



Altering almond protein function through partial enzymatic hydrolysis for creating gel structures in acidic environment

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ABSTRACT

Protein inadequacy is the major problem for most plant-based dairy yoghurt substitutes. This study investigated three limited degree of hydrolysis (DH: 1%, 5%, and 9%) of almond protein and the combined effect of DH and hydrolysed almond protein (HP) to non-hydrolysed almond protein (NP) ratios (HP/NP: 40:60, 20:80, 10:90 and 5:95) on the physicochemical properties of resulting fermentation induced almond-based gel (yoghurt). The gel microstructure, particle size, firmness, pH, water holding capacity (WHC), lubrication, flow, and gelation characteristics were measured and associated with the DH, composition, and SDS-PAGE results. The results show significant differences in gel samples with the same HP/NP (40:60) ratio of protein but different protein DH. A higher DH (9%) resulted in samples with lower hardness (6.03 g), viscosity (0.11 Pa s at 50 s⁻¹), cohesiveness (0.63) and higher friction (0.203 at 10 mm/s) compared to sample with 1% DH with higher hardness - 7.34 g, viscosity at 50 s⁻¹ - 0.16 Pa s, cohesiveness - 0.86 and friction at 10 mm/s - 0.194. Comparing samples with the same DH (5%) but different HP/NP ratios showed smaller coarse microgel particles (21.36 μm) and lower hardness (7.17 g), viscosity (0.14 Pa s at 50 s⁻¹) and friction value (0.189 at 10 mm/s) in samples with high HP/NP (40:60) compared to sample with low HP/NP (5:95) that contained significantly large coarse microgel particles (34.61 μm) with the gel being very hard (9.38 g), highly viscous (0.32 Pa s at 50 s⁻¹), and less lubricating (0.220 at 10 mm/s).

1. Introduction

Almond yoghurts (i.e., the fermentation-induced almond-based gel, referred to as almond-based gel in this study) are becoming increasingly popular as they meet consumers' demands for dairy-free protein, no beany flavour, are lactose-free, with pro-prebiotic like functionalities and are more environmentally friendly than dairy products (Grasso et al., 2020). Almond yoghurt has an excellent nutritional profile (e.g., proteins, fibres, vitamins, minerals) that impart health benefits (e.g., cancer prevention, antioxidants, vegan diet) (Makinen et al., 2016). It was found that almond yoghurt with specific probiotic bacteria strains culture can enhance iron intake and maintain gut health (Bernat et al., 2015). In terms of organoleptic characteristics, He and Hekmat (2014) found that almond yoghurt obtained the highest sensory score in terms of texture, flavour, consistency and overall acceptability compared to the other two commonly consumed non-dairy yoghurt (derived from soy and peanut) and no significant different ($p > 0.05$) was found compared to the control dairy yoghurt. They also proposed that almond milk (to

avoid controversy, we named it as 'almond emulsion' in this study) is suitable for probiotic survival. Thus, fermentation with almond emulsion could be feasible for innovative non-dairy yoghurt substitute studies.

However, most commercial almond-based gels have low protein content (<2.3%) compared with dairy yoghurts (>2.7% protein) (Zhao et al., 2021). The main technical reason for the low protein content in commercial almond-based gels could be that the almond protein has weak hydrophobicity and low molecular weight (basically 20–22 kDa, acidic polypeptides 42–46 kDa), resulting in poor protein dispersibility in almond emulsion and unstable gel formation in almond gel (de Souza et al., 2020; Devnani et al., 2020; Shi et al., 2020). Functional properties such as solubility, gelation, viscosity, foamability, water retention ability and oil binding capacity of almond proteins significantly affect the quality of almond-based foods (Sze-Tao and Sathe, 2000). Enzymatic hydrolysis of plant proteins (such as sourced from almond, soy and corn, sunflower seeds and legumes) changed the general properties (including hydration, surface and structural properties), therefore modifying the

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functional properties (e.g., smoothness, solubility, water holding capacity, viscosity and cohesiveness) of the protein (Akharume et al., 2021). The hydrolysed plant proteins will further determine the quality of plant-based gels.

Plant proteins sourced from soy, pea, wheat, and rice are used as nutritional and functional ingredients in yoghurt production; their benefits lie in nutrition improvement due to abundant amino acid contents and in texture enhancement (e.g. improved gel stability, increased viscosity, desirable consistency) as observed by Akin and Ozcan (2017). Most plant proteins may become less soluble due to the denaturation under extreme pH (close to protein isoelectric point may results in aggregation), high pressure (e.g., 200 MPa, 5 min for peanut protein) and temperature (e.g. 85 °C, 30 min for soy protein and 95 °C, 15 min for pea protein) in the food system (e.g. beverage, yoghurt) or during production (Lin et al., 2017). Almond protein isolate has shown good functionality in foam stability, oil absorption ability and water solubility and considered suitable to be used in an acidic environment (Sze-Tao and Sathe, 2000).

Enzymatic hydrolysis can enhance functionality (e.g., dispersibility, emulsifying, solubility, and foaming properties) of proteins by cleaving the peptide bonds and still retaining their nutritional value (Akharume et al., 2021). Enzymatic treatment notably increased the solubility of plant proteins and therefore alters the functionality, which can be ascribed to the reduced molecular weight, increased hydrophobic regions and released ionizable groups (Wouters et al., 2016). Plant protein hydrolysates sourced from almond, rice, okara, chickpea has been used as nutrition supplement in foods because of their antioxidant effect, abundant amino acid groups and the size reduction of polypeptides (Adebiyi et al., 2008; de Souza et al., 2020; Li et al., 2008; Sbroggio et al., 2016; Sze-Tao and Sathe, 2000; Udenigwe et al., 2013; Yust et al., 2010). Almond protein contains balanced amino acid groups, except for methionine (Sathe (1992)). They hypothesized that the enzymatic treatment releases the methionine. The bitterness generated from the accumulation of hydrophobic amino acid residues is the main challenge in applying plant-based protein hydrolysates. However, it can be prevented by limited hydrolysis with a degree of hydrolysis (DH) of less than 10% (Akharume et al., 2021). Alcalase is found to be an effective proteinase in the hydrolysis of plant proteins such as apricot kernel protein, soy protein and pea protein (Klost et al., 2020; Osman et al., 2016; Zhu et al., 2010).

Enzymatic modification of dairy products has already been studied: Transglutaminase (TGase) is the commonly used enzyme to improve the firmness, elasticity and viscosity of set type yoghurt; protein-glutaminase (PG) treatment breaks casein micelles into smaller particles and reduces the pore size in the gel structure of a set type yoghurt gel; while other enzymes such as peroxidases, oxidative enzymes, lactase, sulphhydryl oxidases and tyrosinase were only investigated for their effect on the milk protein profile (Ercili-Cura et al., 2015). The studies on plant protein hydrolysates sourced from almond, soy, apricot kernel, chickpea are primarily focused on functional properties (e.g. emulsification, fat absorption, and gelling properties) and nutritional benefits like antioxidant properties (Akharume et al., 2021; de Souza et al., 2020; Sun, 2011; Yust et al., 2010; Zhu et al., 2010). There are few studies on utilising plant-based protein hydrolysates (e.g., pea, quinoa) in acidic gels (Galante et al., 2020; Klost et al., 2020). The role of hydrolysed protein to the physicochemical and microstructural properties of high-protein enriched almond-based gels has not been previously reported. Therefore, the main aim of this research is to investigate the role of limited hydrolysis degrees (DH: 1%, 5%, and 9%) of almond protein and the combined effect of partially enzymatically hydrolysed and non-hydrolysed almond proteins on the characteristics of the almond gel. The effect of hydrolysis degree and the hydrolysed almond protein (HP) to non-hydrolysed almond protein (NP), HP/NP ratio on the properties (e.g., micrograph, particle size, firmness, pH, syneresis level and lubrication behaviour) of almond gel were investigated. It is hypothesized that using the reconstituted almond emulsions made from

varying percentage of partially hydrolysed and non-hydrolysed almond protein isolates, almond oil and sugar can improve the solubility of the protein in base almond emulsions that will promote gel network formation during the fermentation process. Limited hydrolysis (DH < 10%) of protein makes high protein almond gel production possible and improve their elastic properties and stability.

2. Materials and methods

2.1. Materials and reagents

Almond protein isolate (API, 90% purity, Xi'an Quanao Biotech Co., Ltd, China), almond oil (90.7% purity, Melrose Laboratories Pty Ltd, AU) and table sugar (99.8% purity, Coles supermarket, AU) were used for sample preparation. The freeze-dried lactic culture YC-X11 (YoFlex®) which for direct vat set yoghurt fermentation was provided by Chr. Hansen Pty. Ltd (AU). Alcalase® 2.4 L FG (2.4 AU-A/g) was purchased from Sigma-Aldrich manufactured from Novozymes Corp. All other reagents used in this research were of analytical grade.

2.2. Preparation of almond protein hydrolysates

In this study, almond protein hydrolysates were produced by partial enzymatic hydrolysis [Alcalase® (2.4 L FG)] of API, with an enzyme to substrate (E/S) ratio of 2:100 (w/w). API (8.9%) solutions were mixed with the enzyme at 55 °C, pH 8 for 1 min, 20 min and 40 min to achieve almond protein hydrolysates with DH of 1%, 5% and 9%, respectively. The pH 8 was maintained with NaOH (1 mol/L) during hydrolysis. The enzyme was inactivated post hydrolysis by heating the solution for 10 min at 90 °C. The degree of hydrolysis (DH) of almond protein hydrolysate was measured by the quick and accurate o-phthalaldehyde (OPA) method as described by Nielsen et al. (2001) and de Almeida et al. (2019) that is based on the reaction between primary amino groups with OPA. The almond protein hydrolysate (400 µL) was first mixed with a 3 mL OPA reagent. The mixture was then rested at room temperature for 2 min before determining the absorbance of the OPA reacted with amino acids and peptides at 340 nm. The standard sample used for the method was L-serine solution (0.9516 meqV/L) and the blank sample was water. The DH of almond protein was determined as Equation (1) below:

$$DH(\%) = h/h_{tot} \times 100 \quad (1)$$

where h is the number of cleaved peptide bonds; h_{tot} is the total number of peptide bonds per protein equivalent. h_{tot} for almond protein is 7.58 eq/Kg (de Almeida et al., 2019).

2.3. Preparation of base emulsion and almond-based gels

The suitable principal components (8% protein, 4% fat and 3% sugar contents) in formulations were selected based on our previous study (Zhao et al., 2021). Preliminary trials verified that samples made from hydrolysed almond protein and non-hydrolysed almond protein (HP/NP) in the ratio higher than 50:50 showed a fluid texture (not in a set gel form) with a visible watery appearance after one-day storage. This observation is similar to a study conducted by Klost et al. (2020). They explained that Alcalase pea protein hydrolysate with a DH higher than 10% cannot form self-supporting gels due to the low molecular weight of globular proteins that did not meet the minimum gelation requirement of 23 kDa. We hypothesized that adding non-hydrolysed protein along with hydrolysed proteins could help with gel formation. Therefore, after preparation of the almond protein hydrolysates solutions with different DH (1%, 5% and 9%), non-hydrolysed API, almond oil, sugar and water were added to produce 12 base almond emulsions made from various hydrolysed almond protein to non-hydrolysed almond protein (HP/NP) ratio (40:60, 20:80, 10:90 and 5:95). The control sample was the one made from only non-hydrolysed almond

proteins. The formulation of each sample with different DH and the control sample is shown in Table 1. Base emulsion was first mixed (1000 rpm, 15 min) followed by a 2-stage homogenisation (1st 30 Bar, 2nd 170 Bar), pasteurisation (85 °C, 30 min), and fermentation (40 °C, until pH drops to 4.4–4.6) to prepare almond-based gel samples. The detailed production process is presented in Fig. 1.

2.4. Physicochemical property characterization

2.4.1. Textural analysis

A TPA device (Micro Stable System Co., UK) was used for hardness and cohesiveness measurement of almond-based gels, as Basiri et al. (2018) described. A 10 mm diameter cylindrical shaped probe was selected to perform the compression test at 1 mm/s speed with a 5 g trigger force applied.

2.4.2. Rheological properties

The rheological properties of each sample were acquired by a rheometer (AR-G2, TA Instruments Ltd, US) as described by Nguyen et al. (2017) and Zhao et al. (2021). The set gel was stored at 25 °C for 45 min and then gently stirred to eliminate the effect from syneresis and temperature during measurement. The 40 mm diameter sandblasted stainless-steel parallel plate was used to eliminate the slip effect with shear rate performed between 0.1 and 1000 (s⁻¹) at 200 µm gap. To mimic the gelation process during fermentation (40 °C, 4 h), the dynamic oscillatory rheological measurement was conducted at the same shear rate range and gap.

2.4.3. Lubrication behaviour

A Rheometer (Discovery Hybrid, TA Instrument) connected with a ring on plate geometry was used to determine the lubrication properties of foods during oral processing. Samples were rested at 25 °C for 45 min. As described by Nguyen et al. (2017), a 3M Transpore™ surgical tape was used to imitate the tongue surface; samples were then shear for 60 s under 0.01 s⁻¹, the rotation speed is between 0.01 and 100 s⁻¹, the operation force is 2N.

2.4.4. Particle size

Particle size measurement of almond-based gels was conducted in a Mastersizer 2000 (Malvern Scientific Instruments Ltd., UK) with sample handling unit HYDRO 2000MU(A) as described by Zhao et al. (2021) but with 1 min ultrasonic waves applied during measurement. Particle size parameters including (D_{3,2}), (D_{4,3}), d(0.1), d(0.5) and d(0.9) were analysed by the Mastersizer 2000 software.

Table 1

Codes and formulation of samples made with different HP/NP ratio and different levels of DH. ^a

Code	Abbreviation	API (8.9%)				Almond oil (%)	Sugar (%)
		HP/NP ratio	DH (%)	HP (%)	NP (%)		
1	1%, 40:60	40/60	1	3.56	5.34	4.4	3
2	1%, 20:80	20/80		1.78	7.12	4.4	3
3	1%, 10:90	10/90		0.89	8.01	4.4	3
4	1%, 5:95	5/95		0.45	8.46	4.4	3
5	5%, 40:60	40/60	5	3.56	5.34	4.4	3
6	5%, 20:80	20/80		1.78	7.12	4.4	3
7	5%, 10:90	10/90		0.89	8.01	4.4	3
8	5%, 5:95	5/95		0.45	8.46	4.4	3
9	9%, 40:60	40/60	9	3.56	5.34	4.4	3
10	9%, 20:80	20/80		1.78	7.12	4.4	3
11	9%, 10:90	10/90		0.89	8.01	4.4	3
12	9%, 5:95	5/95		0.45	8.46	4.4	3
13	Control	0/100	0	0.00	8.90	4.4	3

^a HP, Hydrolysed almond protein; NP, Non-hydrolysed almond protein; DH: degree of hydrolysis in HP; API, Almond protein isolate (90% purity).

2.4.5. Water holding capacity (WHC)

The WHC of each sample was determined as illustrated by Akalin et al. (2012) but with some modification. Base emulsions (20 g) were weighed in a centrifuge tube and then incubated at 40 °C for 4 h. After storage for 24 h under 4 °C, the set gels were centrifuged by the 5702R centrifuge (Eppendorf, AU) at 3000×g for 10 min at 4 °C. The WHC was calculated using Equation (2):

$$WHC (\%) = (Y - W) / Y \times 100 \quad (2)$$

where Y is the mass of yoghurt and W is the weight of separated whey.

2.4.6. Syneresis

Syneresis was determined by a method described by Klost et al. (2020). Briefly, 10 g base emulsion was weighted in a 12 mL centrifuge tube, and it was then fermented at 40 °C for 4 h and kept in the fridge at 4 °C for 24 h. The syneresis (S%) was expressed as the mass of supernatant divided by the total weight of the yoghurt in percentage.

2.4.7. pH determination

The pH value was measured by a handheld AQUA-pH meter (TPS Pty Ltd, AU) during the fermentation period of 4 h (monitored every 30 min). Further, the pH of almond yoghurt was recorded during storage on day 1, 7, 14, and 21. pH 7 and pH 4 buffer solutions were used to calibrate the pH meter before each measurement.

2.4.8. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein profiles were determined by SDS-PAGE as described by Klost et al. (2020) and Song et al. (2008). The running buffer, fixative solution, staining solution, and the de-staining solution was prepared following the procedure described by Fling and Gregerson (1986). Samples of protein concentrate, 5% (w/w) were made with Laemmli Sample Buffer (Bio-Rad Laboratories, USA) and 2-Mercaptoethanol (Sigma, USA). The commercial gel (4–20% Mini-PROTEAN® TGX™ Precast Protein Gels, Bio-Rad Laboratories, Inc., US) was assembled into the electrophoresis cell (Bio-Rad Mini Protean Tetra Cell System) to run for reducing gel with reference markers (precision plus protein™ dual xtra pre-stained protein standards) under 100V for 1 h. The electrophoresis gels were then stained with Coomassie Brilliant G-250, destained and washed. Finally, the gel was read by a Gel Scanner machine.

2.5. Microstructure

Microstructural images of the almond-based gel samples were viewed by confocal laser scanning microscopy (CSLM, Zeiss LSM700, Carl-Zeiss-Promenade, Germany) as described by Grundy et al. (2016) and Zhao et al. (2021). Dyes were prepared with PEG 200 before each measurement. Then, 20 µL sample was mixed with 20 µL 0.02% Nile Red, 20 µL 0.005% Rhodamine B and 10 µL 0.1% of Calcofluor white. The wavelengths of 488 nm, 555 nm and 405 nm laser were selected for excitation of Nile Red, Rhodamine B and Calcofluor, respectively. Fat, protein and polysaccharide polymers (specific to almond cell wall fibres) were stained in green, red and blue colour, respectively. The software Image J was used for the porosity measurement described by Mauko et al. (2009). Images were transferred into binary format before the porosity measurement to analyse the fraction area.

2.6. Statistical analyses

One-way ANOVA was performed to analyse the significant difference (p < 0.05) between samples using a Minitab 17 statistical package. Principal component analysis (PCA) was used to analyse the data from two perspectives – DH and HP/NP ratio; it is carried out to distinguish the similarities and differences between variables including TPA,

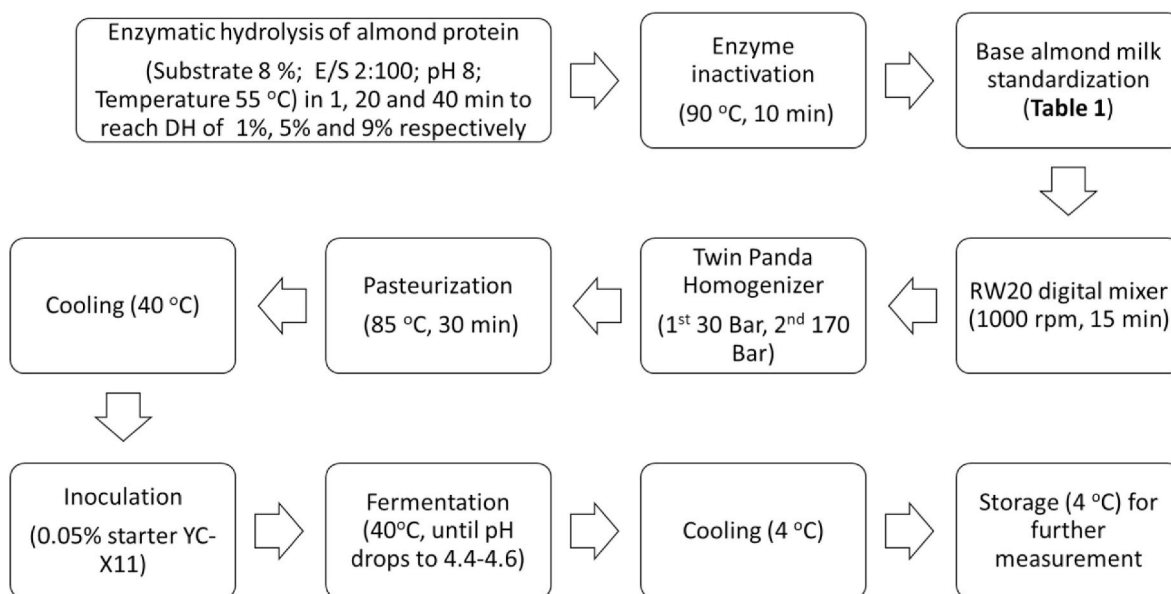


Fig. 1. Flow chart of almond-based gel production from protein hydrolysates with different levels of hydrolysis degree (DH) and non-hydrolysed almond protein substitute.

particle size, pH, flow and tribology behaviours; P-values were set at less than 0.05 to verify the significance. Pearson correlation analysis was performed to investigate the correlations among all tested physico-chemical properties of almond-based gels. All measurements, including emulsion and gel making, were performed in triplicate.

3. Results and discussion

3.1. Effect of partial enzymatic hydrolysis on the almond protein profile (SDS-PAGE)

The SDS-PAGE (Fig. 2A) revealed that the partial enzymatic

hydrolysis of protein significantly changes the almond protein profile as observed by a reduction in bandwidth that indicates molecular weight reduction with an increase in DH. In Fig. 2A, for the non-hydrolysed protein profile, amandin (belongs to the 11S globulin family) is the main seed storage protein with a molecular weight of 62–66 kDa, the bands around 40 kDa (N-terminal subunit) and 20 kDa (C-terminal subunit) correspond to acidic and basic polypeptides as studied by Jin et al. (2009). Upon partial hydrolysis by Alcalase, amandin breaks down to smaller polypeptides with lower molecular weight as the degree of hydrolysis increased from 1%, 5% through to 9% (Fig. 2A), with approximately <20 kDa obtained for almond protein hydrolysates. This indicates that the major polypeptides were broken into smaller fractions.

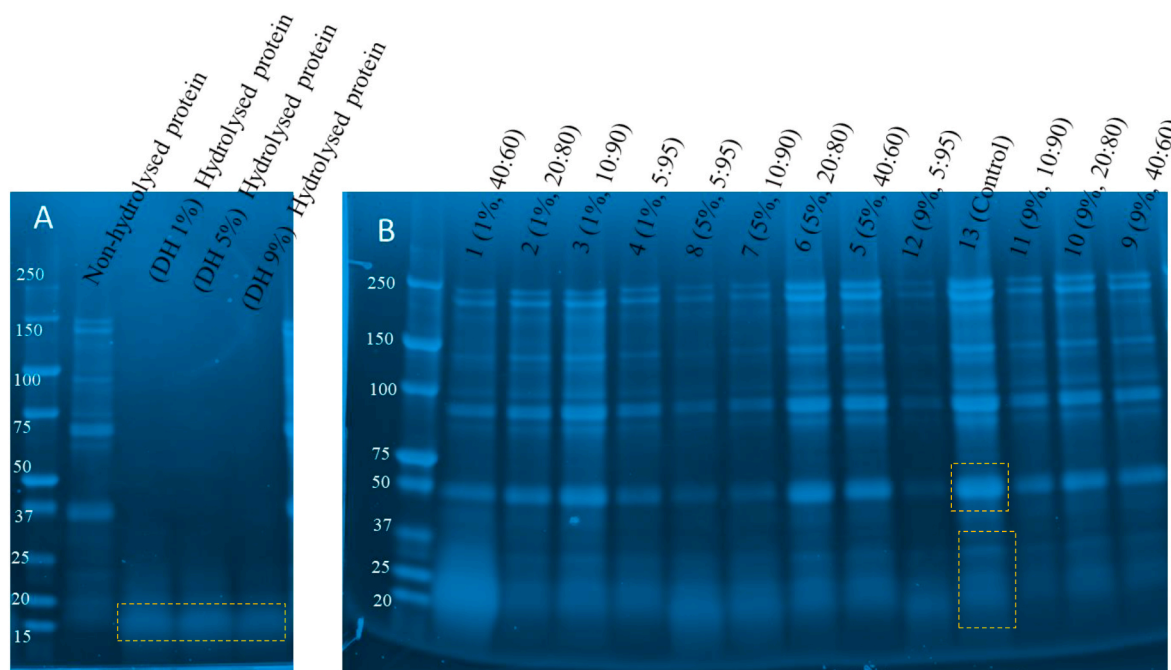


Fig. 2. SDS-PAGE analysis of almond proteins. Molecular weight ladder is included in the far-left lane in image A and image B, numbers indicate molecular weights of respective bands in kDa. (A) almond protein hydrolysates with DH of 1%, 5%, 9% and a control without enzymatic treatment respectively. (B) almond protein profiles in each almond-based gel from sample 1 to 13.

This result agrees with de Almeida et al. (2019) who reported an 80% breakdown of the major polypeptides to less than 14.4 kDa molecular weight upon protease hydrolysis.

When mixing different proportion of hydrolysed almond protein and non-hydrolysed proteins, bands with molecular weight >23 kDa was observed in the electrophoretic profile (Fig. 2B). That means they can form a gel structure (Klost et al., 2020) after incorporation of the non-hydrolysed protein. The non-hydrolysed protein present in the control sample 13 were far denser in some regions based on band position: the region 65–70 kDa refers to the major protein amandin, consistent with Devnani et al. (2020) where amandin appeared at band 60 kDa in their almond milk samples; the bands around 35 kDa should be the known acidic polypeptides of amandin (38–42 kDa), while the band around 20 kDa corresponds to the basic polypeptides (20–22 kDa) that is associated with disulphide bonds (Sathe, 1992). Other rare bands between 90 and 240 kDa at very low levels need further study. Samples incorporated with protein hydrolysates (samples 1–12) all showed similar band positions but were less dense in band positions over 20 kDa than the control sample 13.

3.2. Effect of partial enzymatic hydrolysis (DH and HP/NP ratio) on the pH change of almond-based gel during fermentation and storage period

As an acidifier, lactic acid bacteria have gelling properties during incubation due to the pH drop caused by metabolic activity (Yang et al., 2021). Fig. 3 and Fig. 4 shows the pH change of each sample during fermentation and the 21-day storage period. During the 4-h fermentation, the pH of all samples dropped from 6.3 to 4.5–4.6, indicating the formation of gel structure as almond protein aggregated at the isoelectric point (pI) of 4.5–5.5 (Jeske et al., 2018). The pH of all tested samples is similar to other commercial dairy yoghurt substitutes sourced from the plants (e.g. almond, soy and coconut) (Grasso et al., 2020). Compared to the control sample 13 (without enzymatic treatment), samples with hydrolysed proteins (samples 1–12, Fig. 4) have a lower pH value.

In Figs. 3 and 4, for samples with the same DH, the pH value decreased with an increased HP/NP ratio during the fermentation and storage period. For instance, looking at sample 9 (DH 9%, 40:60) and sample 12 (DH 9%, 5:95), the pH of sample 9 is 5.45 is significantly ($p < 0.05$) lower than the sample 12 (5.56) at 1.5h and show similar trend during storage except for day 21 (Data not shown).

As expected, under the same HP/NP ratio, samples with higher DH showed higher pH values (Figs. 3 and 4) during fermentation and 21-day storage. For example, sample 1 with DH of 1% showed significantly higher pH values (4.53, 4.35, 4.40) compared to sample 9 (4.58, 4.50, 4.44) with a DH of 9%, although they have the same HP/NP ratio of 40:60 on days 1, 7 and 14 (Data not shown). In this case, it can be

deduced that almond-based gels made from more hydrolysed proteins and with a DH of 9% may significantly reduce the fermentation time as more short peptides allow lactic acid bacteria survival. SDS-PAGE results (Fig. 2) confirmed this assumption.

A principal component analysis (PCA) was conducted to explore the relationships between pH change (during fermentation and storage), physical properties and the effect of partial enzymatic hydrolysis based on DH and HP/NP ratio variance. PCA score plot shows clusters of samples based on their similarity, reducing the dataset's dimensionality and exploring the linkage between analysed variables (e.g., DH, hardness, viscosity, WHC, pH, coefficient of friction and particle size). PCA score plot (Fig. 5A) clearly separated grouping based on different HP/NP ratios of all tested samples through physicochemical properties evaluation (e.g., pH, apparent viscosity, hardness, coefficient of friction and particle size), particularly on PC-1. Notably, almost all physicochemical variables are highly contributing to PC-1. From PC-1 with a percentage of 61% (Fig. 5C), the changed pH, hardness, syneresis level, apparent viscosity at 50 s^{-1} , friction at sliding speed 1 mm/s and 10 mm/s, and particles size results are highly positively related to the HP/NP ratio, while WHC is strongly negatively linked to the HP/NP ratio. Different degree of hydrolysis (DH) affects the properties of almond-based gels. PCA score plots in Fig. 5B clearly show the cluster of markers based on different DH through physicochemical evaluation. Notably, almost all physicochemical variables, including changed pH, lubrication behaviour, hardness, cohesiveness, particle size feature, WHC and syneresis level, are highly contributing to PC-2 (10%). In particular, most measured properties are highly contributing to PC-2. Analysis of contributions of individual physicochemical variables to the principal components (Fig. 5D) suggests that the changed pH values showed highly positive contribution, while the apparent viscosity, cohesiveness, hardness, lubrication behaviour and particle size properties show strongly negative contribution. The effect of hydrolysed almond protein to non-hydrolysed almond protein (HP/NP) ratio has more effect than the degree of hydrolysis (DH) on the physical properties of almond-based gels as the loading of PC-1 (61%) is higher than PC-2 (10%). Detailed results are discussed in the following section.

3.3. Effects of hydrolysed almond protein to non-hydrolysed almond protein (HP/NP) ratio and degree of hydrolysis (DH) on the physical properties of the almond-based gel

3.3.1. Water holding capacity (WHC) and syneresis level of almond-based gel

In fermentation-induced gels, part of the water is absorbed or held/attached by the protein particles and the fibres; the rest of the water is held in the gel matrix, small pore structure tends to hold more water. WHC and syneresis levels (S%) are determined by the composition and

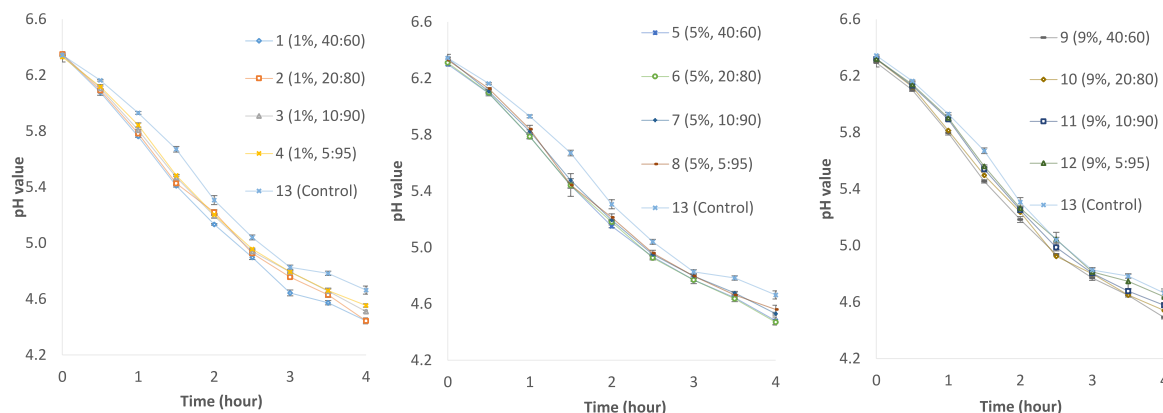


Fig. 3. pH changes of almond-based gels during 4-h fermentation.

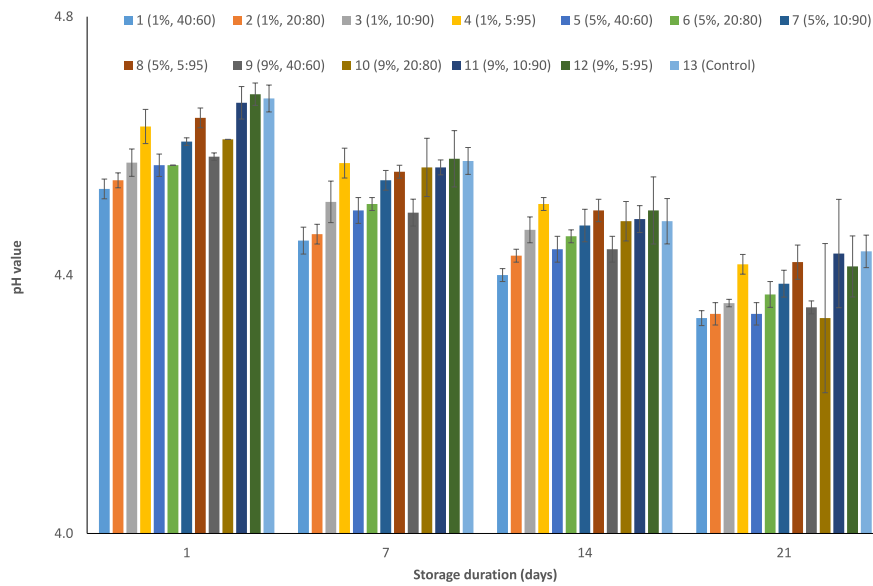


Fig. 4. pH changes of almond-based gels during 21-day storage.

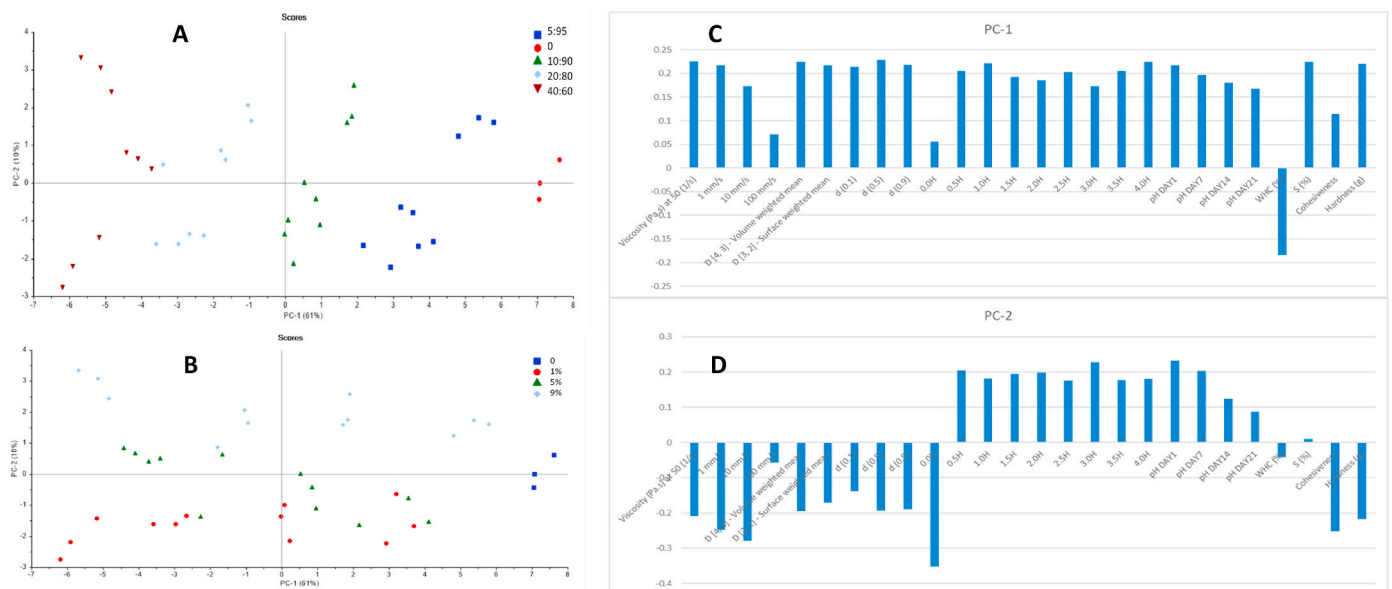


Fig. 5. A. Principal component score plot of all tested samples with different HP/NP ratios (40:60, 20:80, 10:90, 5:95, the control without hydrolysed proteins is labelled as 0) through physicochemical measurements. B. Principal component score plot of all tested samples with different hydrolysis degrees (DH: 1%, 5%, 9%, the control without enzymatic treatment is labelled as 0) through physicochemical measurements. Principal component score plot loadings for PC1 (C.) and PC2 (D.) of all tested samples with different HP/NP ratios and with different hydrolysis degree through physicochemical measurement.

microstructure of almond yoghurts. From Fig. 6, compared to the control sample 13 (WHC-88.91%; S%-2.71%), some samples with hydrolysed proteins in their formulations show higher WHC that can be associated with the enzymatic treatment. As per Akharume et al. (2021), hydrolysis of proteins i.e., the catalytic response between enzymes (Alcalase) and almond protein substrates results in the cleavage of the peptide bonds and formation of short-chain peptides and lower molecular weight of amino acids impacting the functionality of plant proteins, particularly their solubility, emulsifying ability, oil or water absorption and foaming ability. Therefore, the hydrolysed almond proteins form a more stable emulsion or gel matrix that holds more water content. However, samples 11, 4, 8 and 12 showed severe syneresis levels of 2.80%, 2.97%, 3.09% and 3.37%, respectively. Samples with a lower proportion of hydrolysed

protein than non-hydrolysed protein (HP/NP ratio of 5:95) resulted in almond-based gels with better WHC but higher syneresis levels. This indicates that the DH and the HP/NP ratio influences gel stability at an appropriate level.

Higher WHC indicates better stability of almond-based gel. In Fig. 6, samples with the same HP/NP ratio of 40:60 (Sample 1, 5 and 9) show significantly higher WHC (91.38%, 91.02% and 90.65%, respectively), while samples with the same HP/NP ratio of 5:95 (sample 4, 8 and 12) shown significantly lower WHC among all tested samples. It is evident from Fig. 6, that the syneresis of gels decreased as the HP/NP ratio increased from 5:95 to 40:60. For example, sample 4 (1%, 5:95) has a significantly ($p < 0.05$) high S (%) of 2.97% compared to sample 1 (1%, 40:60) with a S (%) of 1.45%. This might be due to the increased amount

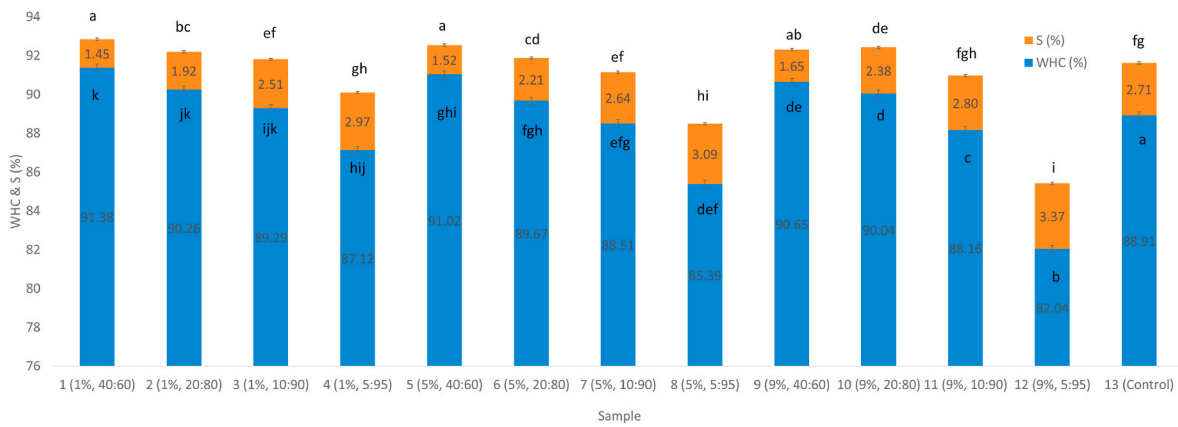


Fig. 6. Water holding capacity (WHC) and Syneresis (S%) of almond-based gels was presented as mean ± SD. Significant differences at 95% of confidence among almond-based gels were labelled with different letters in the same color column. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of protein hydrolysates, as according to Wouters et al. (2016), enzymatic hydrolysis contributed to the release of polar and ionizable groups, thus improving the water holding capacity of plant proteins. The more size reduced peptide chains (Fig. 2B) present in the gel matrix might more evenly extend their branching structure into protein aggregates resulting in even more extensive protein-protein interactions. This will give the yoghurt little syneresis, reflecting better gel stability (Srisuvor et al., 2013).

A significant difference ($p < 0.05$) in WHC was only found in samples with different DH under the HP/NP ratio of 5:95. From Fig. 6, as the DH increased from 1%, 5%–9%, samples 1, 5, and 9, with the same HP/NP ratio of 40:60 show significantly ($p < 0.05$) decreased WHC (91.38%, 90.04% and 88.51%, respectively). The syneresis (S%), by contrast, showed almost opposite trends. Sample 1, 5 and 9 showed increased S (%) (1.45%, 1.53% and 1.65%, respectively). The increase in S (%) with increased DH might be related to the porosity of yoghurt gel (Srisuvor

et al., 2013). A similar trend was observed in pea protein-based yoghurt gels by Klost et al. (2020), they associated the syneresis level with the smaller peptides present in the protein hydrolysates. The micrographs of sample 1 and sample 9 (Fig. 9) showed supportive evidence to this statement regarding the pore size and shape in the gel network formed by protein, fat, polysaccharides (fibre), and water.

3.3.2. Particle size of almond-based gel

Particle size influences the organoleptic features of semi-solid foods like cheese and yoghurt; large-sized particles are responsible for in-mouth graininess (Krzeminski et al., 2014). The measured particles include the floating phase (fat droplets) and sinking phase (mainly solids such as protein aggregate or gel particles) as described by Durand et al. (2003). All almond yoghurt samples showed a similar bimodal pattern of the particle size distribution (figure not shown). The bimodal pattern (two peaks) is assumed to be associated with two groups of particles

Table 2
TPA, tribology and particle size results of almond-based gels.

Items	Textural properties			Coefficient of friction at speed			Particle size (in μm)				
	Viscosity 50s^{-1} (Pa.s)	Hardness (g)	Cohesiveness	1 mm/s	10 mm/s	100 mm/s	D _{4,3}	D _{3,2}	d (0.1)	d (0.5)	d (0.9)
1 (1%, 40:60)	0.16 ± 0.01 ^{hi}	7.34 ± 0.00 ^{bc}	0.86 ± 0.03 ^{bc}	0.196 ± 0.00 ^{ef}	0.194 ± 0.00 ^{cde}	0.265 ± 0.02 ^a	22.02 ± 2.65 ^{de}	5.03 ± 0.51 ^{de}	2.03 ± 0.39 ^{de}	16.46 ± 2.28 ^{de}	47.18 ± 0.51 ^d
2 (1%, 20:80)	0.21 ± 0.00 ^f	7.68 ± 0.05 ^{cd}	0.82 ± 0.03 ^{abc}	0.206 ± 0.00 ^{cd}	0.210 ± 0.00 ^{abcd}	0.273 ± 0.00 ^a	26.70 ± 3.73 ^{bcde}	5.04 ± 0.46 ^{de}	1.99 ± 0.40 ^{de}	17.67 ± 2.34 ^{cde}	61.42 ± 3.86 ^{bcd}
3 (1%, 10:90)	0.29 ± 0.00 ^{de}	8.26 ± 0.13 ^e	0.83 ± 0.01 ^{abc}	0.210 ± 0.00 ^{bc}	0.209 ± 0.02 ^{abcd}	0.261 ± 0.04 ^a	31.19 ± 4.10 ^{bc}	5.78 ± 0.41 ^{bcd}	2.77 ± 0.45 ^{cd}	20.88 ± 2.20 ^{bc}	71.47 ± 2.43 ^{bc}
4 (1%, 5:95)	0.36 ± 0.01 ^b	9.95 ± 0.16 ^h	0.92 ± 0.12 ^{bc}	0.220 ± 0.00 ^a	0.214 ± 0.00 ^{abc}	0.289 ± 0.00 ^a	34.80 ± 4.72 ^{ab}	6.54 ± 0.27 ^{ab}	3.94 ± 0.27 ^a	24.02 ± 1.41 ^{ab}	77.70 ± 2.15 ^{ab}
5 (5%, 40:60)	0.14 ± 0.00 ⁱ	7.17 ± 0.11 ^b	0.89 ± 0.06 ^{bc}	0.194 ± 0.00 ^f	0.189 ± 0.01 ^{de}	0.269 ± 0.03 ^a	21.36 ± 2.67 ^e	5.34 ± 0.24 ^{cd}	2.46 ± 0.21 ^{cd}	16.26 ± 1.43 ^{de}	45.50 ± 0.39 ^d
6 (5%, 20:80)	0.20 ± 0.01 ^{fg}	7.57 ± 0.05 ^{bcd}	0.82 ± 0.03 ^{abc}	0.205 ± 0.00 ^{cd}	0.198 ± 0.01 ^{bcde}	0.262 ± 0.03 ^a	26.28 ± 3.06 ^{cde}	5.83 ± 0.49 ^{abcd}	3.13 ± 0.65 ^{abc}	17.49 ± 1.44 ^{cde}	57.62 ± 3.50 ^{bcd}
7 (5%, 10:90)	0.26 ± 0.01 ^e	7.91 ± 0.09 ^{de}	0.77 ± 0.02 ^{ab}	0.209 ± 0.00 ^{cd}	0.206 ± 0.01 ^{abcde}	0.269 ± 0.02 ^a	30.10 ± 6.23 ^{bcd}	6.04 ± 0.71 ^{abc}	3.11 ± 0.76 ^{abc}	20.22 ± 3.86 ^{bcd}	67.92 ± 2.54 ^{bc}
8 (5%, 5:95)	0.32 ± 0.02 ^c	9.38 ± 0.22 ^g	0.95 ± 0.07 ^{bc}	0.217 ± 0.00 ^{ab}	0.220 ± 0.01 ^a	0.294 ± 0.00 ^a	34.61 ± 0.64 ^{ab}	6.54 ± 0.32 ^{ab}	3.71 ± 0.34 ^{ab}	23.72 ± 1.02 ^{ab}	77.39 ± 1.47 ^{ab}
9 (9%, 40:60)	0.11 ± 0.01 ^j	6.03 ± 0.14 ^a	0.63 ± 0.16 ^a	0.202 ± 0.01 ^{de}	0.203 ± 0.01 ^{abcde}	0.289 ± 0.02 ^a	19.32 ± 2.10 ^e	4.28 ± 0.38 ^e	1.46 ± 0.19 ^e	13.90 ± 1.81 ^e	43.53 ± 2.05 ^d
10 (9%, 20:80)	0.18 ± 0.00 ^{gh}	7.48 ± 0.06 ^{bcd}	0.82 ± 0.10 ^{abc}	0.207 ± 0.00 ^{cd}	0.201 ± 0.01 ^{abcde}	0.257 ± 0.01 ^a	24.29 ± 3.70 ^{cde}	5.39 ± 0.22 ^{cd}	2.32 ± 0.13 ^{cd}	19.22 ± 2.54 ^{bcd}	51.93 ± 2.62 ^{cd}
11 (9%, 10:90)	0.22 ± 0.01 ^f	7.75 ± 0.00 ^{cd}	0.80 ± 0.03 ^{abc}	0.184 ± 0.00 ^g	0.186 ± 0.01 ^e	0.266 ± 0.03 ^a	27.41 ± 3.98 ^{bcde}	5.90 ± 0.39 ^{abc}	3.03 ± 0.42 ^{bc}	20.07 ± 2.23 ^{bcd}	60.12 ± 8.88 ^{bcd}
12 (9%, 5:95)	0.29 ± 0.01 ^{cd}	8.81 ± 0.05 ^f	0.80 ± 0.03 ^{abc}	0.212 ± 0.00 ^{bc}	0.207 ± 0.00 ^{abcde}	0.289 ± 0.00 ^a	34.53 ± 7.19 ^{ab}	6.59 ± 0.45 ^{ab}	3.76 ± 0.46 ^{ab}	23.19 ± 3.51 ^{ab}	76.76 ± 3.48 ^{ab}
13 (Control)	0.43 ± 0.01 ^a	10.48 ± 0.23 ⁱ	0.99 ± 0.03 ^c	0.224 ± 0.00 ^a	0.219 ± 0.00 ^{ab}	0.272 ± 0.01 ^a	40.60 ± 5.25 ^a	6.66 ± 0.40 ^a	3.86 ± 0.41 ^{ab}	25.58 ± 2.39 ^a	93.83 ± 10.77 ^a

Values are expressed as means ± SD; values with different letters in the same columns show significant differences at 95% of confidence.

related to protein particles and fat droplets. From Table 2, samples containing hydrolysed protein showed significantly smaller particle sizes compared with the control (sample 13). For example, 10%, 50% and 90% of particles in sample 13 are 3.86 μm , 25.58 μm , and 93.83 μm respectively, while the particle size $d(0.1)$, $d(0.5)$ and $d(0.9)$ of sample 9 (9%, 40:60) were 1.46 μm , 13.90 μm and 43.53 μm , respectively. This finding indicates that hydrolysed protein in formulation contributes to less graininess of almond yoghurt gels (Krzeminski et al., 2014).

The enzymatic hydrolysis of almond protein isolates changes the protein profile, influencing the particle size and therefore influencing the tribology, rheology, hardness and stability of yoghurt products. In order to study the correlations among tested physicochemical properties of almond yoghurt that affected by the enzymatic treatment of almond proteins, Pearson correlation analysis was conducted. According to Pearson correlation results (Table S1, in supplementary material), the particle size data are highly positively correlated with the hardness, cohesiveness, viscosity, friction and syneresis level, while highly negatively correlated with WHC.

In Table 2, as the HP/NP ratio increased from 5:95 in sample 4 (DH of 1%) to 40:60 in sample 1 (DH of 1%), the $(D_{4,3})$ value which corresponds to coarse microgel particles was significantly ($p < 0.05$) reduced from 34.80 μm to 22.02 μm ; significant size reduction for 90% of particles was also observed (77.70 μm and 47.18 μm , respectively). The smaller particle size in samples with more HP indicated less volume of particles aggregated in the gel system, which confers softer gel formation. Yang et al. (2021) explained that smaller particle sizes contribute to fewer protein particle aggregation, resulting in a lower degree of cross-linking, leading to a gel with a poor hardness value. This is in line with the Pearson correlation results (Table S1, in supplementary material); all particle size variables are highly correlated with the hardness value.

The higher the DH value of samples, the smaller the particles presented in gel systems. Nevertheless, no significant difference ($p < 0.05$) was found between samples with different levels of DH (Table 2). The surface weighted mean diameter ($D_{3,2}$) reflects fine gel particles in the sample. With increased DH from 1% to 9%, samples 1 and 9 showed decreased ($D_{3,2}$) values of 5.03 μm –4.28 μm , respectively. The reduced particle size caused by increased DH may lead to better lubrication within a mixed regime (sliding speed ≥ 10 mm/s) (Krzeminski et al., 2014). This is in line with the tribology results (Table 2).

3.3.3. Hardness and cohesiveness of almond-based gel

In Table 2, control sample 13 shows a significantly higher hardness value of 10.48 g among all tested samples. This can be explained by the higher molecular weight of protein peptides (Fig. 2) in sample 13, corresponding to more branching and stronger interactions between protein-protein molecules. The DH and the HP/NP ratio did not significantly affect the cohesiveness of yoghurt gels. The low cohesiveness value of 0.63 in sample 9 (9%, 40:60) is significantly ($p < 0.05$) lower in comparison with the sample 13 (0.99) (Table 2). The cohesion of a semi-solid food (e.g., yoghurt, mayonnaise) are determined mainly by the hydrogen bonds attraction between water molecules thus the low cohesiveness value in sample 9 could be attributed to the significantly ($p < 0.05$) higher WHC (88.51%) compared with sample 13 (82.04%, respectively). Moreover, the cohesiveness value is possibly affected by the particle size caused by the interactions at the surface between each particle (e.g., protein particles, fat droplets and fiber fragments). Pearson correlation results (Table S1, in supplementary material) provides evidence, the $D(4,3)$ and $D(3,2)$ value is highly negatively correlated with WHC (r^2 0.634 and 0.703 respectively), while they are highly positively ($p < 0.05$) correlated with S% (r^2 0.785 and 0.812 respectively).

According to one-way ANOVA results, the hardness of yoghurt gels was significantly ($p < 0.05$) affected by the HP/NP ratio (Table 2), the more hydrolysed protein used in gel preparation, the lower hardness value was measured. The control sample 13 had the highest hardness of 10.48g, followed by the samples with the low HP/NP ratio of 5:95

(samples 4, 8 and 12). By contrast, samples with the high HP/NP ratio of 40:60 (samples 1, 5 and 9) show a significantly ($p < 0.05$) lower hardness value of 7.34 g, 7.17 g and 6.03 g, respectively. The hardness of yoghurt gel decreased with increased HP/NP ratio, as is evident in samples 8, 7, 6, and 5, all made from 5% DH of hydrolysed protein (Table 2); however, the hardness of the sample gradually decreased from 9.38 g, 7.91 g, 7.57 g–7.17 g as the HP/NP ratio increased from 5:95, 10:90, 20:80 to 40:60.

As shown in Table 2, samples with low DH showed a high hardness value under the same HP/NP ratio. Samples 4, 8 and 12 were made from the same amount of hydrolysed protein. However, sample 4, made from 1% DH of protein, showed a significantly ($p < 0.05$) higher hardness value of 9.95 g compared to sample 12 (8.81 g) with DH of 9%. The control sample 13 had the highest hardness value of 10.48 g, while sample 9 had the lowest hardness (6.03 g), the significant difference ($p < 0.05$) could be due to the more (40:60) and higher DH (9%) of hydrolysed proteins in samples 9 results in small particle size (Table 2), and therefore weakens the protein-protein connection, which in turn form a softer gel network with lower hardness. This can also be verified by the micrographs of the control sample 13 and sample 9 in Fig. 9.

3.3.4. The viscosity and flow behaviour of almond-based gel

From Fig. 7, the viscosity steadily decreased with increased DH, and HP/NP ratio. To draw comparison, the viscosity at shear rate 50s^{-1} was obtained from Fig. 7 and presented in Table 2.

For samples made from the same hydrolysis degree of hydrolysed almond protein, their flow behaviour shows a similar trend: the viscosity significantly decreased with the increased HP/NP ratio (Fig. 7). At shear rate 50 s^{-1} (Table 2), the viscosity significantly decreased ($p < 0.05$) from 0.32 Pa s in sample 8 to 0.14 Pa s in sample 5 with the increased HP/NP ratio from 5:95 to 40:60. The apparent viscosity is highly correlated with the friction values at sliding speed 1 mm/s (r^2 0.929) and 10 mm/s (r^2 0.773) as shown in Table S1 (Data not shown, in supplementary material).

Similarly, as the DH increased from 1% in sample 1, 5% in sample 5–9% in sample 9, the viscosity at 50 s^{-1} gradually decreased from 0.16, 0.14 to 0.11 Pa s respectively (Table 2). Fig. 7 shows that the viscosity steadily decreased with the increased DH. The samples made from high DH of protein hydrolysates had lower apparent viscosity. This can be explained by the gel network of yoghurts in Fig. 9. Detailed discussion on this is provided later in section 3.4 while discussing the effects of hydrolysed almond protein to non-hydrolysed almond protein (HP/NP) ratio and degree of hydrolysis (DH) on the gel properties of almond-based gels.

3.3.5. The gelation behaviour of almond-based gel

The measurement of elastic properties of acid gels is expressed as storage modulus (G'), reflecting the solid-like behaviour of gels. The gelation of protein is mainly based on protein-protein interactions such as hydrophobic, hydrogen bonds, and electrostatic forces (Klost et al., 2020). As shown in Fig. 2, the protein profile is influenced by the DH or varying the HP/NP ratio, which affects the protein-protein interactions during gel formation. Fig. 8 presents the gelation behaviour of 13 yoghurt samples made with different HP/NP ratios and varying degree of hydrolysis of almond protein isolates within the fermentation period. During the fermentation stage (4 h at 40 °C), all samples showed a linear increase in G' value as the HP/NP ratio (Fig. 8A) and DH (Fig. 8B) changed. This indicates extra protein-protein bonds were formed and protein matrix re-arranged in gel network during fermentation (Lee and Lucey, 2010). The gel point (the time at which G' value becomes relatively stable) for each sample is different, which can be associated with the nature of the proteins (the size of polypeptides), as protein hydrolysates interfere with gelation of yoghurts. Furthermore, it may also affect the metabolism of culture strains, as they interfere with the reduced polypeptides caused by enzymatic hydrolysis.

During the fermentation stage (4 h at 40 °C), under the same DH,

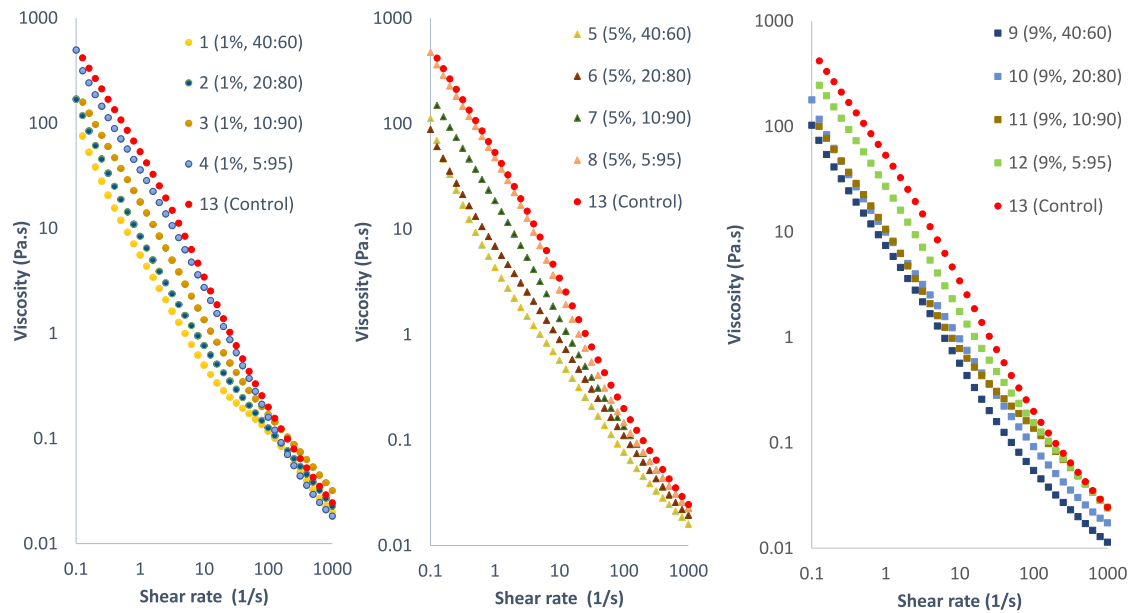


Fig. 7. Flow behaviour of each almond-based gel.

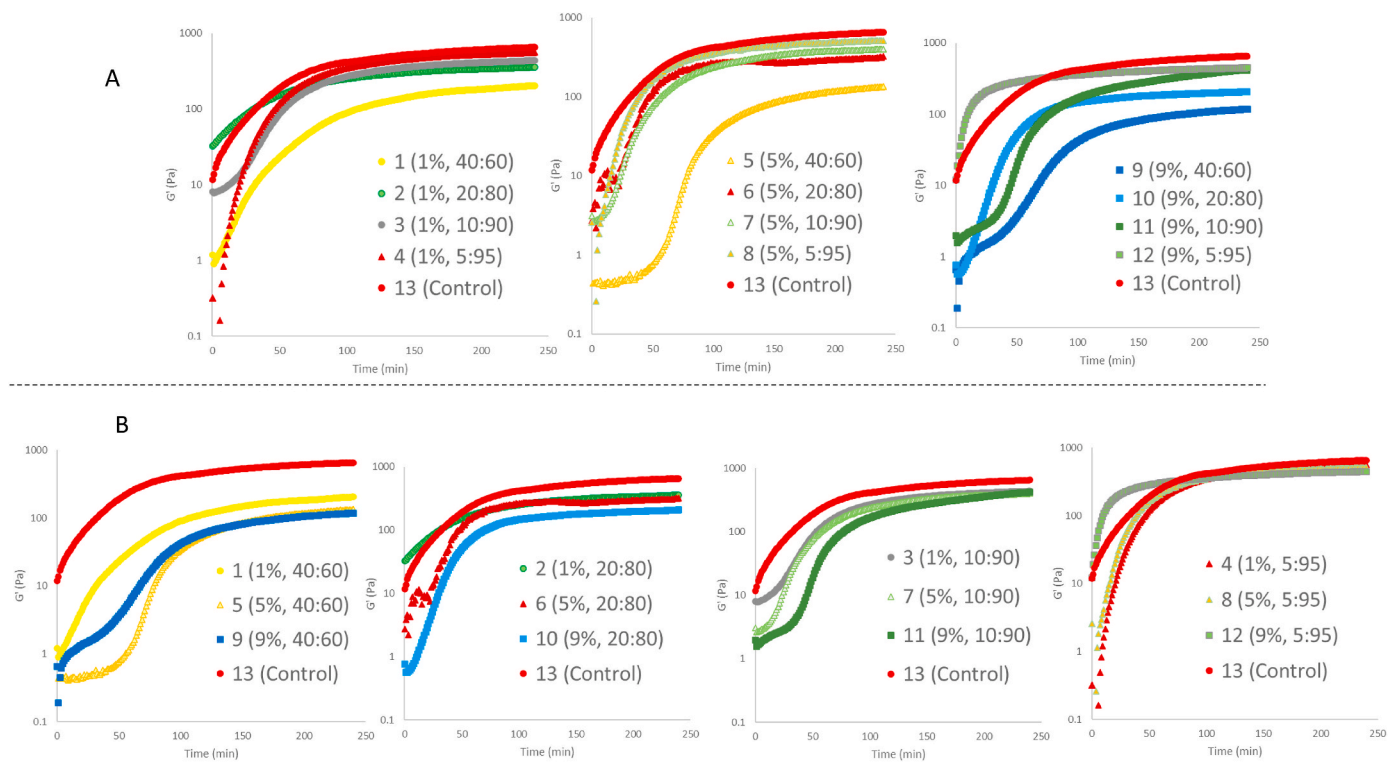


Fig. 8. A. Effect of HP/NP ratio on gelation behaviour of almond-based gels during 4 h fermentation B. Effect of DH on gelation behaviour of almond-based gels during 4 h fermentation.

increased HP/NP ratio from 5:95 to 40:60 resulted in lower G' values of almond-based gels (Fig. 8A). That could be due to the higher HP/NP ratio responsible for smaller particles (Table 2) being present in the gel network that decreased branching within the protein matrix, resulting in lower G' value of the gels (Jørgensen et al., 2019). The higher HP/NP ratio corresponds to more hydrolysed protein, the more shortened polypeptides in the gel structure may affect the hydrophobic interactions between protein-protein bindings and protein-fat

connections, thus weakening the gel network structure (Lucey, 2002). During the gelation period, under the same HP/NP ratio, with the increase of DH from 1%, 5%–9%, the G' value decreased (Fig. 8B). This result is in line with the hardness value (Table 2). The softer gel formation with increased DH of hydrolysed almond proteins can be explained by the higher DH of hydrolysed almond proteins bringing more shortened polypeptides (Fig. 2), that decreased the branching in the protein matrix and changed the molecular forces, thus weakening

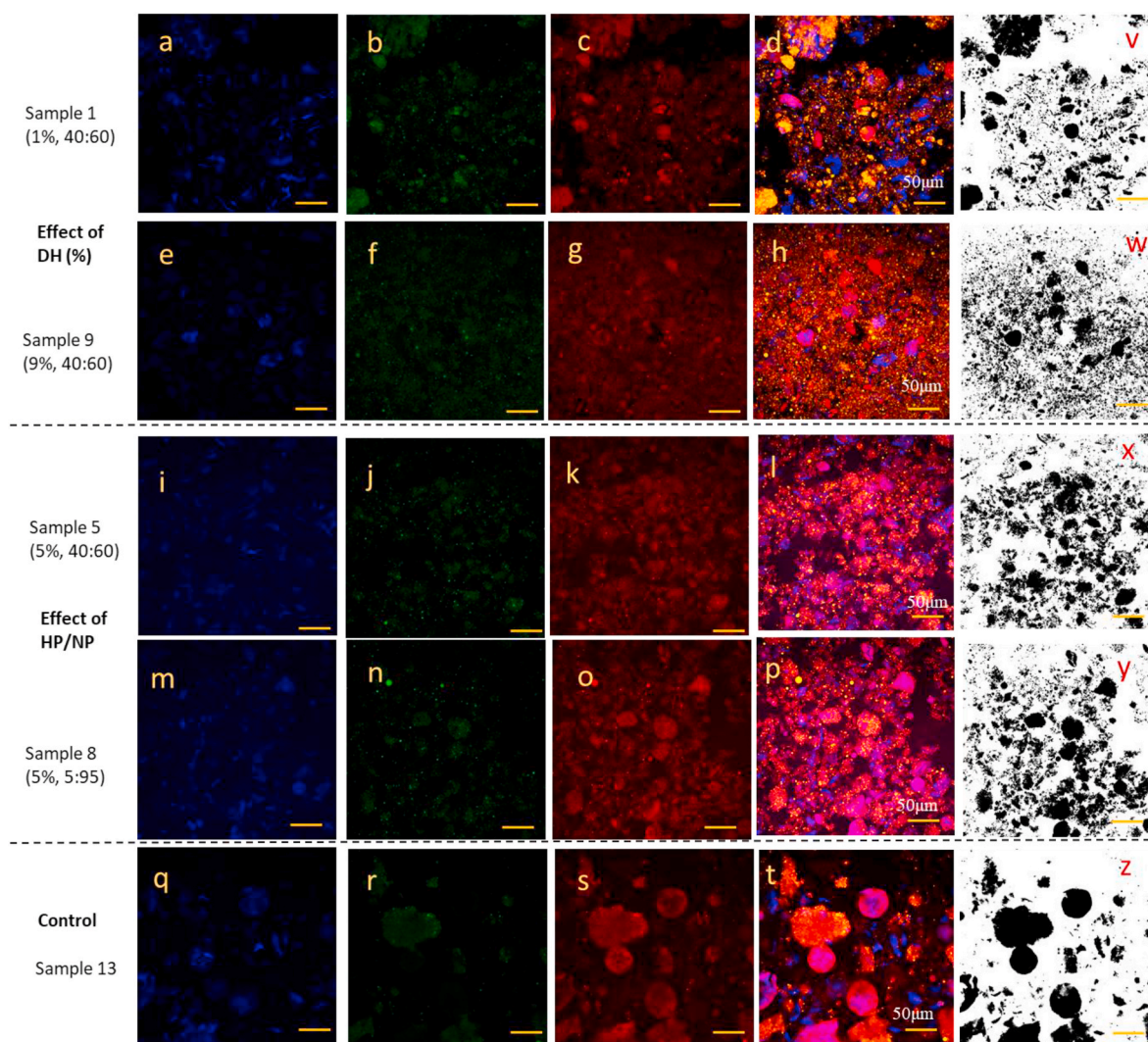


Fig. 9. Confocal images of almond-based gels. Polysaccharide (a, e, i, m, q), fat (b, f, j, n, r) and protein (c, g, k, o, s) contents were stained in blue, green, and red color, their combination images were shown in d, h, l, p and t. The software Image J shows the porosity of samples in image v, w, x, y and z. Results shown that the average particle size and porosity for each analysed image are 71 μm , 77% (image v), 52 μm , 70% (image w), 120 μm , 69% (image x), 116 μm , 68% (image y) and 275 μm , 74% (image z). All scale bars are in 50 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the hydrophobic strength and electrostatic charges between protein-protein molecules (Klost et al., 2020; Wouters et al., 2016). In Fig. 8B, the sample 9 with smaller particle sizes showed a lower G' value during the whole gelation period than sample 1. The formulation's softer gel formation with increased DH can also be ascribed to the fine particle size. As its evidence from Table 2 that the $(D_{3,2})$ value was significantly decreased from 5.03 μm in sample 1 (1%, 40:60) to 4.28 μm in sample 9 (9%, 40:60).

3.3.6. The lubrication behaviour of almond-based gel

The tribometer results implicate foods' lubrication/friction sensation during oral processing. The coefficient of friction (COF) provides essential information on the oral tribology performance prediction of acid gels. It is closely related to the sensory perception of smoothness and creaminess, which cannot be interpreted by TPA and rheology results (Prakash, 2017; Shewan et al., 2019). The COF of the yoghurt gels at sliding speed 1 mm/s, 10 mm/s and 100 mm/s reflects the tribology behaviour of almond-based gel samples is presented in Table 2.

At sliding speed 1 mm/s, sample 4 and 8 have similar COF to control sample 13 (0.220, 0.217 and 0.224, respectively), while others have a significantly lower value. As the sliding speed increased to 10 mm/s,

samples with different DH and HP/NP ratios showed a similar trend. No statistically significant difference ($p < 0.05$) was found in COF of all tested samples when sliding speed reached 100 mm/s, indicating hydrolysed protein has no effects on the smoothness of yoghurt gels at the end of consumption of yoghurt at 35 $^{\circ}\text{C}$ (Table 2). These results suggest that the higher DH and HP/NP ratio contribute to higher lubrication of almond yoghurts at sliding speeds 1 mm/s and 10 mm/s, but no significant effects were observed at 100 mm/s.

At the initial sliding speed of 1 mm/s, with the HP/NP ratio increased from 5:95 in sample 4 to 40:60 in sample 1, the coefficient of friction (COF) value significantly decreased from 0.220 to 0.196, indicating improved lubrication behaviour with more HP used in yoghurt formulation (Table 2). At the initial sliding speed of 1 mm/s, with increased DH from 1% in sample 3–9% in sample 11, the COF value significantly decreased ($p < 0.05$) from 0.210 to 0.184. With further increased speed to 10 mm/s, the COF of each sample continuously significantly decreased ($p < 0.05$) in sample 3 (0.209) and sample 11 (0.186). The lower friction in yoghurt gels caused by the smaller particle size (Table 2) is in good agreement with (Krzeminski et al., 2014). Pearson correlation also supports evidence that the r^2 between friction values and particle size results are highly correlated with $p < 0.05$ (Table S1, in

supplementary material).

3.4. Supporting evidence from microstructure of almond-based gel

The physicochemical results demonstrate that a higher HP/NP ratio or a higher DH speed up the fermentation process. On the other hand, the more the protein hydrolysates are used in almond-based gel production, the softer the gel structure formed with lower hardness, less viscous, less elastic strength, better lubrication, stronger WHC, less wheying-off phenomenon and a finer porosity of gel network. The above finding is further supported by the micrographs obtained from CSLM.

The micrographs (Fig. 9) revealed the gel structure and presented the distribution of fat, protein and other particles in the almond-based gel matrix. It showed that the fat droplets (green) were coated on the protein aggregates (red), while almond cell wall fibres (blue) were entrapped between the protein-fat matrix as part of the gel network. Srisuvor et al. (2013) described a similar gel network in a dairy yoghurt fortified with banana fibre, where proteins are covered by the fats and polysaccharides (fibres) that stabilize the protein matrix. Obviously, samples (1, 9, 5 & 8) made from hydrolysed protein show smaller protein aggregates and loose gel networks than the control sample 13 (Fig. 9). According to the Image J results: the average particle size (includes all visually particles and aggregates shown in the image) and porosity for samples 1, 9, 5, 8, 13 are 71 µm, 77% (image v), 52 µm, 70% (image w), 120 µm, 69% (image x), 116 µm, 68% (image y) and 275 µm, 74% (image z), respectively. This indicates that hydrolysed proteins can reduce the particle size and reduce the porosity in gel structures to some extent. The use of enzymatic hydrolysis in pore size reduction in the gel structure was also reported by Ercili-Cura et al. (2015) in a set-type dairy yoghurt that they attribute to the broken casein micelles by the enzyme PG.

The HP/NP ratio effect can be observed from sample 5 and sample 8 in Fig. 9. Sample 5 and sample 8 all made from the same DH (5%) of hydrolysed protein, however high HP/NP ratio (40:60) in sample 5 results in smaller protein aggregates (Fig. 9k) coated by fat droplets (Fig. 9j), more evenly entrapped fibres (Fig. 9i) and retain lesser water content (Fig. 9l) compared to the sample 8 with a HP/NP ratio of 5:95 in Fig. 9m-p. The higher HP/NP ratio increases the amount of protein hydrolysates, brings more size reduced polypeptides (Akharume et al., 2021), which will lose gel structure integrity and thus reduce viscosity, hardness of almond yoghurt. However, the finer porosity tends to retain more water, leading to a stronger WHC and less syneresis. The increased WHC with decreased viscosity and hardness in this study conflicts with Srisuvor et al. (2013) finding in a dairy-based set type yoghurt, possibly due to the nature of proteins used in their research is different from almond proteins. Moreover, the fibre contents coated in a loose gel network (visualised in Fig. 9) may help in explaining the adverse effect on WHC and syneresis levels. Klost et al. (2020) studied that the increased water holding stability and less syneresis in plant-based yoghurts (pea-based) may be also ascribed to the high concentration of protein (10%).

The effects of degree of hydrolysis (DH) are illustrated by the micrographs of almond yoghurts, such as porosity, particle size, protein matrix and components of the gel matrix. The CLSM images of sample 1 (Fig. 9a-d) and sample 9 (Fig. 9e-h) presenting the same HP/NP ratio (i. e., 40:60) clearly showed the effects of DH on the microstructure of almond yoghurts. High DH sample 9 (9%) showed smaller protein particles (Fig. 9c) compared to sample 1 with a low DH (1%) in Fig. 9g. The protein matrix coated even finer fat droplets (Fig. 9f) and had less space for entrapped fibres (Fig. 9e) and water (Fig. 9h). The different gel structure between sample 1 and sample 9 is caused by different DH of protein hydrolysates used in their formulation. The functional properties (e.g., solubility, surface-active properties, water- and fat-holding capacities, gelation) of protein hydrolysates are not determined by the DH itself, but the structure of resulting polypeptides as it changes the nature of protein such as amino acid composition, covalent bonds and hydrogen

bonds (Wouters et al., 2016). In addition, the smaller porosity gel structure in sample 9 (Fig. 9h) compared to in sample 1 (Fig. 9d) can help to explain the better lubrication properties associated with small particles (Table 2). A similar phenomenon was also found in a set type yoghurt by Nguyen et al. (2017) but sourced from dairy.

4. Conclusion

This research fills the gap between the degree of hydrolysis and HP/NP ratios of interactions participating in the gel formation and the quality determination of plant protein-based fermentation-induced gels. The increased hydrolysed almond protein's DH from 1%, 5%–9% by the enzymatic treatment reduces the particle size of yoghurts. The presence of smaller particle size enhances the stability of almond-based gel (better water holding capacity and less severe wheying-off phenomenon) and contributes to a softer gel formation with less viscous, lower firmness and good lubrication properties. The increased HP/NP ratio from 5:95, 10:90, 20:80, to 40:60 shows a similar trend but have a more significant effect on the properties of the almond-based gel. Studying how protein functionality modification by enzymatic hydrolysis supports almond-based gel formulation provides fundamental knowledge for further plant proteins application in food product development or designing a product with target functionality such as viscosity by modulating the proteins. This method can guide the manufacture of high protein plant-based gels and allow the tailoring of clean labels for high protein dairy substitutes to suit customers' desire for sustainability, dairy-free, health concern or a vegan lifestyle. Future studies on organoleptic properties, product stability during storage and protein stabilized low fat almond-based fermentation-induced gel, are currently being pursued.

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CRediT authorship contribution statement

Jia Zhao: Investigation, Data curation, Writing – original draft, Writing – review & editing. **Bhesh Bhandari:** Writing – review & editing. **Claire Gaiani:** Writing – review & editing. **Sangeeta Prakash:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2022.03.012>.

References

- Adebijoye, A.P., Adebijoye, A.O., Yamashita, J., Ogawa, T., Muramoto, K., 2008. Purification and characterization of antioxidative peptides derived from rice bran protein hydrolysates. *Eur. Food Res. Technol.* 228 (4), 553–563. <https://doi.org/10.1007/s00217-008-0962-3>.
- Akalin, A.S., Unal, G., Dinkci, N., Hayaloglu, A.A., 2012. Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. *J. Dairy Sci.* 95 (7), 3617–3628. <https://doi.org/10.3168/jds.2011-5297>.
- Akharume, F.U., Aluko, R.E., Adedeji, A.A., 2021. Modification of plant proteins for improved functionality: a review. *Compr. Rev. Food Sci. Food Saf.* 20 (1), 198–224.
- Akin, Z., Ozcan, T., 2017. Functional properties of fermented milk produced with plant proteins. *LWT - Food Sci. Technol.* 86, 25–30. <https://doi.org/10.1016/j.lwt.2017.07.025>.

- Basiri, S., Haidary, N., Shekarforoush, S.S., Niakousari, M., 2018. Flaxseed mucilage: a natural stabilizer in stirred yogurt. *Carbohydr. Polym.* 187, 59–65. <https://doi.org/10.1016/j.carbpol.2018.01.049>.
- Bernat, N., Chafer, M., Chiralt, A., Gonzalez-Martinez, C., 2015. Development of a non-dairy probiotic fermented product based on almond milk and inulin. *Food Sci. Technol. Int.* 21 (6), 440–453. <https://doi.org/10.1177/1082013214543705>.
- de Almeida, N.M., Dias, F.F.G., Rodrigues, M.I., de Moura Bell, L.N., M, J., 2019. Effects of processing conditions on the simultaneous extraction and distribution of oil and protein from almond flour. *Processes* 7 (11). <https://doi.org/10.3390/pr7110844>.
- de Souza, T.S.P., Dias, F.F.G., Oliveira, J.P.S., de Moura Bell, J., Koblit, M.G.B., 2020. Biological properties of almond proteins produced by aqueous and enzyme-assisted aqueous extraction processes from almond cake. *Sci. Rep.* 10 (1), 10873 <https://doi.org/10.1038/s41598-020-67682-3>.
- Devnani, B., Ong, L., Kentish, S., Gras, S., 2020. Heat induced denaturation, aggregation and gelation of almond proteins in skim and full fat almond milk. *Food Chem.* 325, 126901. <https://doi.org/10.1016/j.foodchem.2020.126901>.
- Durand, A., Franks, G., Hosken, R., 2003. Particle sizes and stability of UHT bovine, cereal and grain milks. *Food Hydrocolloids* 17 (5), 671–678. [https://doi.org/10.1016/S0268-005X\(03\)00012-2](https://doi.org/10.1016/S0268-005X(03)00012-2).
- Ercili-Cura, D., Huppertz, T., Kelly, A.L., 2015. Enzymatic modification of dairy product texture. In: *Modifying Food Texture*, pp. 71–97. <https://doi.org/10.1016/b978-1-78242-333-1.00004-8>.
- Fling, S.P., Gregerson, D.S., 1986. Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea. *Anal. Biochem.* 155 (1), 83–88. [https://doi.org/10.1016/0003-2697\(86\)90228-9](https://doi.org/10.1016/0003-2697(86)90228-9).
- Galante, M., De Flaviis, R., Boeris, V., Spelzini, D., 2020. Effects of the enzymatic hydrolysis treatment on functional and antioxidant properties of quinoa protein acid-induced gels. *LWT (Lebensm.-Wiss. & Technol.)* 118. <https://doi.org/10.1016/j.lwt.2019.108845>.
- Grasso, N., Alonso-Miravalles, L., O'Mahony, J.A., 2020. Composition, physicochemical and nutritional properties of commercial plant-based yogurts. *Foods* 9 (3). <https://doi.org/10.3390/foods9030252>.
- Grundy, M.M., Carriere, F., Mackie, A.R., Gray, D.A., Butterworth, P.J., Ellis, P.R., 2016. The role of plant cell wall encapsulation and porosity in regulating lipolysis during the digestion of almond seeds. *Food Funct.* 7 (1), 69–78. <https://doi.org/10.1039/c5fo00758e>.
- He, S., Hekmat, S., 2014. Sensory evaluation of non-dairy probiotic beverages. *J. Food Res.* 4 (1) <https://doi.org/10.5539/jfr.v4n1p186>.
- Jeske, S., Zannini, E., Arendt, E.K., 2018. Past, present and future: the strength of plant-based dairy substitutes based on gluten-free raw materials. *Food Res. Int.* 110, 42–51. <https://doi.org/10.1016/j.foodres.2017.03.045>.
- Jin, T., Albillos, S.M., Guo, F., Howard, A., Fu, T.J., Kothary, M.H., Zhang, Y.Z., 2009. Crystal structure of prunin-1, a major component of the almond (*Prunus dulcis*) allergen *amandin*. *J. Agric. Food Chem.* 57 (18), 8643–8651. <https://doi.org/10.1021/jf9017355>.
- Jørgensen, C.E., Abrahamsen, R.K., Rukke, E.-O., Hoffmann, T.K., Johansen, A.-G., Skeie, S.B., 2019. Processing of high-protein yoghurt – a review. *Int. Dairy J.* 88, 42–59. <https://doi.org/10.1016/j.idairyj.2018.08.002>.
- Klost, M., Giménez-Ribes, G., Drusch, S., 2020. Enzymatic hydrolysis of pea protein: interactions and protein fractions involved in fermentation induced gels and their influence on rheological properties. *Food Hydrocolloids* 105. <https://doi.org/10.1016/j.foodhyd.2020.105793>.
- Krzeminski, A., Prell, K.A., Busch-Stockfisch, M., Weiss, J., Hinrichs, J., 2014. Whey protein–pectin complexes as new texturising elements in fat-reduced yoghurt systems. *Int. Dairy J.* 36 (2), 118–127. <https://doi.org/10.1016/j.idairyj.2014.01.018>.
- Lee, W., Lucey, J., 2010. Formation and physical properties of yogurt. *Asian-Australas. J. Anim. Sci.* 23 (9), 1127–1136. <https://doi.org/10.5713/ajas.2010.r.05>.
- Li, Y., Jiang, B., Zhang, T., Mu, W., Liu, J., 2008. Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chem.* 106 (2), 444–450. <https://doi.org/10.1016/j.foodchem.2007.04.067>.
- Lin, D., Lu, W., Kelly, A.L., Zhang, L., Zheng, B., Miao, S., 2017. Interactions of vegetable proteins with other polymers: structure-function relationships and applications in the food industry. *Trends Food Sci. Technol.* 68, 130–144. <https://doi.org/10.1016/j.tifs.2017.08.006>.
- Lucey, J., 2002. Formation and physical properties of milk protein gels. *J. Dairy Sci.* 85 (2), 281–294. [https://doi.org/10.3168/jds.S0022-0302\(02\)74078-2](https://doi.org/10.3168/jds.S0022-0302(02)74078-2).
- Makinen, O.E., Wanhalinna, V., Zannini, E., Arendt, E.K., 2016. Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products. *Crit. Rev. Food Sci. Nutr.* 56 (3), 339–349. <https://doi.org/10.1080/10408398.2012.761950>.
- Mauko, A., Muck, T., Mirtič, B., Mladenović, A., Kreft, M., 2009. Use of confocal laser scanning microscopy (CLSM) for the characterization of porosity in marble. *Mater. Char.* 60 (7), 603–609. <https://doi.org/10.1016/j.matchar.2009.01.008>.
- Nguyen, P.T.M., Kravchuk, O., Bhandari, B., Prakash, S., 2017. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids* 72, 90–104. <https://doi.org/10.1016/j.foodhyd.2017.05.035>.
- Nielsen, P., Petersen, D., Dambmann, C., 2001. Improved method for determining food protein degree of hydrolysis. *J. Food Sci.* 66 (5), 642–646. <https://doi.org/10.1111/j.1365-2621.2001.tb04614.x>.
- Osman, A., Goda, H.A., Abdel-Hamid, M., Badran, S.M., Otte, J., 2016. Antibacterial peptides generated by Alcalase hydrolysis of goat whey. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 65, 480–486. <https://doi.org/10.1016/j.lwt.2015.08.043>.
- Prakash, S., 2017. From rheology to tribology: applications of tribology in studying food oral processing and texture perception. In: *Advances in Food Rheology and its Applications*, pp. 65–86. <https://doi.org/10.1016/b978-0-08-100431-9.00004-8>.
- Sathe, S., 1992. SOLUBILIZATION, electrophoretic characterization and IN vitro digestibility OF almond (*prunus AMYGDALUS*) PROTEINS1,2. *J. Food Biochem.* 16 (4), 249–264. <https://doi.org/10.1111/j.1745-4514.1992.tb00450.x>.
- Sbroglio, M.F., Montilha, M.S., Figueiredo, V.R. G.d., Georgetti, S.R., Kurozawa, L.E., 2016. Influence of the degree of hydrolysis and type of enzyme on antioxidant activity of okara protein hydrolysates. *Food Sci. Technol.* 36 (2), 375–381. <https://doi.org/10.1590/1678-457X.000216>.
- Shewan, H.M., Pradal, C., Stokes, J.R., 2019. Tribology and its growing use toward the study of food oral processing and sensory perception. *J. Texture Stud.* <https://doi.org/10.1111/jtxs.12452>.
- Shi, H., Kraft, J., Guo, M., 2020. Physicochemical and microstructural properties and probiotic survivability of symbiotic almond yogurt alternative using polymerized whey protein as a gelation agent. *J. Food Sci.* 85 (10), 3450–3458. <https://doi.org/10.1111/1750-3841.15431>.
- Song, Y.-S., Frías, J., Martínez-Villaluenga, C., Vidal-Valverde, C., de Mejia, E.G., 2008. Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chem.* 108 (2), 571–581. <https://doi.org/10.1016/j.foodchem.2007.11.013>.
- Srisuvar, N., Chinprahast, N., Prakitchaiwattana, C., Subhimaros, S., 2013. Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana purée. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 51 (1), 30–36. <https://doi.org/10.1016/j.lwt.2012.10.018>.
- Sun, X.D., 2011. Enzymatic hydrolysis of soy proteins and the hydrolysates utilisation. *Int. J. Food Sci. Technol.* 46 (12), 2447–2459. <https://doi.org/10.1111/j.1365-2621.2011.02785.x>.
- Sze-Tao, K., Sathe, S., 2000. Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate. *Food Chem.* 69 (2), 153–160. [https://doi.org/10.1016/S0308-8146\(99\)00244-7](https://doi.org/10.1016/S0308-8146(99)00244-7).
- Udenigwe, C.C., Je, J.-Y., Cho, Y.-S., Yada, R.Y., 2013. Almond protein hydrolysate fraction modulates the expression of proinflammatory cytokines and enzymes in activated macrophages. *Food Funct.* 4 (5), 777–783. <https://doi.org/10.1039/C3FO30327F>.
- Wouters, A.G.B., Rombouts, I., Fierens, E., Brijs, K., Delcour, J.A., 2016. Relevance of the functional properties of enzymatic plant protein hydrolysates in food systems. *Compr. Rev. Food Sci. Food Saf.* 15 (4), 786–800. <https://doi.org/10.1111/1541-4337.12209>.
- Yang, X., Ke, C., Li, L., 2021. Physicochemical, rheological and digestive characteristics of soy protein isolate gel induced by lactic acid bacteria. *J. Food Eng.* 292 <https://doi.org/10.1016/j.jfoodeng.2020.110243>.
- Yust, M.d.M., Pedroche, J., Millán-Linares, M.d.C., Alcaide-Hidalgo, J.M., Millán, F., 2010. Improvement of functional properties of chickpea proteins by hydrolysis with immobilised Alcalase. *Food Chem.* 122 (4), 1212–1217. <https://doi.org/10.1016/j.foodchem.2010.03.121>.
- Zhao, J., Bhandari, B., Gaiani, C., Prakash, S., 2021. Physicochemical and microstructural properties of fermentation-induced almond emulsion-filled gels with varying concentrations of protein, fat and sugar contents. *Curr. Res. Nutr. Food Sci.* 4, 577–587. <https://doi.org/10.1016/j.crfns.2021.08.007>.
- Zhu, Z., Qiu, N., Yi, J., 2010. Production and characterization of angiotensin converting enzyme (ACE) inhibitory peptides from apricot (*Prunus armeniaca* L.) kernel protein hydrolysate. *Eur. Food Res. Technol.* 231 (1), 13–19. <https://doi.org/10.1007/s00217-010-1235-5>.