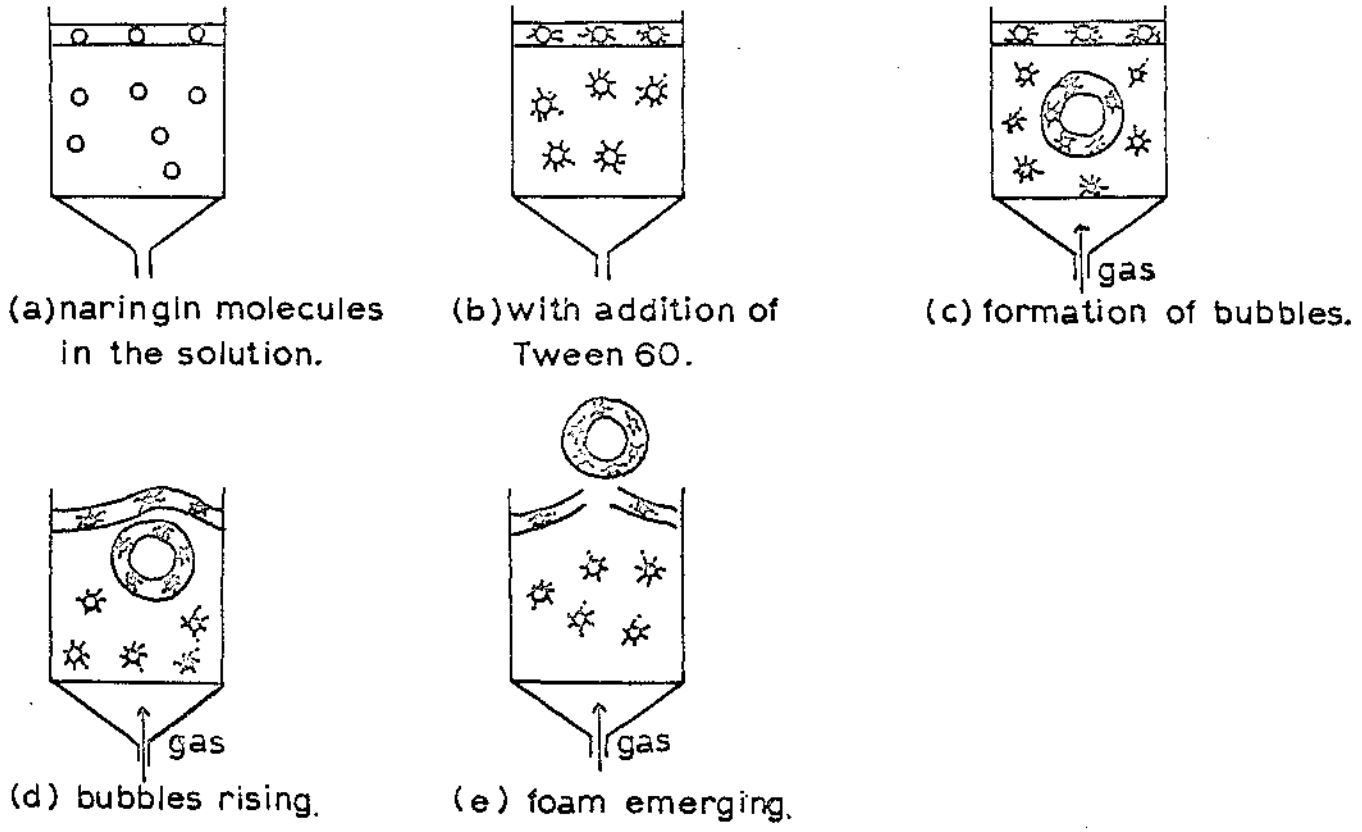
Thus when a surfactant is added in the fluid food system, it is reasonable to assume that there may be some interactions between the surfactant and the particularly undesirable substance through their hydrophilic or lipophilic groups. The extent of interaction will of course, depend on the affinity of the undesirable substance for the surfactant and vice versa. The affinity for association in turn, will be determined by the hydrophilic or lipophilic property of both the surfactant and the undesirable substance involved. For example, a hydrophilic substance would have a greater tendency to associate with a hydrophilic surfactant.

And on the basis of all the work done hitherto, it appears that, solubility of the substance is the most important single factor in determining the efficiency of its removal by frothing operation. That is, the less soluble the substance, the more efficient is its removal.

Hence, the efficiency of a surfactant to remove the undesirable substances in question may be judged on the solubility basis. This would enable one to have some valuable clues in selecting a suitable surfactant for a particular substance. To select such suitable surfactant, two general rules may be given:-

(1) For a Hydrophilic Substance, a hydrophilic surfactant is necessary, in order to establish a firm association between the surfactant and the undesirable substance. However, the optimum hydrophilic surfactant is not necessarily the most hydrophilic, but one which is hydrophilic enough to interact with the undesirable substance and yet yielding a combined surfactant - undesirable substance product which has the minimum solubility. If this is achieved then upon frothing, the undesirable substance has a great tendency to dissociate from the bulk water and concentrate at the air-liquid interface.

A practical example based on the present experimental work can be illustrated here. In the study of pure naringin solution using surfactant (see p 93), Span 40, a lipophilic surfactant was found to be ineffective in bringing out the naringin from the solution. This is possibly because naringin is hydrophilic, hence the association with Span 40 is very poor, consequently, very few molecules of naringin were carried out with Span 40 froths. On the other hand, Tween 60, a hydrophilic surfactant was found to be effective as a naringin-carrier. This suggests that naringin has an affinity for Tween 60 surfactant, therefore, when Tween 60 molecules were being adsorbed into the air-liquid interface, they tend to drag the naringin molecules along, resulting in a higher naringin concentration in the froth. However, Tween 20, a surfactant even more hydrophilic than Tween 60, was found to be not as effective as Tween 60. This phenomenon can be explained by the fact that Tween 20 is a much more hydrophilic surfactant, therefore, the combined Tween 20 - naringin will be more hydrophilic than the Tween 60 - naringin pair. In consequence, the former would have greater tendency to remain in the bulk aqueous phase. As a result, less naringin would be obtained in the froth fraction.



(a) naringin molecules in the solution.
(b) with addition of Tween 60.
(c) formation of bubbles.
(d) bubbles rising.
(e) foam emerging.

FIGURE 21. The proposed mechanism of frothing of naringin solution with Tween 60 surfactant.

The mechanism of naringin adsorption using Tween 60 can be further illustrated in the schematic diagram in Figure 21.

Figure 21 (a) shows a naringin solution prior to addition of Tween 60. In this juncture, for the sake of simplicity naringin molecules are represented by circles, and they can be seen fully dispersed throughout the aqueous phase with few molecules being adsorbed at the air-liquid surface. When Tween 60 was added into the naringin solution, interaction between Tween 60 and naringin will take place. The exact mechanism of association and orientation between the naringin and Tween 60 molecules are still unknown, it could be that, each molecule of naringin is associated with a single Tween 60 molecule according to the appropriate orientation, or several Tween 60 molecules associate with only a naringin molecule or vice versa. However, if we assume that, in this case, several surfactant molecules are associated with only one naringin molecule and the Tween 60 surfactant is represented by  (where o represents hydrophilic groups and represents lipophilic groups), the association between Tween 60 and naringin can be shown in Figure 21 (b). It can be seen that the links between naringin and Tween 60 are mainly through the hydrophilic groupings. However, there may be some Tween 60 molecules attracted by the lipophilic groups of naringin, thus leaving

(a)showing the lipophilic substance dispersing in the system before adding of any surfactant.

(b) showing the interaction between the lipophilic substance and the added surfactant.

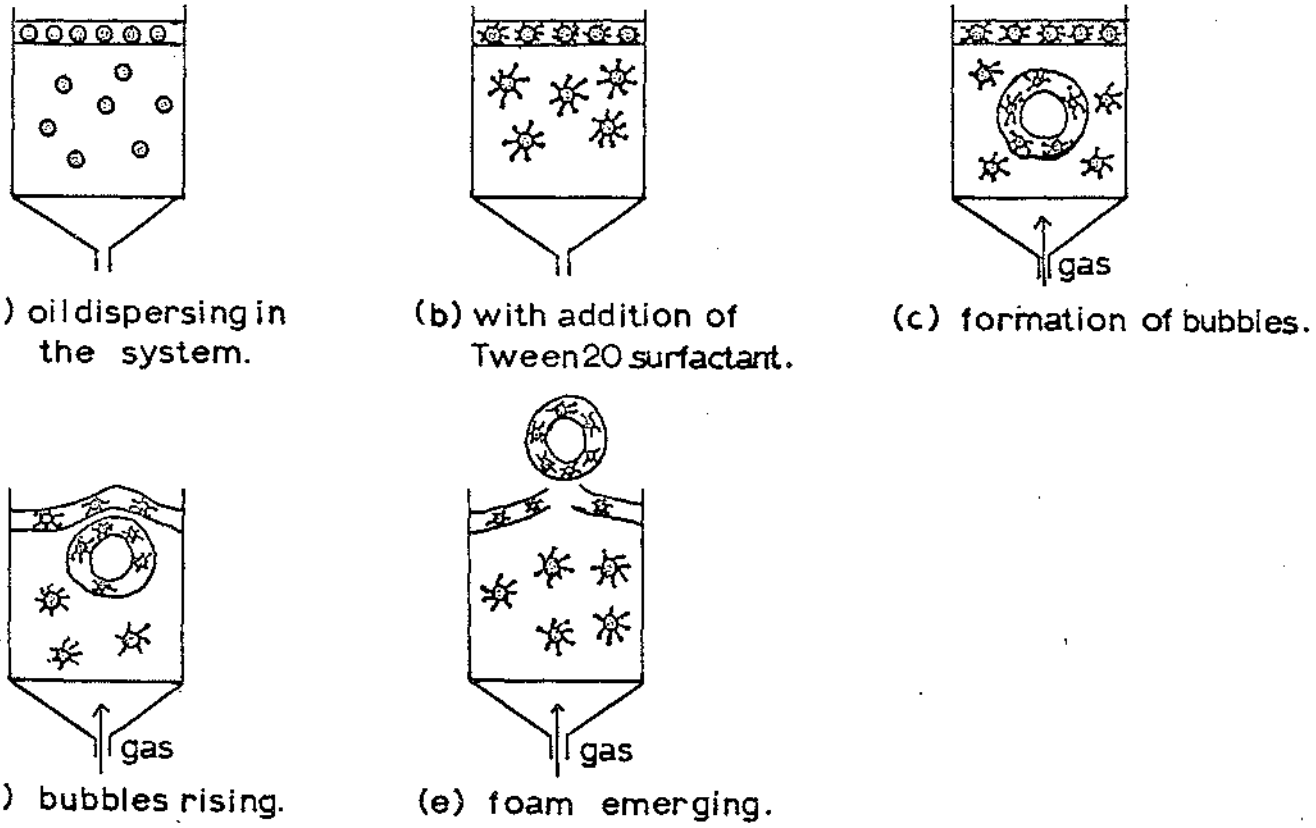
FIGURE 22 Diagrams showing the association of the lipophilic substance with the added surfactant.

some hydrophilic groups facing the bulk liquid. When gas was introduced into the system, bubbles will be formed and the combined naringin-Tween 60 will be adsorbed at the air-liquid interface, as shown in Figure 21 (c). The bubble gradually rose to the surface adsorbing more naringin-Tween 60 combinations on the way and finally emerged as foam, as can be illustrated by Figure 21 (d) and (e).

(2) <u>If the Undesirable Substance in the Fluid Food Product is Lipophilic</u>. Then, according to the solubility theory, the logical choice of surfactant will be lipophilic surfactant. Since, it would associate well with the lipophilic substance and the overall solubility of the combined products will be less than when hydrophilic surfactant is used. This can be demonstrated diagrammatically in Figure 22 in which the lipophilic undesirable substance was represented by a circle. In Figure 22 (a), the lipophilic substance was shown dispersing in the aqueous phase prior to adding of surfactant. When a lipophilic surfactant is introduced, the association between the surfactant and the lipophilic substance takes place. The lipophilic groups of the surfactant will naturally interact with the predominantly lipophilic groups in the undesirable substance, leaving

the hydrophilic parts facing towards the bulk aqueous solution. However, owing to the comparatively low percentage of hydrophilic groups in the surfactant, the overall solubility of the combined product is still very low. Therefore, they would tend to concentrate greatly at the air-liquid interface upon frothing. On the other hand, if a hydrophilic agent is introduced, the manner in which the surfactant associates with the lipophilic substance will be exactly the same as shown in Figure 22. However, because they contain more hydrophilic groups, they will confer more hydrophilic character to the combined products making them more soluble. Thus, it is reasonable to expect less efficient removal of the lipophilic substance upon frothing process.

However, from evidence obtained in the present work, it seems that for a highly lipophilic substance such as essential oil, the choice of the surfactant is not as critical as in the case of naringin, and hydrophilic surfactant may be used quite satisfactorily for its removal. For example, in the studies involving frothing of essential oil in dispersion with carotene (see p 113), Tween 20, a hydrophilic surfactant was found to be quite effective for removal of essential oil. This may be due to the fact that essential



(a) oil dispersing in the system.

(b) with addition of Tween 20 surfactant.

(c) formation of bubbles.

(d) bubbles rising.

(e) foam emerging.

FIGURE 23. Diagrams showing the proposed mechanism of the frothing of carotene/essential oil system with Tween 20 surfactant.

oil is so highly lipophilic that they are present in the aqueous phase as dispersing droplets. An addition of a hydrophilic agent like Tween 20 may contribute slightly more hydrophilic property to the dispersed oil droplets, non the less, the overall solubility of the combined product is still so low that they are greatly adsorbed and removed with the outgoing froths.

The frothing of a solution containing carotene, essential oil and Tween 20 surfactant can be illustrated diagramatically in Figure 23.

Figure 23 (a) shows the oil droplets dispersed in the aqueous phase with carotene (represented by red dots) soluble in them. It is reasonable to assume that, there was a considerable essential oil-rich layer on the surface.

When Tween 20 was introduced into the system, Tween 20 molecules will associate with the oil droplets in such a way that most of their lipophilic groups would emerse in the oil phase, leaving the hydrophilic parts facing the bulk aqueous solution. (see Figure 23 (b)) Hence the stability of the dispering droplets was considerably increased. Upon introduction of gas, bubbles were formed (only one such bubble is shown in Figure 23 (c)), resulting in enormous increases in the air-liquid interface. Due to their low

solubility in the aqueous phase, the combined Tween 20 - essential oil would tend to concentrate at these interfaces as shown in Figure 23 (c). The bubble rose slowly and at the same time picking up more Tween 20 - essential oil droplets. The bubble reached the surface, possibly attracting more oil droplet from the surface layer and finally emerged as froth, as can be shown in Figure 23 (d) and (e).

Conclusion

A theory to account for the removal of undesirable substances has been put forward. By selecting the appropriate surfactants, according to the hydrophilic or lipophilic property of undesirable substances, an efficient removal may be resulted.

IV. FIELDS FOR FURTHER RESEARCH

Certain aspects of this work on frothing processes, particularly with respect to its application in the citrus field was somewhat hampered by raw material availability and thus it was not possible at certain time of the year to follow through studies as in great a detail as would have been wished. For this reason a number of further lines for research are suggested as follows:

(1) In the frothing study of pure naringin solutions (see p 94), it was found that, using Tween 60, a hydrophilic surfactant, a significant amount of naringin could be removed with the froth. And when Tween 60 was applied to grapefruit juice(see p 138), the naringin concentrations in froth fractions were higher than those obtained from juices without the surfactant. This suggests that, if a suitable surfactant is chosen, naringin may be efficiently removed by frothing techniques. However, Tween 60 may still not be the best surfactant. Moreover, its slightly bitter taste makes it hardly suitable for practical purposes. Further research should therefore be directed to this field so that those surfactants suitable for removal of naringin can be discovered.

(2) Because analytical method for limonin is complicated and lengthy, detailed studies of the effect of frothing on limonin content in citrus juices have not been undertaken. However, it was suggested that, (see p 126), because limonin present in citrus juices is normally in the form of suspension, it is most probable that, a large proportion of limonin would be removed with the outgoing froth Further work is thus needed to confirm this.

(3) So far, no study has been made on the effect of frothing on nutrient concentrations (particularly vitamin C contents) of citrus juice. Such a study may be necessary if frothing technique is to be adopted as a possible method for improving the flavour of citrus juices.

V. BIBLIOGRAPHY

1. ABRIBAT, M. - Actualites Scientifiques et Industrielles. Hermann et C. Paris. 932: 123-175. 1942; C.R. 209: 244-6. 1939.
U.S. Patent 2,313,007. March 2, 1943.

2. AMERINE, M.A., - Roessler, E.B., and Filipello, F. Hilgardia Vol 28, P 525, (1959).

3. ANDREWS, G., and SCHUTZ, F. - Biochem J. (London) 39, li (1945).

4. ARRAZOLA, J.M., - Ion (Madrid), 2, 119(1942); Chem Abstr. 37, 1301^{9}(1943).

5. ASACHINA, Y., and INUBUSE, M. - J. Pharm. Soc. Japan 49: 128-34. 1929.

6. BADER, R. et al. - Nature, 154, 183 (1944)

7. BAILEY, M.I., - Ind. Eng. Chem. 27: 973(1935)

8. BARMORE, M.A., - Colo. Agr. Expt. Sta., Tech. Bull. 9, 58 pp (1934); Chem. Abstr, 38, 2979^{9} (1944)

9. BERKMANN, S., and EGLOFF, G. - Emulsion and Foams, Reinhold, New York, 1941.

10. BICKERMANN, J.J., - Foams, Thoery and Industrial Applications, Reinhold, New York, 1953.

11. BLAIR, J.S., GODAR, E.M. MASTERS, J.E. and RIESTER, D.W., - Food Research. 17, 235-260 (1952).

12. BOYD, J.M., and PETERSON, G.T., - Indus. and Engin. Chem. 37: 370-373 (1945).

13. BURDICK, E.M., and ALLEN, J.S., - Analy. Chem. Vol 20. 539-541 (1948).

14. BURDICK, E.M., and MAURER, R.H. - U.S. Patent 2, 510, 797 (June 6, 1950).

15. Calif. Citrog. 26(12): 360. 380, 382.

16. CHANDLER, B.V., and KEFFORD, J.F., - Journal of the Science of Food and Agriculture, Vol 17, No.5. May, 1966 pp 193-197.

17. CLAYTON, W. - "Colloid Aspects of Food Chemistry and Technology". J. and A. Churchill London (1932).

18. CLEVENGER, J.F. - Journal of the A.O.A.C. 17, 371-372 (1934) or U.S. Dept. Agriculture. U.s. Standards for grades of Canned Grapefruit Juice. Production and Marketing Administration .

19. CRUICKSHANK, E.M. - U.S. Egg and Poultry Mag. 47: 22, 64 (1940).

20. CURL, A.L. - Journal of the A.O.A.C. 30, 567-575 (1947).

21. DAVIS, S.G., FELLERS, C.R. and ESSELER, W.B. - Food Research, 14, 417 (1949).

22. DAVIS, W.B. - Analytical Chemistry 19, p 476-478 (1947).

22a. Dean, F.M. - "Naturally occurring oxygen ring compounds p 22, Butterworths, London (1963).

23. DOGNON, A. - Actualites Scientifiques et Industrielles. Hermann et C. Paris 932: 157-172, 1942; Bull. Soc. Chim Biol. 23: 249-63, 1941; Rev. Sci. 79: 613-19, 1941.

24. DOGNON, A & H - Dumontet, C.R. Soc Biol 135: 884, 1941.

25. EL-RAREY, M.S., and RICHARDSON, G.A. - J. Dairy Sci., 19-13, (1944).

26. FOULK, C.W., and MILLER, J.N. - Ind. Eng. Chem. 23, 1283, (1931).

27. FREUNDLICH, H. - "Colloids and Capillary Chemistry" translated by C. Barger, Methuen & Co., and E.P. Dutton & Co., 1924.

28. FREUNDLICH, H. - "The Element of Colloidal Chemistry" translated by C. Barger, Methuen & Co., and E.P. Dutton & Co., 1924.

29. GIBBS, J.W. - Trans. Conn. Acad, 3, 439 (1876).

30. GLASSTONE, S., and LEWIS, D. - Element of Physical Chemistry, 2nd ed., London, Macmillan, 1960.

31. GRAY, P.P., and STONE, I. - Wallerstein Labs Communs, 3, 159 (1940); Chem. Abstr 35, 3029^{5} (1941).

32. HALL, D.H. - Chemistry and Industry 57, 473 (1938).

33. HANNING, F.M. - J. Sci. 20: 10 (1945).

34. HENRY, R.E., and CLIFCORN, L.E. - Canning Trade 70, (31): 7-8, 22, (1948).

35. HENRY, W.C., and BARBOUR, A.D. - Ind. Eng. Chem. 25: 1054 (1933).

36. HESS, K., and HESS (c) - Ber, 81, 354 (1948).

37. HIGBY, R.H., - Amer. Chem. Soc. Jour. 60: 3013-3018 (1938).

38. HIGBY, R.H. - U.S. Patent 2, 421, 061, May 27, 1947. Chem. Abstr. 41, 6000, 1947.

39. JENNESS, R., and PATTON, S. - "Principles of Dairy Chemistry. John Wiley & Sons Inc., New York. Chapman & Hall Limited, London.

40. KARRER, P., and JUCKER, E. - "Carotenoids". Translated and revised by Ernest A. Brande p 5, (1950).

41. KESTERSON, J.W. and HENDRICKSON, R. - Naringin a bitter principle of grapefruit. Fla. Agr. Exp. Sta. Tech. Bull 511, 1-35, 1953.

42. KITCHENER, J.A. and COOPER, C.F. - Quart. Revs (London), 13, 71 (1959).

43. LONDON, M., COHEN, M., HUDSON,P.B. - J. Am. Chem. Soc., 75, 1746 (1953).

44. LONDON, M., COHEN, M., and HUDSON, P.B. - Biochem et Biophys. Acta, 13, 11 (1954).

45. LONGWORTH, L.G., CANNAN, R.K. and MacINNES - J. Amer. Chem. Soc. 62: 2580 (1940).

46. LOWE, B. - Experimental Cookery, 4th Ed. p 337 John Wiley & Sons Inc., New York. Chapman & Hall Limited, London.

47. McCOLLOCH, R.J. - Calif. Citrog. 35: 290, 292 (1950).

48. MEYER, W. - Centr. Zuckerind 48, 449 (1940) Chem. Abstr. 36, 5375 (1942).

49. MILLER, J.M., RICE, R.G. AND OTHERS - Jour. Agr. and Food Chem. 1: 510-512 (1953).

50. MORGAN, D.A., VELDHUIS, M.K. ESQUE, R.K. and PHILLIPS, G.W.M. - Food Tech. 7, 332-336 (1953).

51. NISSEN, B.H., and ESTES, C. - Am. Soc. Brewing Chemists Proc, 23, 1940.
Chem. Abstr. 35, 3029^{8} (1941).

52. OSIPOW, L.I. - Surface Chemistry: Theory and Industrial Application. N.Y., Reinhold, 1964.

53. OSTWALD, Wo., and SIEHR, A. - Kolloid Z, 76, 33-46 (1936).
Chem Abstr. 30, 6620^{4}, 1936.

54. OSTWALD, Wo., and MISCHKE, W. - Kolloid Z, 90, 205 (1940).

55. PERRI, J.M., and HAZEL, F. - Ind. Eng. Chem. 38, 549 (1946).

56. RAMSDEN, W. - Arch Physiol p 1517 (1894);
Proc. Roy. Soc. London, B72, 156 (1903)
Z. physik. chem., 47, 336 (1904).

57. RICE, R.G., KELLER, G.J., and BEAVENS, E.A. - Food Tech. 6: 35=39 (1952).

58. ROMANOFF, A.L., and ROMANOFF A.J. - The Avian Egg. John Wiley & Sons. 1949.

59. RUBIN, E., GADEN, E.L. Jr. - "Foam Separation"
New Chemical Engineering Separation Techniques.
Edited by Herbert M. Schoen.
Interscience, New York - London 1962 pp 319-385.

60. SCHNEPF, R.W., GADEN, E.L. - J. Biochem and Microbiol. Tech. and Eng., 1, No. 1, 1 (1959).

61. SCOTT, W.C. - Journal of the A.O.A.C. 24, 165-170 (1941).

62. SCOTT, W.C. and VELDHUIS, M.K. - Journal of the A.O.A.C. Vol 49, No. 3, pp 628-633, (1966).

63. SHEDLOVSKY, L. - Annals of the New York Academy of Sciences Vol 49, pp 279-294 (1948).

64. SMITH, A.K., NASH, A.M., ELDRIDGE, A.C., and WOLF, W.J. - Journal of Agri. and Food Chem. 10, p 302 (1962).

65. SPENGLER, O. and DORBELDTZ, W. - Chem. Abstr. 37, 6923 (1943).

66. THE ATLAS HLB SYSTEM - Atlas Chemical Industries Inc.

67. THOMAS, D.W., SMYTHE, C.V. and LABBEE, M.D. - Food Research. Vol 23, p 591, 1958.

68. TING, S.V. - J. Agri. and Food Chem. 6, 546 (1958).

69. United States Department of Agriculture, Washington, D.C. "Chemistry and Technology of Citrus, Citrus Products and by-Products". Agriculture Handbook No. 98, Nov. 1956.

70. U.S. Patent No. 2, 357, 895, (1944).

71. VELDHUIS, M.K. et al. - U.S. Patent No. 2, 510, 138 (1950).

71a. Van SLYKE, L.L., and PRICE,W.V. - "Cheese". Orange Judd Publishing Co. New York p 58 (1932).

72. WEISER, H.B. - "A text book of Colloid Chemistry" 2nd Ed. John Wiley & Sons Inc., New York. Chapman & Hall, Limited, London (1949).

73. WOLFORD, R.W., ALBERDING, G.E., ATTAWAY,J.A. - Journal of Agri. and Food Chem. Vol 10, p 297, 1962.

74. WU, H. and LING, S.M. - Chinese J. Physiol, 1, 407 (1927).

75. YOUNG, E.G. - J. Biol. Chem. 120: 1 (1937).

76. ZEMPLEN, G., and REZSO BOGNAR - Chemischen Gesell 76: 773-775, 1943.