

# UHT Treatment on the Stability of Faba Bean Protein Emulsion †

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**Abstract:** Faba beans are one of the most consumed legumes and an emerging source of edible legume-based protein. Efficacy of faba bean protein concentrate (available protein ~ 63%) during UHT processing of homogenised O/W emulsion was assessed in the present study by using four different concentrations viz., FPC5 ~ 5%, FPC6 ~ 6%, FPC7 ~ 7%, and FPC8 ~ 8%. Additionally, the physicochemical properties of emulsion at various processing stages viz., coarse, homogenised, and UHT were also measured. Overall, this study showed the potential use of faba bean protein as a replica of soy protein in UHT processed legume-based beverages.

**Keywords:**

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## 1. Introduction

The use of plant proteins for human consumption foods begins since ancient times and appears especially in many traditional home dishes and cuisines in the Asian and Indian diets (such as soy beverages, tofu, tempeh, dosas) [1]. Industrialised food production has allowed mass production of plant proteins and new product innovation has transformed many of the traditional plant proteins products. A wide range of commercial plant proteins-based foods and beverages, such as plant based meat and milk analogues are commercially available [2,3]. In these food applications, the use of plant proteins extends beyond the nutritional values and provides functional purposes such as solubility, emulsification, gelation, water and fat binding capacities [4]. These functionalities are critical to the successful application of plant proteins and the ultimate stability, quality and acceptability of the finished products [5].

Plant-based beverage is normally an oil-in-water (O/W) emulsion, where water is the aqueous phase and oil is the dispersed phase. The two phases are generally immiscible and are thermodynamically unstable, where they tend to separate overtime via creaming, flocculation, coalescence and/or aggregation [3]. One of the critical functionalities of plant proteins is their ability to adsorb at the oil-water interface and form stabilising protein films around the oil droplets. Plant proteins that are readily adsorbed at the interface, be able to unfold their structures and form a cohesive film around the oil droplets should generally be considered as effective emulsifier [6]. Many studies have evaluated the emulsifying properties of plant proteins such as soy [7], chickpea [8], peas [9], and beans [10], and have demonstrated the potential of plant proteins to serve as alternative proteins for stabilising O/W emulsions. In terms of plant protein based beverages, common processing technologies such as high shear mixing, homogenisation and ultra-heat treatment (UHT)

can create pressure and thermal effects which can lead to changes in the stability of the protein emulsions [11].

Among the plant proteins, faba bean protein remains an industrially underexploited and represents an emerging alternative source of plant protein. Faba bean, also known as fava bean or broad bean is a legume rich in protein (250 g protein/kg seed). The major storage proteins consist of two globulin proteins, 11S (legumin) and 7S (vicilin) [12]. Both globulin proteins exhibit different physiochemical and functional properties [13]. Several studies have reported the emulsifying potential of faba beans in various emulsion systems. Felix, et al. [14] evaluated the emulsifying properties of faba bean protein isolates (~89% purity) in an O/W emulsion system consisting of sunflower oil dispersed in the aqueous phase. The greatest stability and the smallest droplet sizes were obtained for the emulsion system created at pH 8.0 by high-shear mixing (18000 rpm) and two-stages homogenisation (800 and 80 bar). Raikos et al. [15] reported that the emulsifying ability and stability of faba bean flour (composed of 300 gkg<sup>-1</sup> protein) were most superior when the emulsion was blended and homogenised (12,000 rpm) with flaxseed oil at pH 10. Liu et al. [16] studied the emulsifying properties of faba bean protein isolates by creating an emulsion system consisting of rapeseed oil dispersed in aqueous dispersion of hydrolysed faba bean protein isolates. The polypeptides produced (at 4% degree of hydrolysis by Alcalase) had suitable molecular mass, increased surface net charge and hydrophobicity, favouring emulsifying activity as evidenced by the homogenous droplet sizes and the high surface coverage of proteins at the interfacial layer. Karaca et al. [17] prepared a series of emulsions by blending flaxseed oil with respective aqueous solutions of faba bean, chickpea, lentil, pea and soy protein isolates prepared with isoelectric precipitation and salt extraction. While all protein isolates prepared by isoelectric precipitation had superior emulsifying properties, the study also found similar emulsifying properties of faba bean protein isolates compared to soy and lentil protein isolates. Tsoukala et al. [18] reported superior stability, droplet size distribution and protein surface load, of emulsions consisted of corn oil blended with native faba bean meals, although modified faba bean meals (through induced autolysis) led to less stable emulsion systems over time. The authors deduced that the modified legume proteins with reduced solubility were not efficiently dissolved and not able to create a rigid film around the oil droplets for long term emulsion stability, though they have a less rigid structure adsorbed at a faster rate.

In this study, O/W emulsions were prepared by blending sunflower oil, sunflower lecithin and faba bean protein concentrates at a controlled pH condition. The emulsions created by high shear mixing, homogenisation and ultra-heat thermal processing were evaluated for their particle size,  $\zeta$ -potential, (protein and lipid) droplet size, surface hydrophobicity, flocculation, coalescence and creaming index.

## 2. Materials and Methods

### *Materials*

Faba bean protein concentrate (VITESSENCE™ Pulse CT 3602 Protein) with 63% purity was obtained from Link Trading Queensland Pty Ltd., QLD, Australia. The composition and caloric value of the faba protein concentrate (per 100 g) are carbohydrate (27 g), total fat (3 g), protein content (63 g), moisture content (3 g), total ash (4 g) and calories (410 KCal). Sunflower oil and soy lecithin (food grade) were purchased from a local grocery store. Faba bean protein isolate (available protein ~ >85%) and soy protein isolate (available protein ~ 83%) were produced in CSIRO pilot plant (Werribee, VIC, Australia). All chemicals including potassium phosphate (food grade), sodium dodecyl sulfate (SDS) 8-anilino-1-naphthalenesulfonic acid (ANS), methanol and Nile red dye were purchased from Sigma-Aldrich, Castle Hill NSW, Australia.

### 3. Preparation of Emulsion

#### 3.1. Mixing

Four different concentrations of faba bean concentrates (FPC5 ~ 5%, FPC6 ~ 6%, FPC7 ~ 7%, and FPC8 ~ 8%) were used in this study. Oil in water emulsion (approximately, 3000 g) was prepared in 5 mM phosphate buffer, pH 8.0 by adding appropriate amount of the protein concentrate, sunflower oil (1%), sunflower lecithin (0.2%), and mixing with a multi-mixer at 1000 rpm for 10 min. The pH was then re-adjusted to 8.0 with NaOH prior to preparing the coarse emulsions (cFPC5, cFPC6, cFPC7, and cFPC8) by homogenising the lipidic and aqueous phases using multi-mixer at 10,000 rpm for 20 min and vacuum filtering to eliminate large undissolved material.

#### 3.2. Homogenisation of the Emulsion

The coarse protein emulsions obtained by mixing with multi-mixer were further subjected to homogenisation treatment (hFPC5, hFPC6, hFPC7, and hFPC8). Each coarse emulsion was homogenised by single pass in an EmulsiFlex-C5 (Avestin, Ottawa, ON, Canada) operating at ~80 MPa.

#### 3.3. Ultra-High Temperature Processing of the Emulsion

The homogenised samples were subjected to UHT processing using a bench-top UHT plant (pre-heated to 105°C for 3 s and high heat-treated to 135 °C for 3 s). The UHT plant was operated at a flow rate of 3 mL/s. The UHT treated emulsions viz., uhtFPC5, uhtFPC6, uhtFPC7 and uhtFPC8.

### 4. Physicochemical Properties of the Emulsion

#### 4.1 Particle size Distribution

The average particle size distribution of the protein emulsions was determined using a Malvern Mastersizer. The continuous phase of the experiment was Milli-Q water (Refractive Index: 1.33) whereas, the dispersed phase was sunflower oil and plant protein. Particle size measurements was obtained around 12.5% laser obscuration and the proportion of droplet size distribution at 10%, 50% and 90% was  $d(0.1)$ ,  $d(0.5)$  and  $d(0.9)$ , respectively. The uniformity, surface weighted mean,  $D^{3,2}$  and volume weighted mean  $D^{4,3}$  was also obtained from the particle size distribution graphs [19]. The polydispersity index (PDI) was also calculated according to the equation 1.

$$PDI = d(0.9) - d(0.1)/d(0.5) \quad (1)$$

#### 4.2. Flocculation index (FI) and Coalescence Index (CI)

Flocculation refers to the process where undissolved particles clump together to form aggregates, making the emulsion unstable. Coalescence is the process by which two or more particles merge during contact to form a single large particle [20]. Flocculation index (FI) and Coalescence index (CI) of the protein emulsions were measured through  $D^{4,3}$  values using the method of Felix, et al. [21]. Emulsions were dispersed at a ratio of ~1:10 either in deionized water (DI) or 1% sodium dodecyl sulfate (SDS) and  $D^{4,3}$  values of freshly prepared and stored (after 24 h) emulsions were obtained from the Mastersizer. For CI measurement, all emulsions were diluted only in 1% SDS solution.

The FI and CI was calculated by Equations (2) and (3), respectively.

$$FI (\%) = [D^{4,3} \text{ in water} - 1] \times \frac{100}{D^{4,3} \text{ in SDS}} \quad (2)$$

$$CI (\%) = [D^{4,3} (24 \text{ h}) - 1] \times \frac{100}{D^{4,3} (0 \text{ h})}$$

where  $D^{4,3}$  in water is sample diluted in water;  $D^{4,3}$  in SDS is sample diluted in sodium dodecyl sulfate (SDS);  $D^{4,3}$  (24 h) is sample stored for 24 h in the refrigerator ( $\sim 4$  °C) and  $D^{4,3}$  (0 h) is freshly prepared samples.

#### 4.3. Confocal Laser Scanning Microscopy

The microscopic analysis of emulsion was done by labelling fat drop using Nile red followed by confocal laser scanning microscopy (CLSM) as described by Qamar, Bhandari and Prakash [11]. Sample of protein emulsion (0.5 mL) and 2  $\mu$ L of Nile red (0.02 % *w/v* in polyethylene glycol) was mixed properly using vortex mixer and kept in dark for 5 min. A drop of the mixture was placed on a glass slide and used for measurement of lipid droplet size in the sample.

#### 4.4. $\zeta$ -Potential

The  $\zeta$ -potential of the samples was measured using Marlvén zetasizer as described by Li, et al. [22]. Freshly prepared, homogenised and UHT treated samples were pre-diluted 10-folds in a 5 mM solution of phosphate buffer prepared with Milli-Q water (to avoid the coagulation effect due to high lipid concentration) and transferred to  $\zeta$ -potential cell. The  $\zeta$ -potential was measured at 25°C at a refractive index of 1.33.

#### 4.5. Surface Hydrophobicity Index ( $S_o$ )

The  $S_o$  of selected samples of protein emulsion in aqueous solution was measured using 8-anilino-1-naphthalene sulfonic acid (ANS) as a fluorescent probe on a spectrofluorometer as described by Nwachukwu and Aluko [23]. Four different serial dilutions (0.006, 0.003, 0.0012 and 0.0009%) of each stock solution of protein emulsion samples were prepared. Each dilution was further divided into two batches (with and without ANS solution). 20  $\mu$ L of ANS (8.0 mM prepared in 5 mM phosphate buffer) were added in 5 mL of the first batch of diluted protein samples while diluted protein samples without ANS was used as a blank. The ANS-protein conjugation was measured at 370 nm (excitation) and 490 nm (emission) wavelengths using a plate reader (Varioskan Flash, Thermo Scientific, Waltham, MA, USA). The  $S_o$  was obtained through linear regression analysis of the plot between fluorescence intensity (FI) and protein concentrations (as the slope of fluorescence intensity-protein concentration).

### 5. Creaming Index

The creaming index of the freshly prepared emulsions was determined by centrifuging 5 mL of the emulsion in a plastic centrifuge tube (at 20 °C and 4000 rpm for 20 min) using modified method of Liu et al. [24]. Each tube was then stored in the refrigerator ( $\sim 4$  °C) for 7 days. After 7 days of quiescent storage, the total height of emulsion ( $H_t$ ) and height of cream ( $H_c$ ) was measured. The percentage of creaming layer was calculated by using the equation 4.

$$\text{Creaming index} = \left( \frac{H_t}{H_c} \right) \times 100 \quad (4)$$

#### 5.1. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing conditions. Mixtures of faba bean protein concentrate (FPC) having  $\sim 63\%$  protein, faba bean protein isolate (FPI) having  $\sim >85\%$  protein, and soy protein isolate (SPI) having  $\sim 83\%$  protein or reconstituted markers, were mixed with sample buffer and mercaptoethanol at a ratio 1:1:0.1, respectively and heated in boiling water for 3 min followed by cooling in ice. Then, 4  $\mu$ L of treated samples/markers were loaded on gels and the electrophoresis was carried out at 60 Vh for 40 min. Protein bands were stained using Coomassie Brilliant Blue. The molecular weights (MWs) of unknown proteins were estimated via regression between the log of standard MWs and the relative mobility of the protein markers.

### 5.2. Headspace Gas Chromatography-Mass Spectrometry (GC-MS)

Aliquots (~5 g) of selected samples of protein emulsions prepared fresh and UHT samples stored for a month at refrigerated temperature (~4 °C) were weighed in amber 22 mL headspace vials and analysed for volatile organic compounds (VOCs). The analysis was performed using an Agilent gas chromatography mass spectrometry system (GCMS- 6890N model GC and 5975B model MSD; Agilent Technologies Australia Pty Ltd., Mulgrave, Victoria, Australia) equipped with a CombiPAL robotic autosampler (CTC Analytics AG, Zwingen, Switzerland). Volatiles in the sample vial was extracted/concentrated from headspace on SPME fibre (carboxen/polydimethylsiloxane, CAR/PDMS; 85 µm film thickness; 10 mm long; Supelco, Bellefonte, PA) for 30 min at 50 °C. SPME-sampled volatiles were thermally desorbed in the GC inlet and chromatographed on a VF-WAXms column (30 m × 0.32 mm × 1.0 µm; Agilent Technologies) using a temperature gradient. Eluted compounds were detected by the MS. GCMS system was interfaced with the computer using MassHunter Workstation software (version 10.0; Agilent Technologies). Details on the set-up of GCMS, identification and quantification of compounds were as detailed by [25].

### 5.3 Statistical Analysis

All experiments were performed in triplicate and the results are expressed in means ± standard deviations. Experimental data was assessed by one-way analysis of variance (ANOVA) with Minitab 19 (Minitab® for Windows Release 19, Minitab Inc, Chicago) to determine the significant differences. The data was then analysed using Tukeys pair-wise comparison, at 5% level of significance, to compare the results between different treatments.

## 6. Results and Discussion

### *UHT Processing of Faba Bean Protein Emulsions*

The homogenised emulsions of faba bean protein concentrate viz., hFPC5, hFPC6, hFPC7, and hFPC8, when subjected to UHT processing did not foul for the duration of the run (~1 h). Fouling or depositing results in reduced flow rate, flow pressure and outlet temperature, which was not observed in the UHT processing of faba bean protein emulsions. Flow rate, flow pressure and outlet temperature were maintained at  $3 \pm 1$  mL/se,  $200 \pm 20$  kPa, and  $135 \pm 1$  °C.

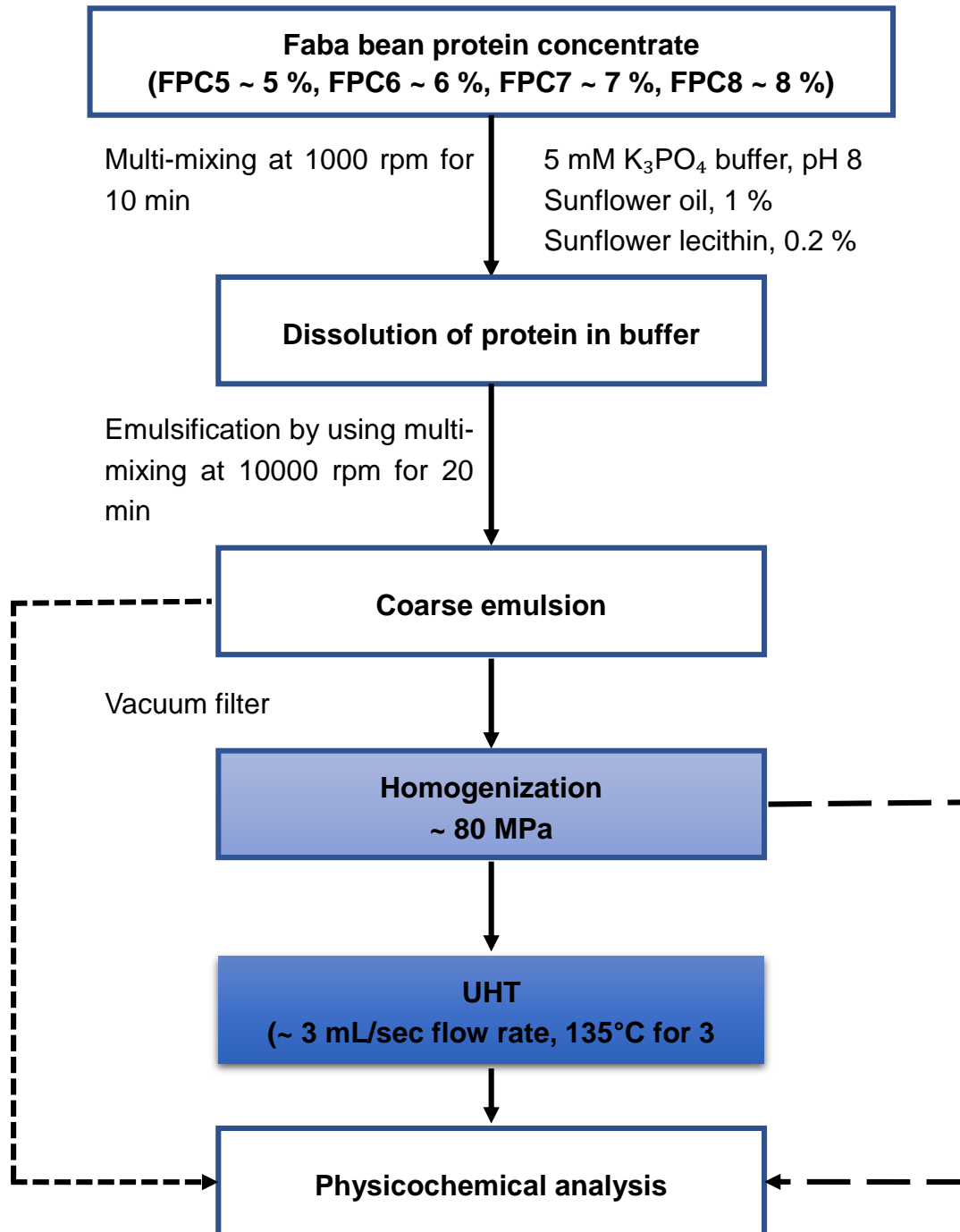
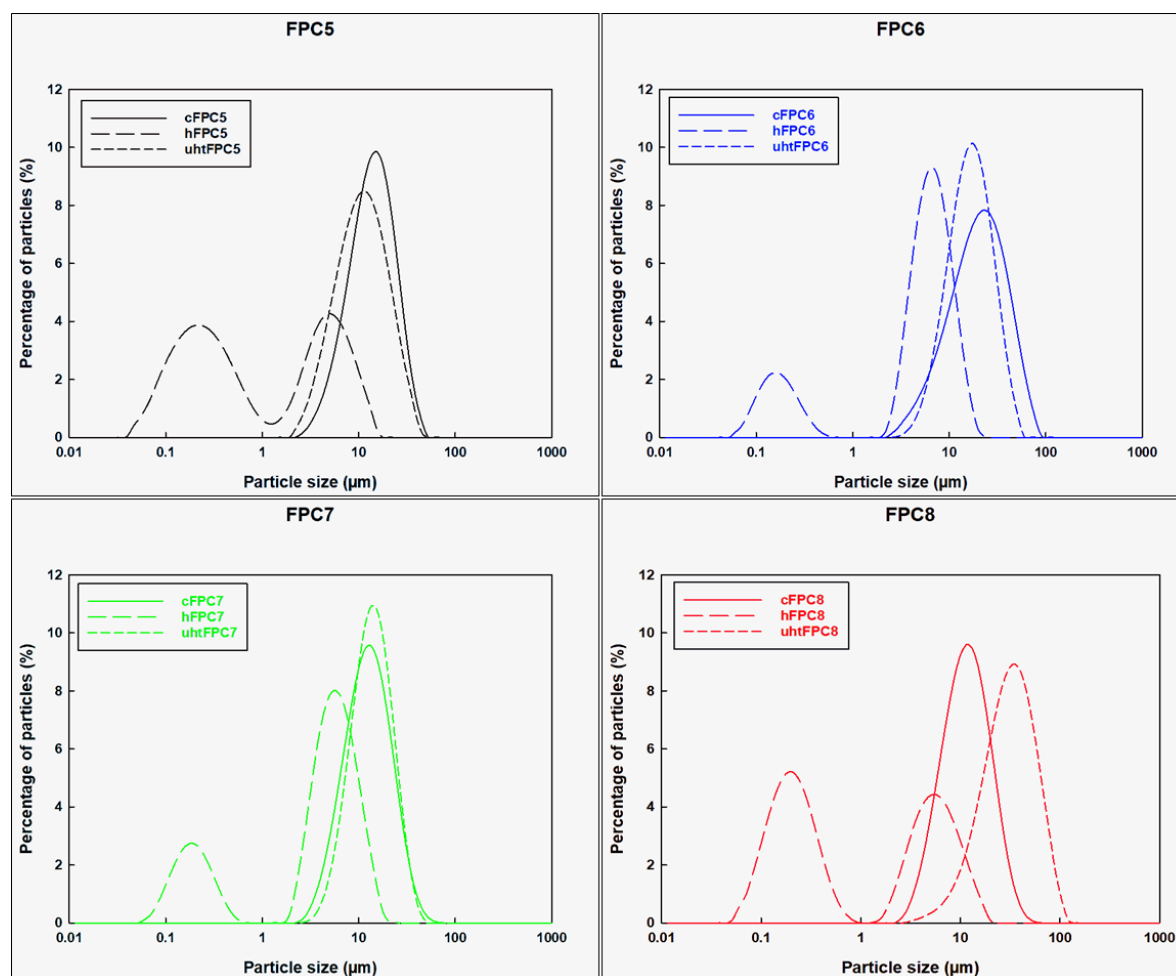


Figure 1. Experimental design.

## 7. Physicochemical Properties of the Emulsions

### 7.1. Particle Size Distribution

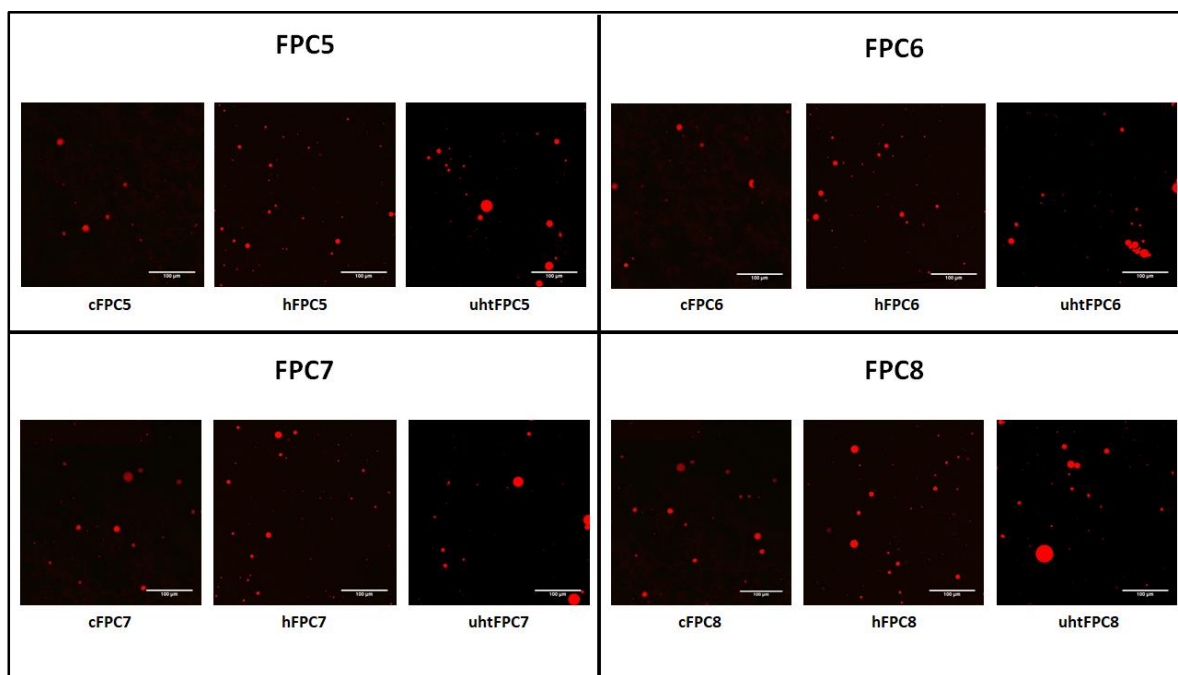
The results of particle size distribution of coarse (cFPC5, cFPC6, cFPC7, and cFPC8), homogenised (hFPC5, hFPC6, hFPC7, and hFPC8), and UHT (uhtFPC5, uhtFPC6, uhtFPC7, and uhtFPC8) treated emulsions of faba bean protein concentrate are presented in Figure 2. Results showed that the UHT treatment resulted in increased particle size possibly due to thermal induced aggregation. The limitation of particle size distribution analysis is to distinguish between disperse particles, as the detection range of the equipment used is between 0.1 and 1000  $\mu\text{m}$ . In this range, it is possible that the particle size analysis is including not only fat globules but also some protein aggregates and even protein-fat globule and globule aggregates [26].



**Figure 2.** Particle size distribution curves of O/W emulsions with various concentrations of faba bean protein concentrate at various stages during UHT processing.

### 7.2. Flocculation Index (FI) and Coalescence Index (CI)

Flocculation and coalescence usually occur due to the attractive interactions or aggregation between the depleted surfactant ions resulting in a liquid-solid transition state. This will result in increased particle size and reduced emulsion stability [27]. Thus, the FI and CI are the indicative assessment of emulsion stability. The results of FI and CI for coarse (cFPC5, cFPC6, cFPC7, and cFPC8), homogenised (hFPC5, hFPC6, hFPC7, and hFPC8), and UHT (uhtFPC5, uhtFPC6, uhtFPC7, and uhtFPC8) treated emulsions of faba bean protein concentrate are presented in Table 1. Results showed that the FI and CI of emulsion varied with not only the protein concentration but also the processing stage. Increase in the protein concentration and thermal treatment during UHT processing caused an increased droplet flocculation [28]. The possible interactions between absorbed proteins within a droplet or between droplets might affect the viscosity of the emulsions. The formation of bridges between droplets as well as presence of droplets with large aggregates on the surface would also increase the viscosity of the emulsion [29]. Rheological experiments were not carried out on coarse, homogenised and UHT emulsions with varying protein contents as particle size analysis already indicated the presence of aggregated oil droplets. Similar findings were observed in confocal laser scanning micrographs (CLSM) of coarse (cFPC5, cFPC6, cFPC7, and cFPC8), homogenised (hFPC5, hFPC6, hFPC7, and hFPC8), and UHT (uhtFPC5, uhtFPC6, uhtFPC7, and uhtFPC8) treated emulsions of faba bean protein concentrate are presented in Figure 3.



**Figure 3.** Confocal laser scanning micrographs of O/W emulsions with various concentrations of faba bean protein concentrate at various stages during UHT processing.

**Table 1.** Effect of faba bean protein concentration on the physicochemical properties viz., polydispersity index (PDI), flocculation index (FI), coalescence index (CI), ζ-potential, and creaming index of O/W emulsions at various stages during UHT processing. \*

Treatment	Emulsion Type	PDI (%)	FI	CI	ζ-Potential (mV)	Creaming Index
FPC5	coarse	24.38 ± 0.01 <sup>c</sup>	88.81 ± 0.35 <sup>d</sup>	101.26 ± 5.24 <sup>cd</sup>	-27.60 ± 3.25 <sup>d</sup>	12.45 ± 0.35 <sup>b</sup>
	homogenised	6.88 ± 0.24 <sup>e</sup>	4.79 ± 0.19 <sup>e</sup>	63.93 ± 0.34 <sup>ef</sup>	-24.63 ± 1.50 <sup>cd</sup>	7.00 ± 0.28 <sup>s</sup>
	UHT	21.37 ± 0.71 <sup>cd</sup>	105.06 ± 3.66 <sup>d</sup>	142.34 ± 9.92 <sup>b</sup>	-23.10 ± 0.96 <sup>bc</sup>	9.25 ± 0.21 <sup>de</sup>
FPC6	coarse	41.52 ± 5.45 <sup>b</sup>	160.82 ± 17.80 <sup>c</sup>	101.57 ± 2.24 <sup>cd</sup>	-23.10 ± 2.10 <sup>bc</sup>	13.05 ± 0.21 <sup>b</sup>
	homogenised	10.44 ± 0.13 <sup>de</sup>	11.10 ± 2.87 <sup>e</sup>	47.81 ± 0.49 <sup>f</sup>	-21.20 ± 0.80 <sup>b</sup>	7.20 ± 0.42 <sup>s</sup>
	UHT	29.03 ± 1.81 <sup>c</sup>	194.98 ± 0.15 <sup>ab</sup>	175.32 ± 4.81 <sup>a</sup>	-21.47 ± 1.10 <sup>bc</sup>	10.25 ± 0.50 <sup>cd</sup>
FPC7	coarse	22.81 ± 0.33 <sup>c</sup>	182.22 ± 12.42 <sup>bc</sup>	110.95 ± 5.30 <sup>c</sup>	-0.02 ± 0.02 <sup>a</sup>	14.55 ± 0.21 <sup>a</sup>
	homogenised	9.17 ± 0.41 <sup>e</sup>	10.83 ± 3.06 <sup>e</sup>	83.41 ± 7.59 <sup>de</sup>	0.08 ± 0.03 <sup>a</sup>	7.95 ± 0.21 <sup>fg</sup>
	UHT	22.89 ± 1.45 <sup>c</sup>	212.14 ± 3.91 <sup>ab</sup>	191.81 ± 11.82 <sup>a</sup>	-0.12 ± 0.07 <sup>a</sup>	10.40 ± 0.28 <sup>c</sup>
FPC8	coarse	20.89 ± 0.18 <sup>cd</sup>	199.74 ± 18.27 <sup>ab</sup>	109.06 ± 1.30 <sup>c</sup>	0.01 ± 0.07 <sup>a</sup>	14.95 ± 0.21 <sup>a</sup>
	homogenised	7.84 ± 0.34 <sup>e</sup>	16.10 ± 1.27 <sup>e</sup>	78.98 ± 0.32 <sup>de</sup>	0.01 ± 0.20 <sup>a</sup>	8.40 ± 0.14 <sup>ef</sup>
	UHT	59.49 ± 7.40 <sup>a</sup>	226.50 ± 0.26 <sup>a</sup>	197.43 ± 7.08 <sup>a</sup>	0.24 ± 0.11 <sup>a</sup>	15.15 ± 0.21 <sup>a</sup>

\* Values (mean ± S.D.) are in triplicates (n = 3) and the values within a column followed by the same letter are not significantly different at 95% confidence level ( $P < 0.05$ ).

### 7.3. ζ-Potential

ζ-potential is also an indication of emulsion stability. It is now quite well understood that the emulsions having low electronegativity are unstable and easily flocculate or coagulate during storage while the emulsions with more electronegativity are comparatively more stable due to more repulsive forces than attractive forces [30]. The ζ-potential of coarse (cFPC5, cFPC6, cFPC7, and cFPC8), homogenised (hFPC5, hFPC6, hFPC7, and hFPC8), and UHT (uhtFPC5, uhtFPC6, uhtFPC7, and uhtFPC8) treated emulsions of faba bean protein concentrate are presented in Table 1. Results showed that the ζ-potential not only depends on the concentration of protein in emulsion but also on the processing stage. Low protein concentrations viz., FPC5 and FPC6 showed greater negative ζ-potential and thereby high solubility through enhanced electrostatic repulsions than that of FPC7 and FPC8. Also, processing stage especially UHT treatment resulted in less negative values compared to coarse and homogenised emulsions. This can be related to the formation of protein aggregate mainly



at higher temperature and reduction of solubility. Similar findings were recorded in particle size analysis (Figure 2) and CLSM (Figure 3), where increased particle size distribution was detected.

#### 7.4. Surface Hydrophobicity ( $S_o$ )

Surface hydrophobicity ( $S_o$ ) is one of the most important surface-related properties of emulsion and it is directly related to the emulsifying properties especially emulsion stability [31]. The adsorbing ability of protein at the O/W interface in the emulsion can be determined by  $S_o$  [32]. The  $S_o$  of coarse (cFPC5, cFPC6, cFPC7, and cFPC8), homogenised (hFPC5, hFPC6, hFPC7, and hFPC8), and UHT (uhtFPC5, uhtFPC6, uhtFPC7, and uhtFPC8) treated emulsions of faba bean protein concentrate are presented in Figure 4. Results showed that the increase in protein concentration and thermal application during UHT resulted in increased  $S_o$ . It is evident from the previous studies that the heat treatment has a direct influence on the emulsion [33]. Heat induces the reordering of hydrophobic side chains of protein network [34]. These reordered hydrophobic side chains behave like an elastic gel resulting in increased  $S_o$  [35].

#### 7.5. Creaming Index

Creaming index or gravitational separation is also an indication of emulsion stability [36]. The creaming indexes of coarse (cFPC5, cFPC6, cFPC7, and cFPC8), homogenised (hFPC5, hFPC6, hFPC7, and hFPC8), and UHT (uhtFPC5, uhtFPC6, uhtFPC7, and uhtFPC8) treated emulsions of faba bean protein concentrate are presented in Table 1. Results showed that the creaming index not only depends on the concentration of protein in emulsion but also on the processing stage. Higher protein concentrations viz., FPC7 and FPC8 showed more creaming index compared to lesser protein emulsions viz., FPC5 and FPC6. Also, thermal application during UHT processing induced increased cream separation in all treatments.

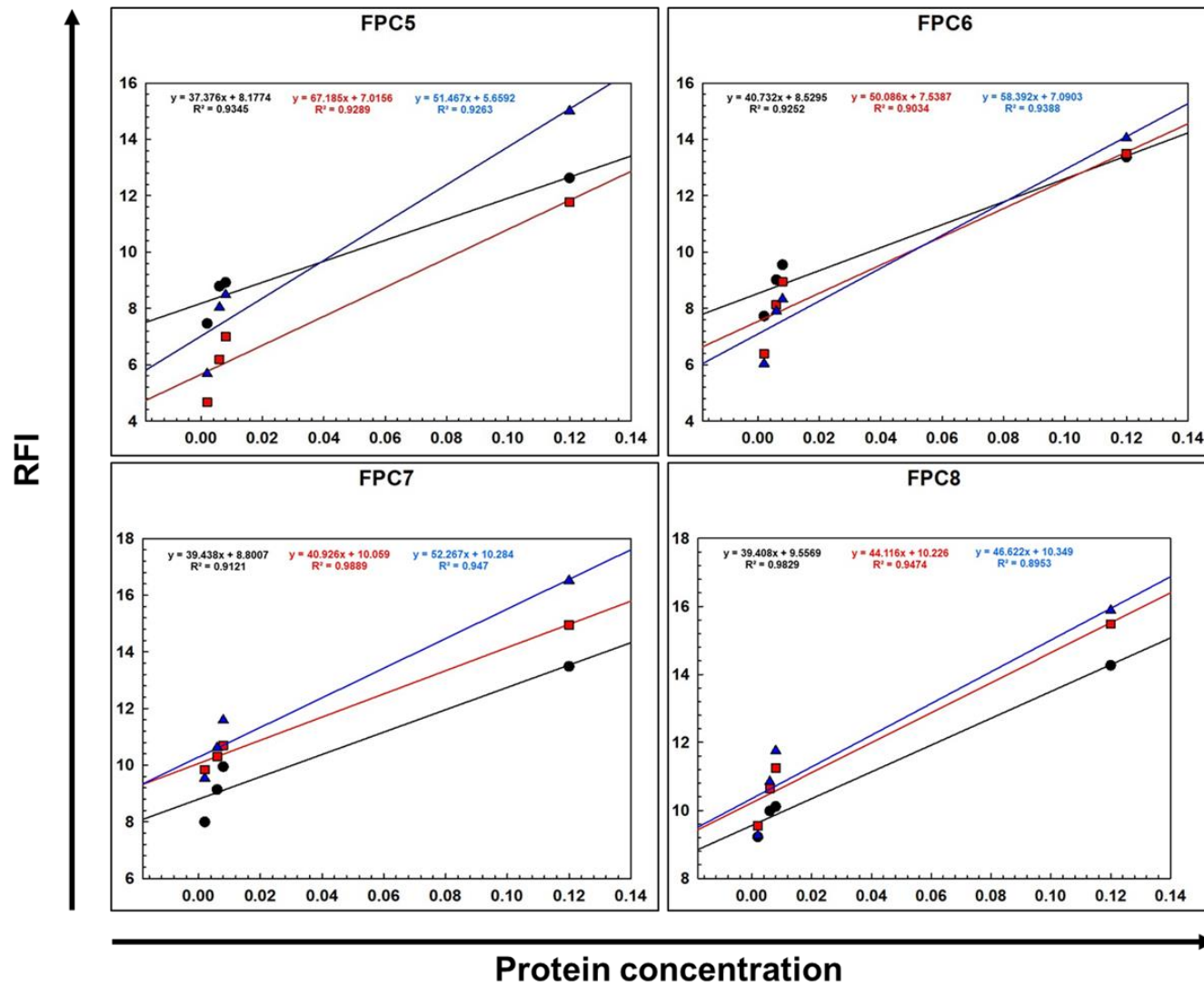
#### 7.6. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE for faba bean protein concentrate (FPC) having ~ 63% protein, faba bean protein isolate (FPI) having ~ > 85% protein, and soy protein isolate (SPI) having ~ 83% protein is presented in Figure 5. Results showed similarities in the protein composition of both faba and soy (mainly 11S globulin ~ Glycinin and 7S globulin ~  $\beta$ -conglycinin). The molecular weights for 7S proteins are between 45 and 97 kDa, and for 11S are below 36 kDa [37].

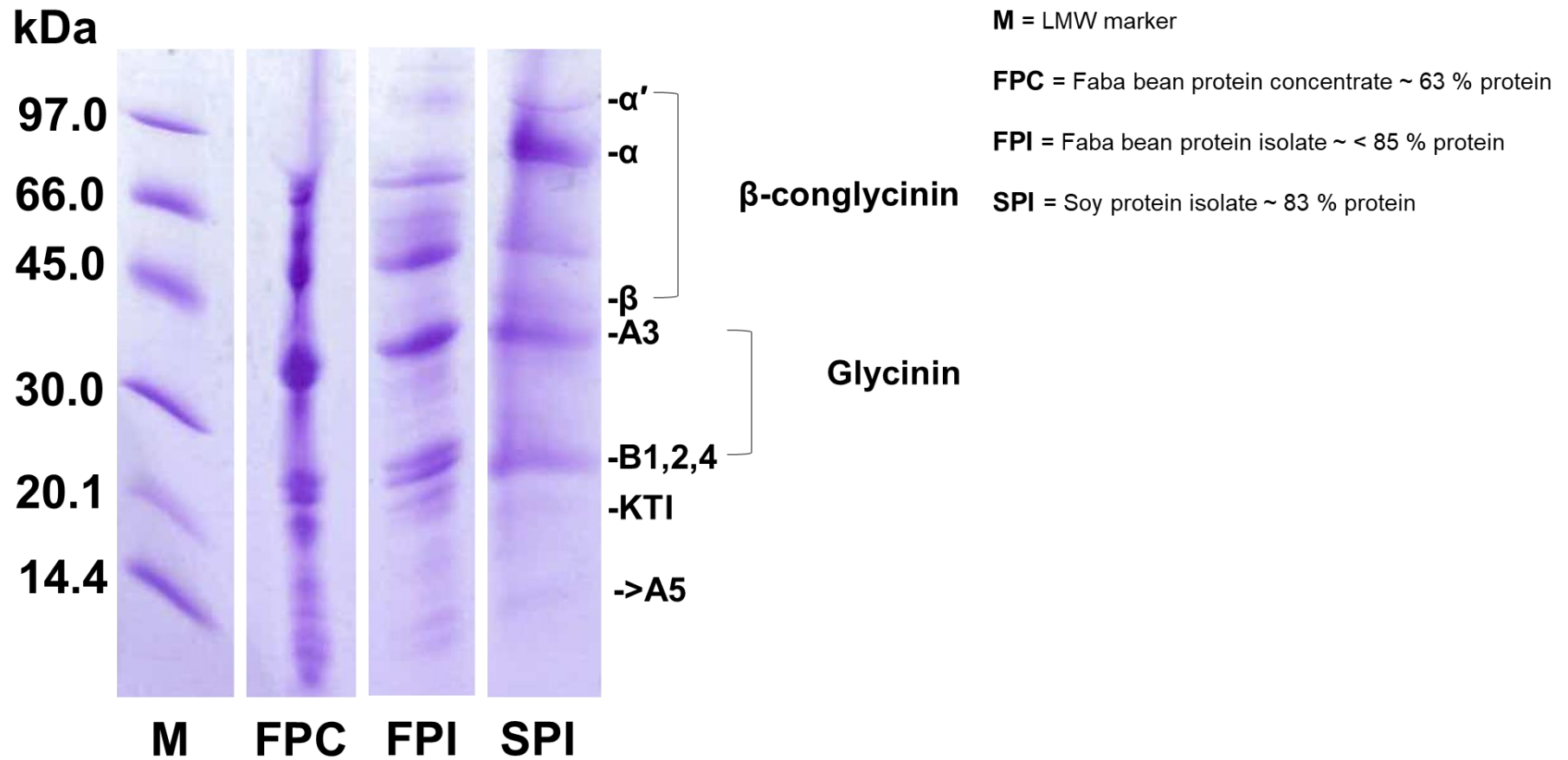
#### 7.7. Headspace Gas Chromatography-Mass Spectrometry (GC-MS)

Volatile compounds profiling of products at each of the above three steps of manufacture of model faba protein-based beverage was performed by headspace GCMS analysis. In total 21 compounds were detected and quantified, representing different chemical classes such as alcohols, aldehydes, ketones, esters, furan and acids. These volatiles have major consequences for the overall flavour chemistry of the model beverage product.

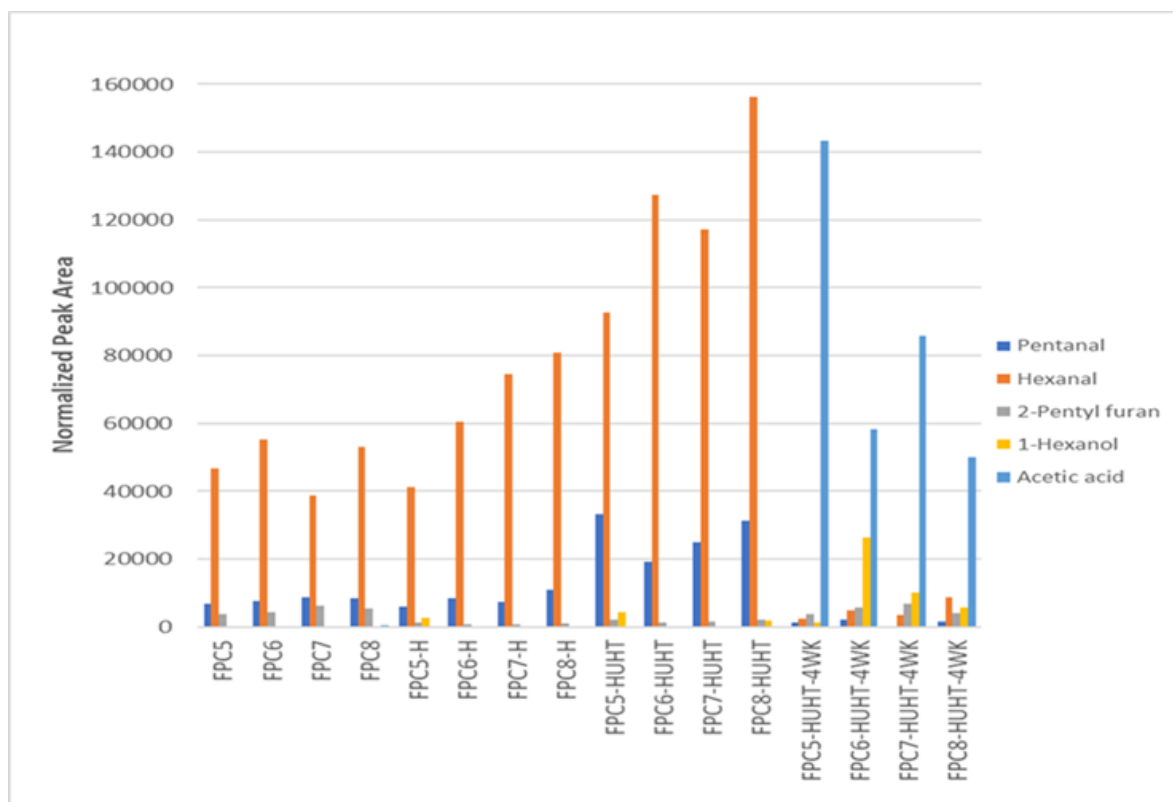
Origins of majority of these volatile organic compounds can be attributed to oxidative deterioration of lipids. Several lipid oxidation derived compounds are known potent odorants with extremely low human sensory detection thresholds, namely pentanal, hexanal, heptanal, nonanal, E-2-heptenal and 2-pentyl furan. Odour notes mainly attributed to these compounds are green, grassy, leafy, cardboard, beany, rancid etc. These compounds are primarily oil soluble and also known to be adsorbed on to the proteins. Concentration of volatiles in general increased at each of the steps involved in the manufacture of the model beverage (Figure 6).



**Figure 4.** Effects of protein concentration and various stages of UHT processing viz., coarse (●), homogenised (■), and UHT (▲) on the surface hydrophobicity ( $S_0$ ).



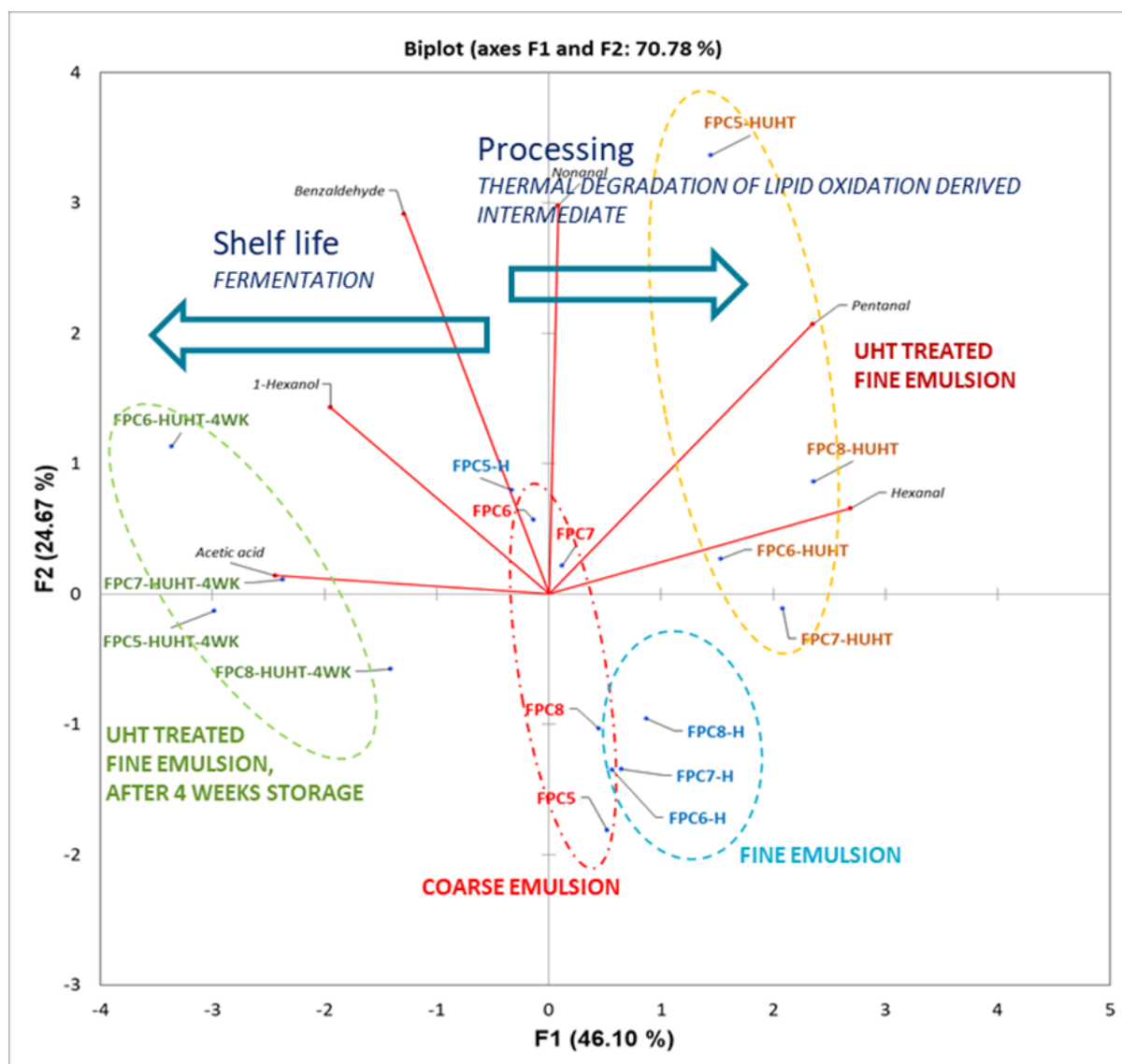
**Figure 5.** SDS-PAGE patterns of low molecular weight marker (M), faba bean protein concentrate (FPC), faba bean protein isolate (FPI), and soy protein isolate (SPI).



**Figure 6.** Changes in the concentration of key volatile organic compounds during manufacture of model faba protein-based beverage and storage. Three distinct steps were: 1- preparation of coarse emulsion (samples FPC5- 8; FPC, faba protein concentrate), 2- homogenization of coarse emulsion (samples FPC7-8-H; H, homogenization) and 3- thermal stabilization model beverage by UHT treatment (samples FPC7-8-HUHT; UHT, ultra-high temperature). Thermally stabilized beverages were stored at 4 °C for 4 weeks.

This increase in the levels of volatiles could be attributed to the thermal degradation of lipid oxidation derived intermediate compounds, namely hydroperoxides of various unsaturated fatty acids. Volatiles profiling of thermally stabilized samples of model beverages stored for 4 weeks at refrigerated temperature clearly showed formation of microbial/fermentation activity with the production of elevated levels of acetic acid. In addition, it was also noted that levels of key lipid oxidation derived volatile aldehydes, e.g., pentanal, hexanal etc, significantly reduced with concomitant increase in the levels of corresponding alcohols like 1-hexanol. This reduction in aldehyde could be attributed to reductive enzymes of microbial cells. Principal component analysis was performed on the quantitative volatile data in order to elucidate underlying trends/pattern (Figure 7). PCA bi plot captures 70% of underlying variation in the data and clearly highlighted key biochemical reaction mechanisms involved production and storage, namely lipid oxidation and fermentation/microbial degradation during production/processing and storage, respectively.

In model plant protein beverage described above, these compounds find their way in from faba protein concentrate where these compounds were most likely produced via enzymatic oxidation of faba bean lipids occurring during the processing and production of protein ingredient from faba beans.



**Figure 7.** Principal component analysis of quantitative data from volatile analysis on model beverages during manufacture of model Faba protein-based beverage and storage.

## 8. Conclusions

Findings of present studies showed that faba bean has similar protein like soybean and can be used as soy replica in plant-based milk alternatives. Faba bean protein emulsions with 8% protein showed induced gelation after UHT. Therefore, further research is planned to overcome this challenge.

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## References

1. Young, V.R.; Pellett, P.L. Plant proteins in relation to human protein and amino acid nutrition. *Am. J. Clin. Nutr.* **1994**, *59*, 1203S-1212S, doi:10.1093/ajcn/59.5.1203S.
2. McClements, D.J.; Newman, E.; McClements, I.F. Plant-based Milks: A Review of the Science Underpinning Their Design, Fabrication, and Performance. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 2047–2067.

3. Nawaz, M.A.; Tan, M.; Øiseth, S.; Buckow, R. An Emerging Segment of Functional Legume-Based Beverages: A Review. *Food Rev. Int.* **2020**, 1–39, doi:10.1080/87559129.2020.1762641.
4. Li-Chan, E.C.Y.; Lacroix, I.M.E. 1-Properties of proteins in food systems: An introduction. In *Proteins in Food Processing*, 2nd ed.; Yada, R.Y., Ed.; Woodhead Publishing: Sawston, UK, 2018; pp. 1–25, doi:10.1016/B978-0-08-100722-8.00002-4.
5. Queirós, R.P.; Saraiva, J.A.; da Silva, J.A.L. Tailoring structure and technological properties of plant proteins using high hydrostatic pressure. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 1538–1556, doi:10.1080/10408398.2016.1271770.
6. Damodaran, S. Protein Stabilization of Emulsions and Foams. *J. Food Sci.* **2005**, *70*, R54–R66, doi:10.1111/j.1365-2621.2005.tb07150.x.
7. Fernandez-Avila, C.; Trujillo, A.J. Ultra-High Pressure Homogenization improves oxidative stability and interfacial properties of soy protein isolate-stabilized emulsions. *Food Chem.* **2016**, *209*, 104–113, doi:10.1016/j.foodchem.2016.04.019.
8. Wang, S.; Chelikani, V.; Serventi, L. Evaluation of chickpea as alternative to soy in plant-based beverages, fresh and fermented. *LWT* **2018**, *97*, 570–572, doi:10.1016/j.lwt.2018.07.067.
9. Chao, D.; Aluko, R.E. Modification of the structural, emulsifying, and foaming properties of an isolated pea protein by thermal pretreatment. *Cyta-J. Food* **2018**, *16*, 357–366, doi:10.1080/19476337.2017.1406536.
10. Qamar, S.; Bhandari, B.; Prakash, S. Effect of different homogenisation methods and UHT processing on the stability of pea protein emulsion. *Food Res. Int.* **2019**, *116*, 1374–1385, doi:10.1016/j.foodres.2018.10.028.
11. Multari, S.; Stewart, D.; Russell, W.R. Potential of Fava Bean as Future Protein Supply to Partially Replace Meat Intake in the Human Diet. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 511–522, doi:10.1111/1541-4337.12146.
12. Kimura, A.; Fukuda, T.; Zhang, M.; Motoyama, S.; Maruyama, N.; Utsumi, S. Comparison of Physicochemical Properties of 7S and 11S Globulins from Pea, Fava Bean, Cowpea, and French Bean with Those of Soybean—French Bean 7S Globulin Exhibits Excellent Properties. *J. Agric. Food Chem.* **2008**, *56*, 10273–10279, doi:10.1021/jf801721b.
13. Felix, M.; Cermeño, M.; FitzGerald, R.J. Assessment of the microstructural characteristics and the in vitro bioactive properties of sunflower oil-based emulsions stabilized by fava bean (*Vicia faba*) protein. *Food Hydrocoll.* **2019**, *97*, 105220, doi:10.1016/j.foodhyd.2019.105220.
14. Raikos, V.; Neacsu, M.; Russell, W.; Duthie, G. Comparative study of the functional properties of lupin, green pea, fava bean, hemp, and buckwheat flours as affected by pH. *Food Sci. Nutr.* **2014**, *2*, 802–810, doi:10.1002/fsn3.143.
15. Liu, C.; Bhattarai, M.; Mikkonen, K.S.; Heinonen, M. Effects of Enzymatic Hydrolysis of Fava Bean Protein Isolate by Alcalase on the Physical and Oxidative Stability of Oil-in-Water Emulsions. *J. Agric. Food Chem.* **2019**, *67*, 6625–6632, doi:10.1021/acs.jafc.9b00914.
16. Karaca, A.C.; Low, N.; Nickerson, M. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Res. Int.* **2011**, *44*, 2742–2750, doi:10.1016/j.foodres.2011.06.012.
17. Tsoukala, A.; Papalamprou, E.; Makri, E.; Doxastakis, G.; Braudo, E.E. Adsorption at the air–water interface and emulsification properties of grain legume protein derivatives from pea and broad bean. *Colloids Surf. B: Biointerfaces* **2006**, *53*, 203–208, doi:10.1016/j.colsurfb.2006.08.019.
18. Rahmati, N.F.; Koocheki, A.; Varidi, M.; Kadkhodae, R. Thermodynamic compatibility and interactions between Speckled Sugar bean protein and xanthan gum for production of multilayer O/W emulsion. *J. Food Sci. Technol.* **2018**, *55*, 1143–1153, doi:10.1007/s13197-017-3030-9.
19. Oliete, B.; Potin, F.; Cases, E.; Saurel, R. Microfluidization as Homogenization Technique in Pea Globulin-Based Emulsions. *Food Bioprocess Technol.* **2019**, *12*, 877–882, doi:10.1007/s11947-019-02265-3.
20. Felix, M.; Cermeño, M.; Romero, A.; FitzGerald, R.J. Characterisation of the bioactive properties and microstructure of chickpea protein-based oil in water emulsions. *Food Res. Int.* **2019**, *121*, 577–585, doi:10.1016/j.foodres.2018.12.022.
21. Li, Q.; Wang, Z.; Dai, C.; Wang, Y.; Chen, W.; Ju, X.; Yuan, J.; He, R. Physical stability and microstructure of rapeseed protein isolate/gum Arabic stabilized emulsions at alkaline pH. *Food Hydrocoll.* **2019**, *88*, 50–57, doi:10.1016/j.foodhyd.2018.09.020.
22. Nwachukwu, I.D.; Aluko, R.E. Physicochemical and emulsification properties of flaxseed (*Linum usitatissimum*) albumin and globulin fractions. *Food Chem.* **2018**, *255*, 216–225, doi:10.1016/j.foodchem.2018.02.068.

23. Liu, N.; Chen, Q.; Li, G.; Zhu, Z.; Yi, J.; Li, C.; Chen, X.; Wang, Y. Properties and Stability of Perilla Seed Protein-Stabilized Oil-in-Water Emulsions: Influence of Protein Concentration, pH, NaCl Concentration and Thermal Treatment. *Molecules* **2018**, *23*, 1533.
24. Torkamani, A.E.; Juliano, P.; Ajlouni, S.; Singh, T.K. Impact of ultrasound treatment on lipid oxidation of Cheddar cheese whey. *Ultrason. Sonochemistry* **2014**, *21*, 951–957, doi:10.1016/j.ultsonch.2013.11.021.
25. Ahmadian-Kouchaksaraei, Z.; Varidi, M.; Varidi, M.J.; Pourazarang, H. Influence of processing conditions on the physicochemical and sensory properties of sesame milk: A novel nutritional beverage. *Lwt-Food Sci. Technol.* **2014**, *57*, 299–305, doi:10.1016/j.lwt.2013.12.028.
26. Ralla, T.; Salminen, H.; Braun, K.; Edelmann, M.; Dawid, C.; Hofmann, T.; Weiss, J. Investigations into the Structure-Function Relationship of the Naturally-Derived Surfactant Glycyrrhizin: Emulsion Stability. *Food Biophys.* **2020**, 1–9.
27. Dickinson, E. Flocculation of protein-stabilized oil-in-water emulsions. *Colloids Surf. B Biointerfaces* **2010**, *81*, 130–140, doi:10.1016/j.colsurfb.2010.06.033.
28. Keerati-u-rai, M.; Corredig, M. Heat-induced changes in oil-in-water emulsions stabilized with soy protein isolate. *Food Hydrocoll.* **2009**, *23*, 2141–2148, doi:10.1016/j.foodhyd.2009.05.010.
29. Pinto, I.; Buss, A.  $\zeta$  Potential as a Measure of Asphalt Emulsion Stability. *Energy Fuels* **2020**, *34*, 2143–2151, doi:10.1021/acs.energyfuels.9b03565.
30. Lee, J.; Jo, S.H.; Lim, J. Effect of surface modification of CaCO<sub>3</sub> nanoparticles by a silane coupling agent methyltrimethoxysilane on the stability of foam and emulsion. *J. Ind. Eng. Chem.* **2019**, *74*, 63–70, doi:10.1016/j.jiec.2019.02.002.
31. Mahmoudi, N.; Axelos, M.A.V.; Riaublanc, A. Interfacial properties of fractal and spherical whey protein aggregates. *Soft Matter* **2011**, *7*, 7643–7654.
32. Zhai, J.L.; Day, L.; Aguilar, M.-I.; Wooster, T.J. Protein folding at emulsion oil/water interfaces. *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 257–271, doi:10.1016/j.cocis.2013.03.002.
33. Cioci, F.; Lavecchia, R. Molecular thermodynamics of heat-induced protein unfolding in aqueous media. *Aiche J.* **1997**, *43*, 525–534, doi:10.1002/aic.690430225.
34. Caplan, M.R.; Schwartzfarb, E.M.; Zhang, S.; Kamm, R.D.; Lauffenburger, D.A. Control of self-assembling oligopeptide matrix formation through systematic variation of amino acid sequence. *Biomaterials* **2002**, *23*, 219–227.
35. Hosseini, A.; Jafari, S.M.; Mirzaei, H.; Asghari, A.; Akhavan, S. Application of image processing to assess emulsion stability and emulsification properties of Arabic gum. *Carbohydr. Polym.* **2015**, *126*, 1–8, doi:10.1016/j.carbpol.2015.03.020.
36. Wu, W.; Zhang, C.; Kong, X.; Hua, Y. Oxidative modification of soy protein by peroxy radicals. *Food Chem.* **2009**, *116*, 295–301, doi:10.1016/j.foodchem.2009.02.049.

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