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Extraction of Polyphenolics from Apple Juice by Foam Fractionation

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Abstract

This research was undertaken to test the feasibility of using the foam technology to separate polyphenols from fruit juices for use as functional food ingredients. The separation performance, expressed as enrichment ratio, selectivity and percentage recovery, was determined as a function of operating variables, namely air or N₂ flow rate, initial feed concentration, bubble size, solution pH and the presence of alcohol to modify the surface tension. Measurements were made of the average bubble size and gas hold-up volume to calculate interfacial area. The bulk phase concentrations of the polyphenolics in the feed and foam fractions were analysed for total phenolic content by Folin assay and phenolic composition by reverse phase HPLC. Enrichment factors of up to 6 were found under optimum conditions of low sugar concentration (6-9 °Brix), low flow rate (0.2-0.6 ml min⁻¹) and acidic pH (3-4). However recoveries were low at around 30% of total phenolics and selectivity was poor. It was concluded that foam fractionation represents a potential low cost technology to recover a proportion of the polyphenolic content in an enriched juice concentrate suitable for use as a functional ingredient.

KEYWORDS: foam fractionation, extraction, polyphenolics, apple juice, bubble size, superficial velocity, interfacial area

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1. Introduction

Foam fractionation is a commercially available technology that can be a cost effective method for large-scale separation of dissolved solutes from liquid process streams. It is an adsorptive bubble separation technique that selectively separates surface active compounds from the bulk solution by partitioning them onto the gas-liquid interface thereby resulting in concentration in the foam phase at the top of the bulk liquid [7,10,15,17].

Foam fractionation is carried out by generating a dispersed phase (gas bubbles) by using mechanism such as a porous plate-type gas diffuser or sparger. The gas bubbles travel through a liquid column and carry adsorbed solute species into the foam phase at the top of the column. The liquid drainage from the thin film (foam lamellae) between air bubbles causes the foam to collapse resulting in a concentrated foamate solution [10,11]. In batch operations, the separation is done by physically separating foam from bulk solution and in continuous operations by collecting overflow and underflow streams [3,9,11].

Adsorptive bubble separation methods have been utilised in removal and concentration of surface-active ions from the dilute aqueous solutions such as waste process streams [10,12,25]. Alternatively, non-surface active species and weakly surface-active ions and molecules such as phenols can be removed by interaction with the surface-active agents [10,11,12]. Crofcheck *et al.* [8] used foam fractionation to successfully recover non surface-active protein from tobacco extract. The protein of interest was tagged with a histidine sequence forming a complex with cobalt ions and a surfactant possessing a chelating functionality. The protein was recovered in foamate in foam fractionation step. In another study, Saleh *et al.* [19] were able to separate bovine serum albumin (BSA) from a multi-component mixture of proteins using semi-batch foam fractionation processing.

Higher gas flow rates result in lower enrichment because of more liquid entrainment in the foam. The height of feed liquid in the column also affects the enrichment because higher residence times for bubbles in the liquid phase result in higher surface concentration of product at the gas-liquid interfaces [26]. Feed solution concentration, solution pH and bubble size are other important parameters affecting product enrichment and recovery. Higher feed concentration lowers the surface tension, resulting in increased wet foam formation. Higher protein concentration in foam lamellae causes higher liquid viscosity, which decreases the rate of liquid drainage from foam [3].

In this present work foam fractionation has been applied to the extraction of polyphenolics from diluted apple juice concentrate. A wide spectrum of polyphenolics is found in pressed apple juice. They contribute to both colour, due to polyphenol oxidase catalysed browning, and bitter or astringent tastes [1,2].

Removal of part or all of the polyphenolic content of the juice aids in the preventing formation of polymerised polyphenolic-protein complexes that can cause undesired clouding in otherwise clear juices [4]. Additionally, polyphenolics have been recognised as the major source of health promoting antioxidants in apple juice [5,14,24]. Concentrates of polyphenolics are therefore being developed as functional food ingredients [22].

Plant polyphenolics have amphiphilic properties usually containing both hydrophobic phenolic groups as well as hydrophilic hydroxyl groups or glycosidic linkages. They can be effectively adsorbed from aqueous solution onto hydrophobic materials [20]. Thus they would be expected to partition to gas-liquid interfaces generated in foam fractionation. The investigation therefore involved the study of various operating parameters such as feed concentration, feed pH, bubble size distribution, gas flow rate and their effect on enrichment and recovery of polyphenols.

2. Materials and Methods

2.1 Materials

Clear apple juice concentrate with an enhanced level of polyphenols (EPAJC) contained 75 °Brix sugar and cloudy apple juice concentrate (CAJC) at 40 °Brix sugar were supplied by ENZAFOODS New Zealand Ltd. Catechin and Folin Ciocalteu's phenol reagent (2N) were from Sigma (Mo., USA), anhydrous sodium carbonate (GR grade) from Merck (Germany), hydrochloric acid (35%, reagent grade) from Scharlau Chemicals (Spain) and sodium hydroxide pellets were from BDH (UK).

2.2 Performance Criteria

The performance of foam fractionation is commonly expressed in two terms: (a) enrichment; and (b) recovery. Enrichment factor (E) is the ratio of the polyphenol concentration in the foam to that in the feed solution, whereas recovery (R) is the percentage of feed polyphenol recovered in the foam [6].

2.3 Analyses

Spectrophotometer analyses were performed with a SpectraMax micro plate reader (Molecular Devices, Ca., USA) and gas flow was measured using a Humonics Model ADM1000 digital flow meter (Humonics, Ca., USA). Chromatographic analyses were performed using a Shimadzu HPLC system with an SPD M10A diode array detector. Reverse phase HPLC separations were

performed on a Phenomenex (NZ) Synergi 4 μ Hydro RP 80 Å column (250X4.6 mm) at 35°C using a 40 μ l injection volume. The binary mobile phase consisted of (A) acetonitrile: water 5:95 v/v containing 0.1% v/v formic acid and (B) acetonitrile containing 0.1% v/v formic acid. Separations were developed at a flow rate of 1 ml/min in the following gradient series: 0-5 min 100-91.3% A, 15-25 min 91.3-83% A, 25-30 min 83-80% A, 30-39 min 80-70% A, 39-43 min 70-50% A, 43-48 min 50-5% A, 55-65 min 100% A. Individual compounds were quantified at 280 nm using calibration curves of known standards. Total phenolics were measured using the Folin method by the procedure of Singleton *et al.* [23] with minor modifications and using a catechin reference standard.

2.4 Experimental Procedures

The foam fractionation column was an 830 mm long glass column having an internal diameter of 30 mm and a plastic elbow fitted to the top end as shown in Fig.1. Compressed air or nitrogen was introduced into the bottom of the column via a stainless steel frit with mean pore size 10 μ m.

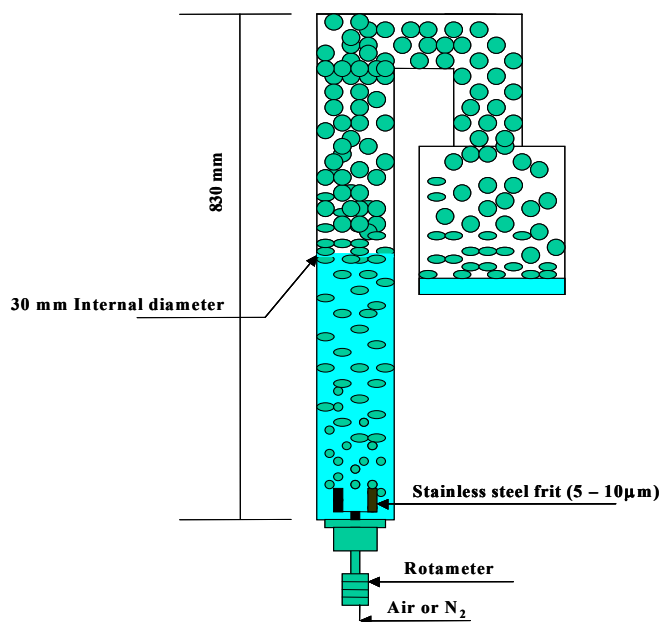


Figure 1: Schematic for a foam fractionation column

A 650 ml feed solution was prepared by mixing EPAJC or CAJC in reverse osmosis water and the pH was adjusted by adding either 1.0 N NaOH or 35 % HCl. The apple juice feed was contacted with gas (air or N₂) bubbles rising from the frit at the bottom of the column. Compressed gas supplied through a pressure

regulator valve and its flow measured by digital flow meter. The foam was allowed to overflow at top of the column through a plastic elbow into a collection vessel and then allowed to collapse. During experiments using N₂, the foam was collected in a glass vessel through plastic tubing and purged with N₂. The mouth of vessel was covered with Parafilm[®] for all experiments to avoid any evaporation losses. The residue volume left in column after completion of the experiment was measured and from that the approximate foam volume was deduced. When the foam had collapsed, the foam volume was measured and the liquid was analysed for total phenolic concentration and HPLC.

2.5 Bubble size distribution, gas hold-up and interfacial area

A feed solution with 4.0 mg l⁻¹ polyphenolics concentration at pH 4 was used to measure the bubble size distribution using 3.54x10⁻⁴ to 1.42x10⁻³ cm s⁻¹ superficial gas velocity. A Nikon Coolpix 4500 digital camera was used to photograph the bubbles from a distance of 100 mm. The bubble size distributions were measured by photographing a foam fractionation column at six locations along the column: 700, 600, 450, 350, 200, 100 mm from the bottom and the average bubble size at each of these locations was estimated. Two projectors and a flash were used to freeze the bubble action, yielding well-focused pictures. The pictures were scanned into a computer, where they were enlarged and the bubble diameters were measured with image analysis software (Image J: <http://rsb.info.nih.gov/ij/>). Around 30–120 bubbles were measured in each photograph.

The percentage gas holdup was measured in a batch of liquid pool by visually observing the drop in level in the liquid pool in response to a sudden shut-off of the rotameter. As the rotameter was shut off, the liquid level first dropped before rising again as a result of the liquid drainage from the foam. The maximum level drop was recorded and interpreted as the gas holdup [16]. Interfacial area (A , cm²) in the liquid pool is related to the percentage gas holdup by Eq. (1),

$$A = \frac{6A_{column}H_L\varepsilon}{\bar{d}_{32}} \quad (1)$$

where H_L is the liquid pool height, A is the cross sectional area of the column, in this case 28.26 cm², ε , dimensionless constant, and \bar{d}_{32} is the average Sauter mean diameter in the liquid pool when it is evenly divided into k sections, in our case it is six.

\bar{d}_{32} is calculated using Eq. (2)

$$\bar{d}_{32} = \frac{k}{\sum_{j=1}^k 1/d_{32,j}} \quad (2)$$

while for each individual section, $d_{32,j}$ is calculated using Eq. (3)

$$\bar{d}_{32} = \frac{\sum_{i=1}^n d_{ij}^3}{\sum_{i=1}^n d_{ij}^2} \quad (3)$$

3. Results and Discussion

3.1 Comparison of EPAJC and CAJC using N₂

3.1.1 Effect of gas flow rate

The effect of gas flow rate on enrichment ratio and recovery is shown in Fig.2. The flow rates used ranged from 0.5 ml min⁻¹ to 2.0 ml min⁻¹ with corresponding superficial gas velocities from 2.95x10⁻⁴ cm s⁻¹ to 1.18x10⁻³ cm s⁻¹. The enrichment ratio decreased and recovery increased with increasing gas flow rate as shown in Fig. 2. A further increase in gas-flow rates resulted in higher volume of wet foam due to the short residence time for the foam to drain the liquid, leading to increase in recovery but decreased enrichment ratios.

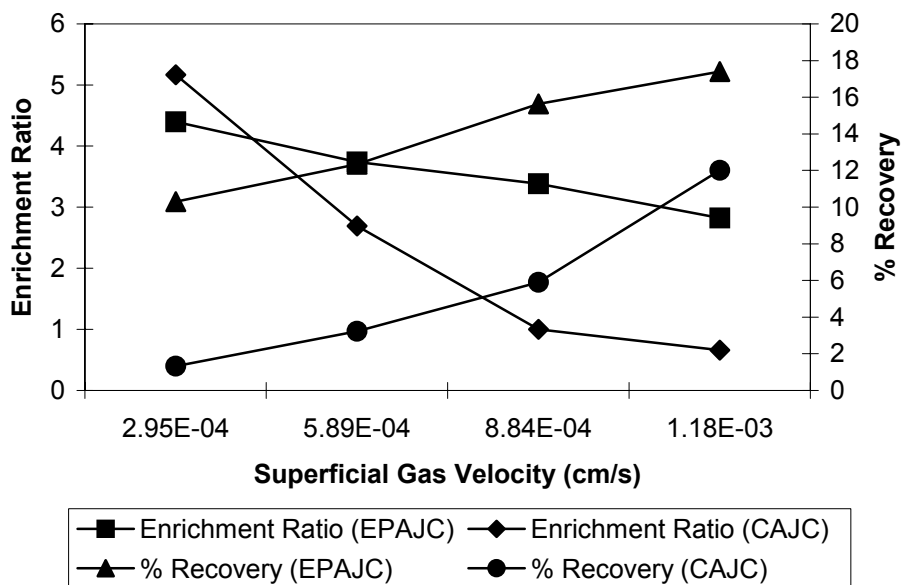


Figure 2: Effect of Gas flow rate on enrichment ratio and recovery at feed concentration 0.017 g l⁻¹ and feed pH 3.0

3.1.2 Effect of feed concentration

The effect of feed concentration is shown in Fig.3. The enrichment ratio increased with a decrease in feed concentration. The wetness of foam decreases with decreasing the feed concentration since surface tension is increased by addition of more water. Dilute solutions prevent the preferential adsorption of higher molecular weight species such as sugars by altering the surface tension. The foam thus formed is very much saturated with surface-active compounds and less stable. Lower protein concentration in foam lamellae results in lower surface liquid viscosity leading to increased rate of liquid drainage from foam. The optimum values of enrichment ratio and percentage recovery were observed at different feed concentrations for different feedstocks examined. For EPAJC, the optimal total phenolic content corresponds to 28 mg of catechin equivalent per litre (dilution factor of 12) whereas for CAJC the optimum value was 47 mg of catechin equivalent per litre (dilution factor of 9).

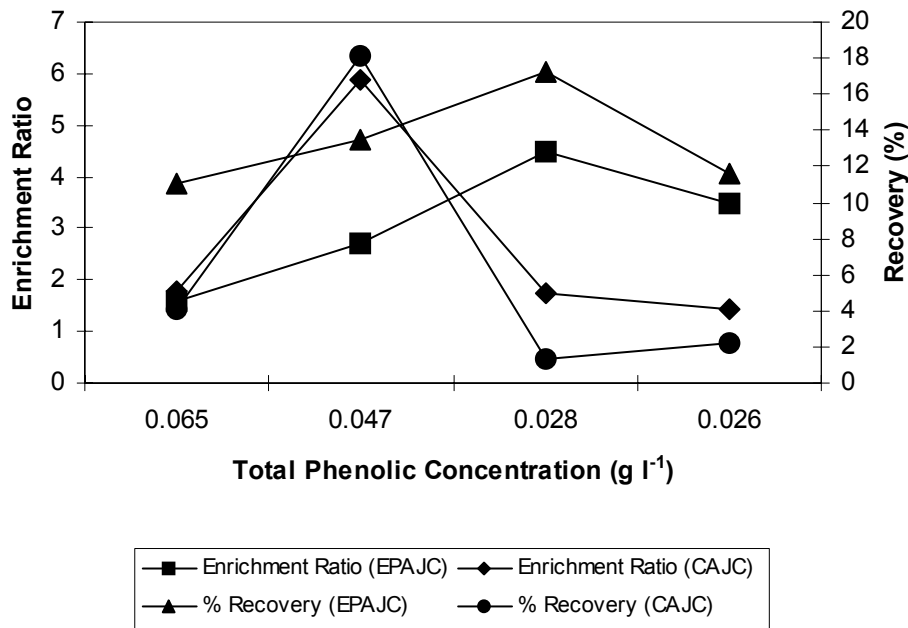


Figure 3: Effect of feed concentration on enrichment ratio and recovery at feed pH 3.0 and gas flow rate 0.5 ml min⁻¹.

3.1.3 Effect of feed pH

The effect of feed solution pH on enrichment and recovery of polyphenols is shown in Fig.4. Effectiveness of foam fractionation was dependent on the pH of the initial feed solution. Enrichment ratio is proportional to the rate of change of

surface tension with pH in the bulk liquid [21]. Variation in pH varies the surface tension and foam properties by altering the adsorption properties of protein-polyphenol complex at gas-liquid interface and hence extent of foam generation during foam fractionation process [13,18]. The maximum enrichment ratio is obtained at the isoelectric point of the protein-polyphenol complexes whereas some substances cannot be foamed at a pH other than the isoelectric point because of poor foam stability [26]. A decrease in enrichment ratio was observed for EPAJC with an increase in pH from 2 to 4. This ratio increased as pH increased from 4 to 5 suggesting the isoelectric point of the polyphenol complex to be somewhere near pH 4. With CAJC the optimum enrichment was observed at pH 5. This may be because it has a relatively higher content of proteinaceous matter as compared to EPAJC.

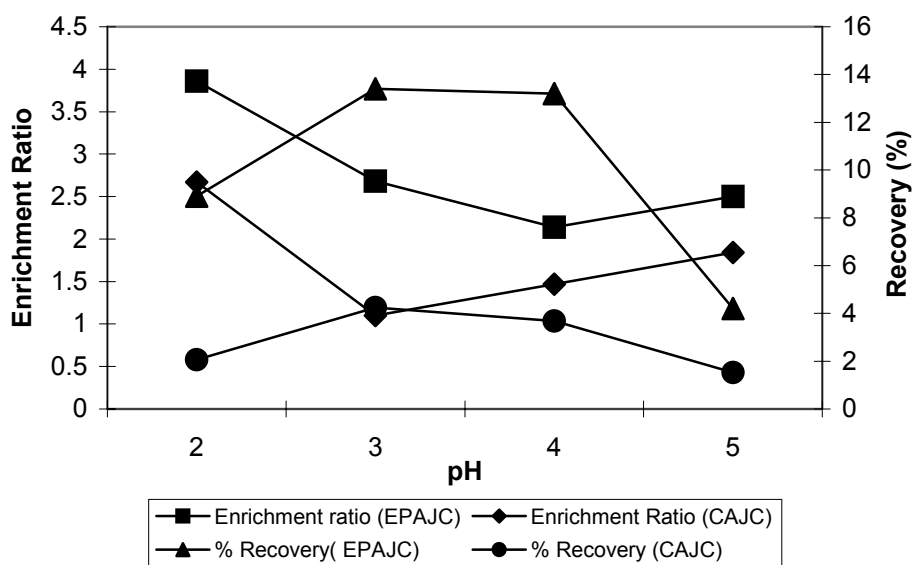


Figure 4: Effect of feed pH on enrichment ratio and recovery at feed concentration 0.017 g l^{-1} and gas flow rate 0.5 ml min^{-1} .

3.2 Performance of EPAJC using N_2 and Air

The comparative results of effect of nitrogen and air flow rate on enrichment and recovery of polyphenols from EPAJC are shown in Fig.5, 6 and 7.

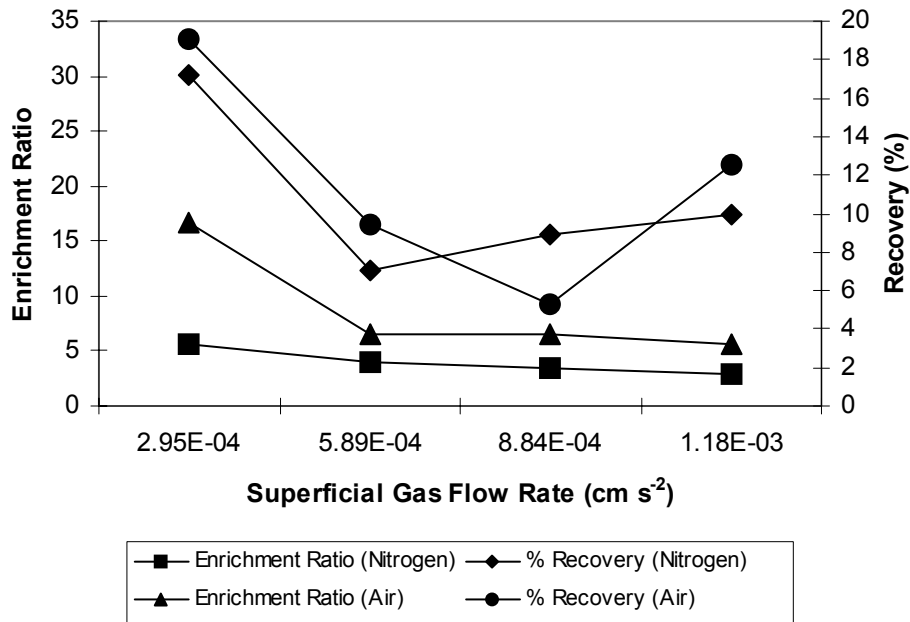


Figure 5: Effect of nitrogen and air on enrichment and recovery at feed pH 3.0 and feed concentration 0.017 g l⁻¹.

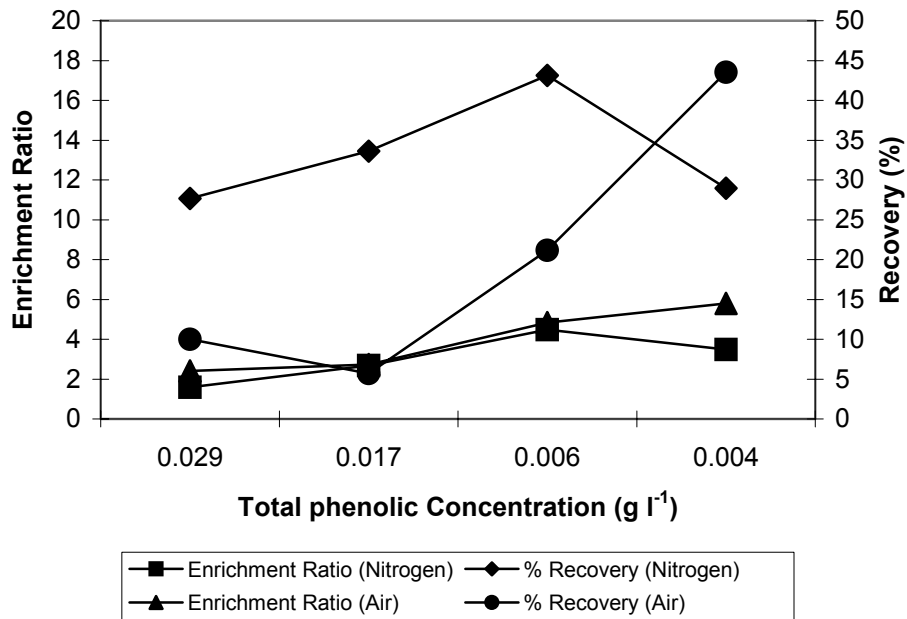


Figure 6: Effect of feed concentration on enrichment and recovery at feed pH 3.0 and gas flow rate 0.5 ml min⁻¹.

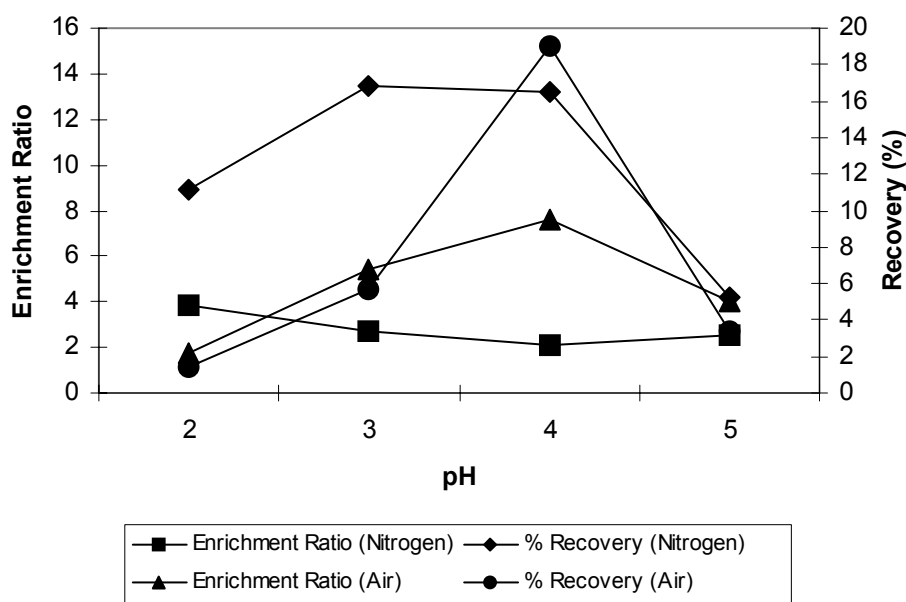


Figure 7: Effect of feed pH on enrichment and recovery at feed concentration 0.017 g l^{-1} and gas flow rate 0.5 ml min^{-1} .

Although, the enrichment trend in all cases is in agreement with the theory i.e. increase in enrichment with increase in dilution or decrease in initial feed concentration and decrease in enrichment at higher superficial gas velocities, the percentage recovery shows an inverse trend when the feed solution was subjected to air. High values of enrichment were obtained when air was used for sparging. The oxygen present in air oxidises low molecular weight polyphenols, which polymerise to higher molecular weight polyphenols (i.e. Tannins). Baseline humps were observed in the HPLC chromatographs, which indicated that tannins were formed and then concentrated in the foam. The problem of oxidation and hence polymerisation associated with use of air was considerably mitigated although not completely prevented by using nitrogen as a gaseous phase.

Some of the apple phenolics were concentrated into the foam. Quercetin galactoside, and *o*-coumaric acid showed 20% and 22% increase respectively in the foamate solution compared to the original bulk liquid. The concentration of other phenolics changed less than 12%.

Recovery was found to be relatively low (up to 40%) in all cases due to the limited concentration of proteins in the feed. The proteins act as a surfactant at the foam liquid interface and generate the foam. Therefore, it is possible to improve the recovery by adding food safe surfactants or proteins to enhance the foaming process.

3.3 Effect of alcohol addition on enrichment and recovery

Additional experiments were carried out with EPAJC and CAJC with 1% and 3% methanol added to the feed solution. Table 1 gives the enrichment and recovery values for both EPAJC and CAJC with addition of alcohol.

Table 1: Effect of alcohol addition on enrichment ratio and % recovery at pH 3.0 Gas flow rate 0.5ml min^{-1} ($2.95\text{E-}04\text{ cm s}^{-1}$) and feed concentration 0.017 g l^{-1}

	EPAJC		CAJC	
	Enrichment	% Recovery	Enrichment	% Recovery
1%	2.14	13.22	1.23	2.17
3%	2.40	9.26	1.41	1.89

The presence of alcohol in the feed solution alters the surface tension characteristics thus affecting enrichment and recovery. It was observed that enrichment value improves with increase in percentage of alcohol addition up to 3% (Table 1).

3.4 Bubble size, gas hold-up, superficial air velocity and interfacial area

A typical plot of the frequency size distribution of the bubbles at a distance 20 mm and 600 mm from the frit is shown in Fig.8.

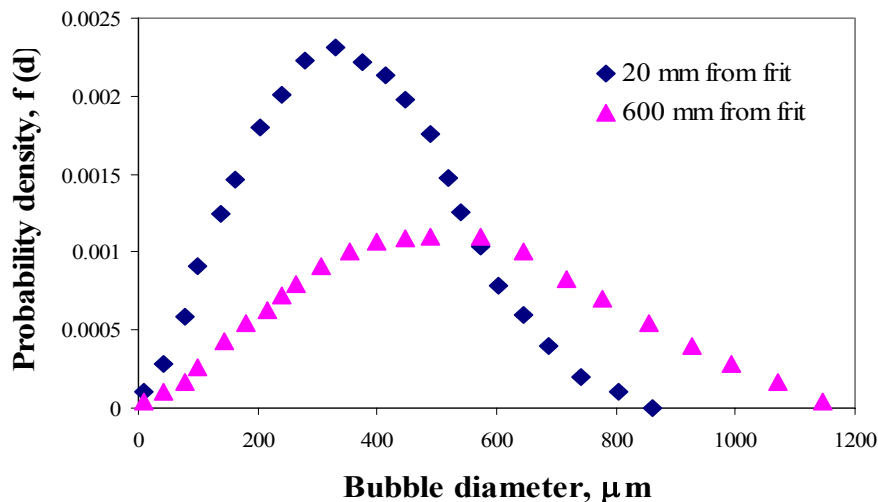


Figure 8: Bubble size distribution at a distance of 20 and 600 mm from the stainless steel frit. Superficial air velocity $3.54 \times 10^{-4}\text{ cm s}^{-1}$, bulk concentration 0.004 g l^{-1} .

The results show that coalescence mainly occurs when bubbles rise up the liquid pool, and bigger bubbles and a wider bubble size distribution were observed at distances around 600 mm further away from the frit sparger. The Sauter mean or the volume-surface mean bubble diameter (d_{32}) was calculated from the bubble size distribution, (Eq. 3) and is plotted as a function of the superficial air velocity and the distance from the bottom of the column in Fig. 9.

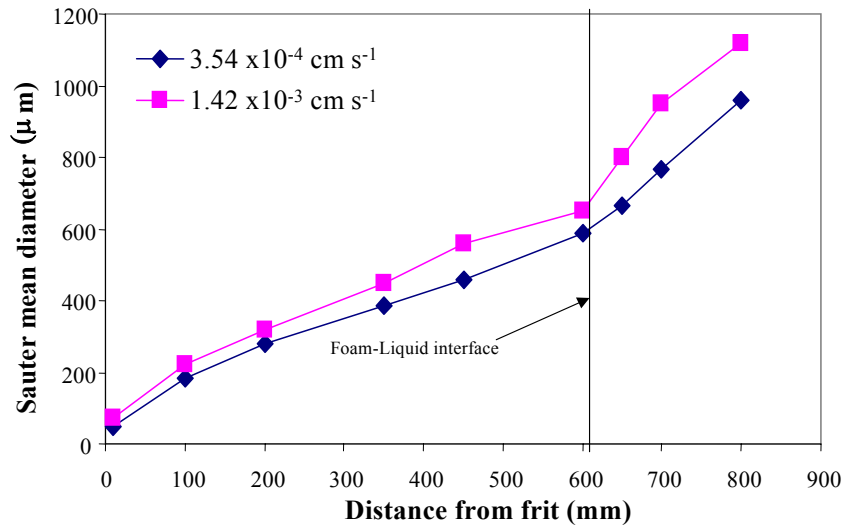


Figure 9: Sauter mean bubble diameter (d_{32}), as a function of the column height at $3.54 \times 10^{-4} \text{ cm s}^{-1}$, $1.42 \times 10^{-3} \text{ cm s}^{-1}$ superficial air velocities.

In addition, the average bubble size increased at higher superficial gas velocities and the effect was more significant in the foam phase. Percentage gas hold-up was measured by varying the superficial gas velocity at two polyphenolics concentrations in the bulk pool as shown in Fig.10. The gas hold-up increased with the increase in the superficial velocity and polyphenolics concentration.

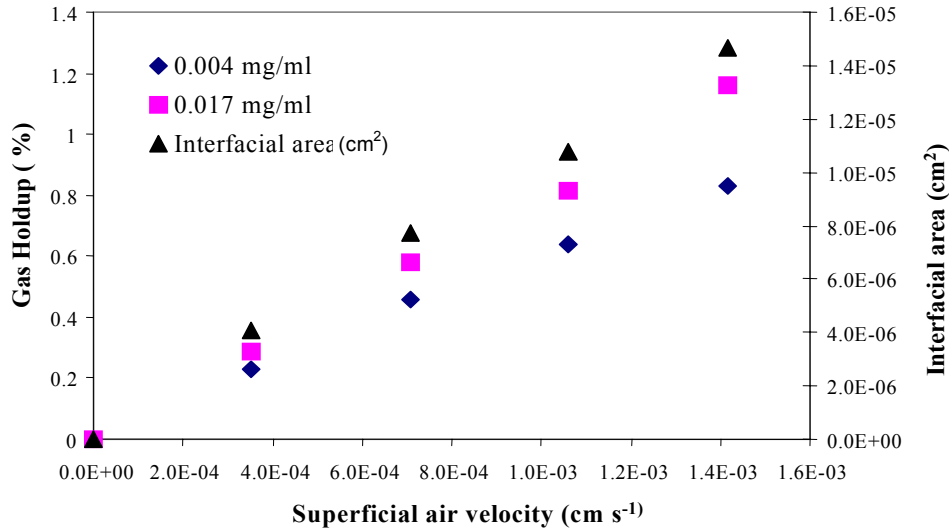


Figure 10: Percentage gas hold-up as a function of superficial air velocity and bulk feed concentration (0.004 g l⁻¹, 0.017 g l⁻¹); pH 4. Interfacial area as a function of superficial air velocity at 0.004 g l⁻¹ polyphenolics concentration; pH 4.

4. Conclusion

A study of separation/concentration of polyphenolics from apple juice led to the following conclusions:

- Foam fractionation can be used to recover and enrich polyphenols from apple juice and, by extension, would work in other juices.
- The enrichment ratio increased with a decrease in feed concentration.
- Increasing the gas-flow rates resulted in higher volume of wet foam due to the short residence time for the foam to drain the liquid, leading to increase in recovery but decreased enrichment ratios.
- The maximum enrichment ratio is obtained at the isoelectric point of the protein-polyphenol complexes.
- Using air as the gas phase showed higher enrichment and recovery values.
- The oxygen present in air oxidises low molecular weight polyphenols, which polymerise to higher molecular weight polyphenols (i.e. Tannins).
- Nitrogen mitigated but did not completely prevent the oxidation.
- It was observed that enrichment value improves with increase in percentage of alcohol addition.

- The average bubble size increased at higher superficial gas velocities and the effect was more significant in the foam phase
- The gas hold-up increased with the increase in the superficial velocity and polyphenolics concentration.
- Further work needs to be done to prevent the adverse effects of oxygen, determine the mass transfer coefficient and optimise the process through the use of food acceptable surfactant to improve the recovery.

5. Acknowledgements

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