Research Article

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Formation and thermal and colloidal stability of oil-in-water emulsions stabilized using quinoa and lentil protein blends

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Abstract

BACKGROUND: The amino acid composition, and rheological, thermal and colloidal stability of plant protein-based oil-in-water emulsion systems containing 1.90, 3.50 and 7.70 g 100 mL^{-1} protein, fat and carbohydrate, respectively, using quinoa and lentil protein ratios of 100:0 and 60:40 were investigated. The emulsion containing lentil protein showed lower initial, peak and final viscosity values (22.7, 61.7 and 61.6 mPa s, respectively) than the emulsion formulated with quinoa protein alone (34.3, 102 and 80.0 mPa s, respectively) on heat treatment.

RESULTS: Particle size analysis showed that both samples had small particle sizes (~1.36 μm) after homogenization; however, the sample with 60:40 quinoa:lentil protein ratio showed greater physical stability, likely related to the superior emulsifying properties of lentil protein. However, upon heat treatment, large aggregates (~100 μm) were formed in both samples, reducing the physical stability of the samples. This physical stability was increased with the addition of 0.20% sodium dodecyl sulfate (SDS), whereas it was negatively affected by the addition of α-amylase. Addition of α-amylase led to lower viscosity for both emulsion samples, with measured values of 41.8 and 46.0 mPa s for the 100:0 and 60:40 samples, respectively. This suggests that the heat-induced increases in particle size were partially due to hydrophobic interactions between the proteins as SDS disrupts hydrophobic bonds between proteins.

CONCLUSION: These results demonstrated that using a mixture of lentil and quinoa proteins positively affected the physical stability of plant protein-based emulsions, in addition to contributing to a more nutritionally complete amino acid profile – both important considerations in the development of plant-based beverages.

Keywords: quinoa; lentil; protein; emulsion; formulation; colloidal stability

INTRODUCTION

Global demand for protein by the 7.3 billion inhabitants of the world is approximately 202 million tonnes annually.[1] The expected continuous growth of the global population to 9.6 billion people by 2050 is creating an ever greater need to identify and develop sustainable solutions for provision of high-quality food protein.[2,3] The consumption of plant protein is increasing in Europe, and this is reflected in the annual growth rates of 14% and 11% for meat and dairy alternatives, respectively.[4]

Quinoa is a gluten-free dicotyledonous grain, referred to as a pseudocereal, with similar nutritional value to cereals such as rice and maize.[5] Quinoa is cultivated in South America, mainly in the Andean region of Peru and Bolivia, often referred to as 'Andean grain',[6] with a protein content of 14–16%,[7,8] the protein content depending on the variety of the cultivar.[9] On the other hand, lentil is considered a legume and has a higher protein content (20.6–31.4%) than cereals or pseudocereals.[10,11] The main producers of lentils are Canada, India, Turkey, the USA and Australia.[12] Quinoa has a high concentrations of essential amino acids, particularly cysteine and methionine, with these being higher than in some common cereals such as rice and maize.[13] Conversely, legumes, especially lentils, are limited in essential amino acids such as tryptophan and threonine.[12] The main protein found in both lentil and quinoa has a similar molecular weight (320–380 kDa and 300–390 kDa, respectively) in both species, and is referred to as globulin in both cases.[10,14]

Oil-in-water emulsion systems formulated with plant proteins are of particular interest to the food industry, as the market for

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plant-based milks, yogurts, spreads, cheeses and infant formula is growing.[4] Adequate amino acid profile and protein content are important attributes and can be achieved by combining cereal/pseudocereal and legume protein sources in order to balance their innate amino acid deficiencies.[15] However, there are challenges when formulating food products with plant proteins (e.g. plant-based beverages), as previous studies have demonstrated the colloidal and physical stability to be low.[16,17] This can be related to the large size of dispersed particles (i.e. fat globules, insoluble particles from raw materials, proteins and starch granules), which make it challenging to obtain a stable product.[17] The stability of plant-based beverages can be improved by reducing the size of the dispersed phase particles using various techniques such as homogenization[18,19] and emulsifiers and stabilizers.[20] In this context, lentil protein has been reported to be an attractive natural emulsifier with good stability to heat treatment and pH changes.[20,21] Furthermore, starch granules, found naturally in grains (e.g. cereals, pseudocereals and legumes), can have emulsifying properties. For example, starch granules in quinoa are relatively small in size – between 0.5 and 3 μm[22,23] – with good emulsifying ability.[24,25] During the production of plant-based products in liquid format, an enzymatic treatment with starch-degrading enzymes (e.g. α-amylase) is often used to reduce the viscosity of the product, with a concomitant increase in physical stability.[17,26]

The objective of this study was to understand how the incorporation of a legume protein source (i.e. lentil protein isolate) can affect the rheological, heat and physical stability of a plant-based oil-in-water emulsion system formulated using a pseudocereal protein ingredient (i.e. quinoa protein concentrate). Lentil protein isolate was selected as a protein ingredient because of its good emulsifying, heat and colloidal stability properties, which have been attributed to its ability to create a thick interfacial layer and strong steric repulsion.[21]

MATERIALS AND METHODS

Ingredients

Lentil protein isolate (LPI) and quinoa protein-rich flour (QPRF) were provided by the Fraunhofer IVV (Freising, Germany), and the macronutrient composition is presented in Table 1. Maltodextrin, with a dextrose equivalent (DE) value of 17, was supplied by Tereos (Lille, France). Sunflower oil was supplied by a local retail outlet (Tesco, Welwyn Garden City, UK). All the reagents used in this study were of analytical grade and supplied by Sigma-Aldrich (St Louis, MO, USA), unless otherwise stated.

Amino acid composition

The amino acid content of the ingredients was analysed following the method of Schuster,[27] described briefly as follows. Samples were hydrolysed in aqueous hydrochloric acid to break peptide bonds and, after hydrolysis, the samples were pH adjusted, brought to volume with a loading buffer and filtered. Amino acids were separated in an amino acid analyser and the detection was carried out using post-column derivatization with ninhydrin reagent at 440 and 570 nm. For quantification, a one-point calibration curve was used and for quality assurance purposes an in-house standard was analysed in every run.

Preparation of emulsions

Emulsions representative of ready-to-feed plant-based infant formula containing 1.90, 3.50 and 7.70 g 100 mL^{-1} of protein, fat and carbohydrate, respectively, were prepared as follows. Two different ratios of quinoa protein:lentil protein (QP:LP) of 100:0 and 60:40, respectively, to prepare the samples were studied. These protein ratios were chosen because they facilitate compliance with the minimum EU regulatory levels of essential amino acids at a protein content of 1.90 g 100 mL^{-1}.[28] The concentrations of carbohydrate, lipid and total solids (13.8%) were maintained constant. These two samples are referred to as 100Q:0L_E and 60Q:40L_E, respectively, throughout the article. In addition, protein-only control samples containing only the quinoa and lentil protein ingredients, without the addition of sunflower oil and maltodextrin, were included for comparison purposes. These are referred to as 100Q:0L_P and 60Q:40L_P, respectively. The quinoa and lentil protein ingredients were dispersed in pre-heated water (70 °C) using a magnetic stirrer at 300 rpm for 1 h at 22 °C, after which the maltodextrin was added to the protein dispersions and mixed for 2 h under the same conditions. The mixture was adjusted to pH 6.8 and allowed to rehydrate at 5 °C overnight while mixing at 300 rpm using magnetic stirring. The temperature of the aqueous phase was adjusted to 22 °C, and the pH was measured and adjusted to 6.8 if necessary. Sunflower oil was added to the aqueous phase in order to achieve a total fat concentration of 3.50 g 100 mL^{-1} and the mixtures were pre-heated to 50 °C before creating coarse emulsions using dispersing equipment (T25 Ultra-Turrax, Staufen, Germany) with a mixing speed of 12 000 rpm for 3 min. The coarse emulsion was then passed immediately through a homogenizer twice, with first- and second-stage pressures of 150 and 30 bar, respectively. The pH of the final emulsions was checked and readjusted, if necessary, to pH 6.8.

Rheological measurements

Thermal treatment using starch pasting cell

Changes in viscosity during heat treatment of the different emulsion samples were determined using an AR-G2 controlled-stress rheometer equipped with a starch pasting cell geometry (TA Instruments Ltd, Waters LLC, Leatherhead, UK); the internal diameter of the cell was 36.0 mm, the diameter of the rotor was 32.4 mm and the gap between the two elements at the geometry

Table 1. Macronutrient composition of lentil protein isolate (LPI) and quinoa protein-rich flour (QPRF)

	Macronutrient composition (%, w/w)					
	Moisture	Ash	Protein	Fat	Starch	Fibre
LPI	4.87	5.46	85.1	4.49	n.d.	4.5
QPRF	5.25	3.60	33.3	12.8	21.4	18.8

n.d., not detected.

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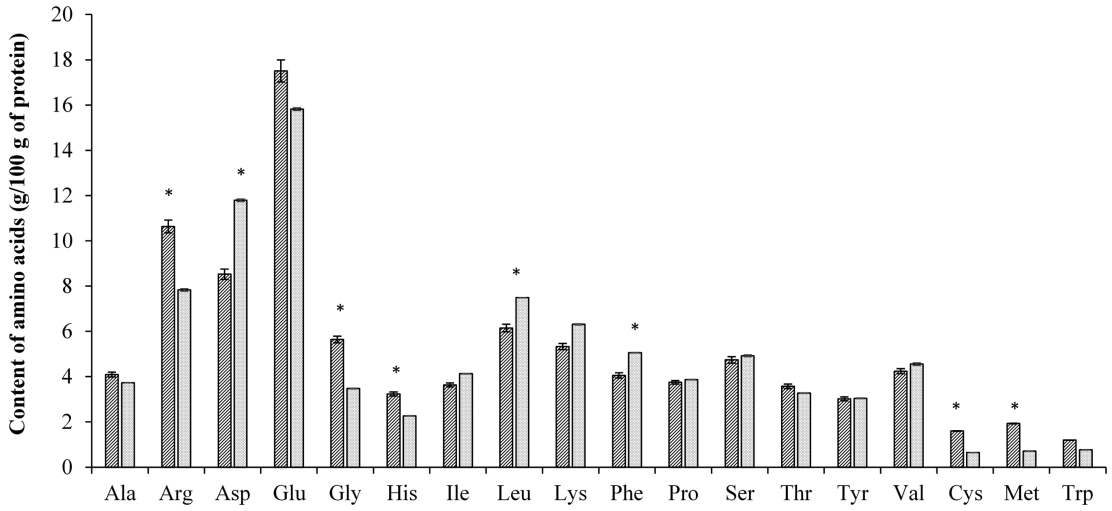

Figure 1. Content of amino acids alanine (Ala), arginine (Arg), asparagine (Asp), glutamine (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), valine (Val), cysteine (Cys), methionine (Met) and tryptophan (Trp) in quinoa protein-rich flour (▨) and lentil protein isolate (▨). *Denotes significant differences between samples for each amino acid.

base was 0.55 mm. All measurements of viscosity were carried out at a fixed shear rate of 15 rad s^{-1}. The sample (28 g) was conditioned and held at 15 °C for 2 and 5 min, respectively, and the temperature increased to 95 °C (10 °C min^{-1}) and held at this temperature for 30 s, after which the temperature was decreased to 15 °C (10 °C min^{-1}) and maintained at this temperature for 5 min. To understand the contribution of starch gelatinization to viscosity during heat treatment, a separate trial involving the addition of α-amylase to the samples was carried out as follows: α-amylase (50 µL) (Megazyme, Wicklow, Ireland) from *Bacillus licheniformis* containing 3000 U mL^{-1} (optimal temperature 75 °C; temperature stability <80 °C and pH stability between 4.5 and 8.0) was added prior to heat treatment in the starch pasting cell to the different samples in order to understand the effects of starch on viscosity and the other analysed parameters (i.e. particle size distribution and physical stability).

Viscosity measurement

Viscosity before and after heat treatment, with and without addition of α-amylase, was measured at 15 °C using a controlled-stress rheometer (TA Instruments Ltd, Leatherhead, UK), equipped with a concentric cylinder geometry and Peltier-controlled heating system. Measurements were performed over a shear rate ramp ranging from 0 to 100 s^{-1} over 4 min, held at 100 s^{-1} for 2 min and

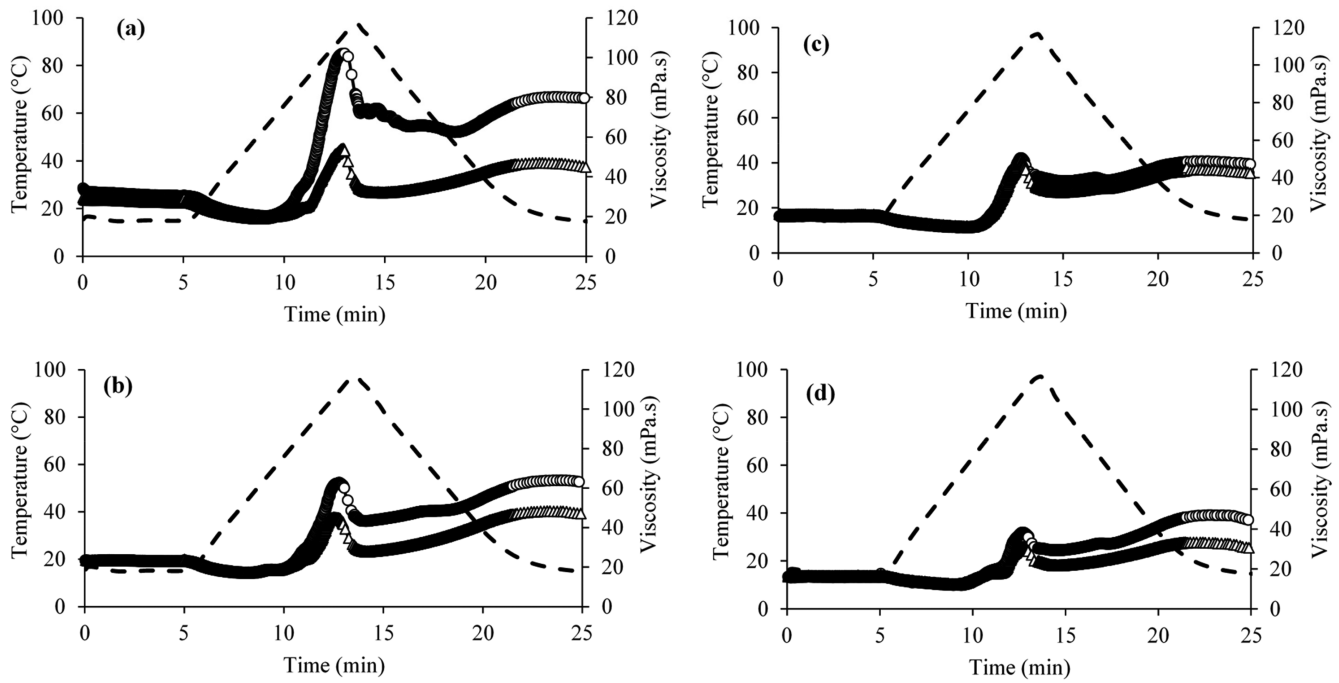

Figure 2. Temperature (dashed line) and viscosity (symbols) at various stages of the pasting regime for emulsion (a, b) and protein-only samples (c, d) formulated with 100:0 (a, c) or 60:40 (b, d) quinoa:lentil protein, respectively, without (–○–) and with (–△–) addition of α-amylase.

Table 2. Viscosity values of emulsions and protein-only control samples with quinoa:lentil protein ratios of 100:0 and 60:40, with or without the addition of α-amylase at different stages of the pasting treatment

	Apparent viscosity (mPa s)			
	Initial	Peak	End of cooling	Final
Emulsion samples				
100Q:0L$_E$	34.3 ± 5.83b	102 ± 13.6b	80.2 ± 5.03b	80.0 ± 2.12c
100Q:0L$_E$ + enzyme	29.0 ± 1.91ab	53.7 ± 6.54a	49.1 ± 1.48a	41.8 ± 2.97a
60Q:40L$_E$	22.7 ± 1.33a	61.7 ± 0.46a	63.4 ± 1.93a	61.6 ± 3.27b
60Q:40L$_E$ + enzyme	23.5 ± 0.37a	44.8 ± 0.65a	48.1 ± 0.85a	46.0 ± 0.87a
Protein samples				
100Q:0L$_P$	19.7 ± 0.41b	50.5 ± 3.82b	48.5 ± 2.30b	45.9 ± 2.40b
100Q:0L$_P$ + enzyme	20.7 ± 0.10b	49.7 ± 0.52b	44.1 ± 0.12b	41.4 ± 0.01b
60Q:40L$_P$	16.2 ± 0.07a	38.2 ± 0.54a	46.7 ± 0.52b	43.3 ± 0.81b
60Q:40L$_P$ + enzyme	16.6 ± 0.40a	31.0 ± 1.34a	33.3 ± 0.06a	29.5 ± 0.20a

Samples not sharing a common letter differ significantly ($P < 0.05$).

followed by a shear rate ramp from 100 to 0 s^{-1} in 4 min. The viscosity values were reported as the mean viscosity at 100 s^{-1}.

Particle size distribution (PSD)

The PSD of the different samples was measured before and after heating the samples at 95 °C for 30 s in the starch pasting cell, with or without the addition of α-amylase. The PSD of the emulsions was measured using a Mastersizer 3000 static laser light diffraction instrument from Malvern Instruments Ltd (Malvern, UK). The refractive index was set at 1.47 and the absorption and dispersant refractive indices used were 0.001 and 1.33, respectively. The emulsion samples were equilibrated at 22 °C and introduced into the dispersing unit using ultrapure water as dispersant until a laser obscuration of 12% was achieved. The PSD was also measured after 0.2% sodium dodecyl sulfate (SDS) was added to the samples after heat treatment in order to understand the interactions generated during heating (i.e. hydrophobic interactions).

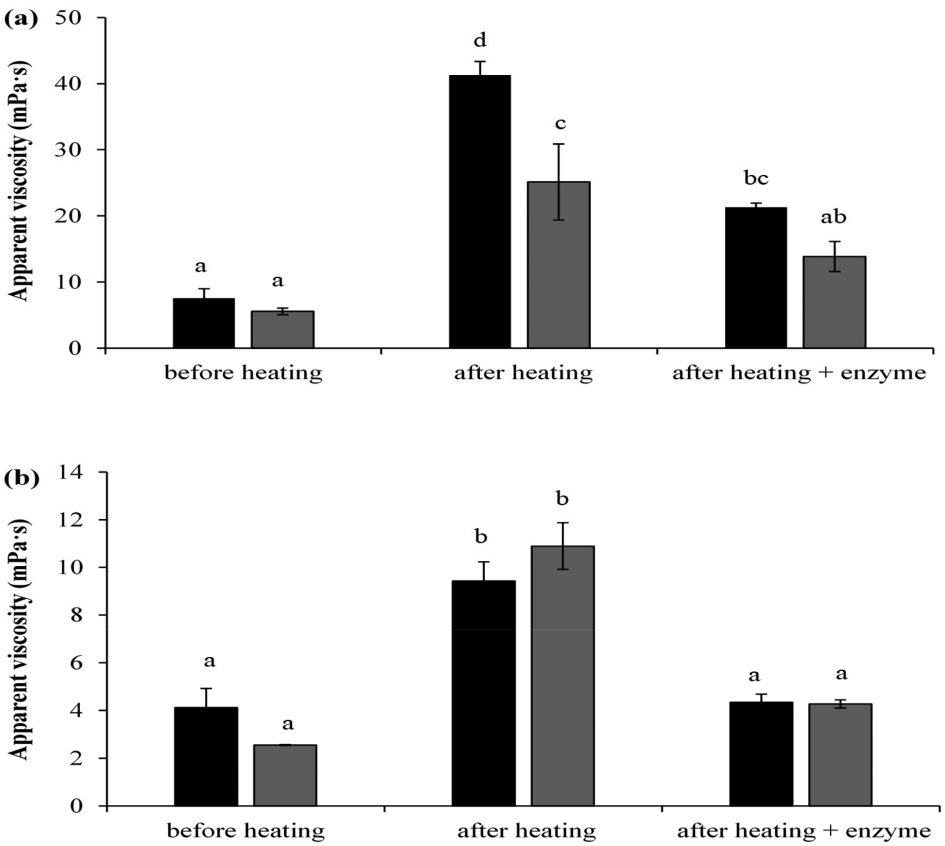

Figure 3. Apparent viscosity of the emulsion (a) and protein-only (b) samples formulated with protein ratios of 100:0 (■) and 60:40 (■) quinoa:lentil protein, respectively, before and after heating, with or without α-amylase, using the starch pasting cell. Different lower case letters above each bar represent statistically significant differences between samples

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Table 3. Particle size distribution parameters for emulsions stabilized using different ratios of quinoa and lentil protein of 100:0 (100Q:0L$_E$) and 60:40 (60Q:40L$_E$), and their respective protein-only solutions, before and after heating at 95 °C for 30 s without or with the addition of α-amylase or 0.2% sodium dodecyl sulphate (SDS) as dissociating agent

	Particle size distribution parameter (μm)							
	Before heating		After heating		After heating + α-amylase		After heating +0.2% SDS	
Emulsion samples	100Q:0L$_E$	60Q:40L$_E$	100Q:0L$_E$	60Q:40L$_E$	100Q:0L$_E$	60Q:40L$_E$	100Q:0L$_E$	60Q:40L$_E$
Dv(10)	0.30 ± 0.01a	0.30 ± 0.01a	0.29 ± 0.01a	0.30 ± 0.01a	0.27 ± 0.00a	0.27 ± 0.01a	0.31 ± 0.00a	0.31 ± 0.01a
Dv(50)	0.83 ± 0.02a	0.84 ± 0.03a	1.00 ± 0.05b	1.04 ± 0.05b	0.93 ± 0.02ab	0.98 ± 0.05b	1.00 ± 0.00b	1.06 ± 0.06b
Dv(90)	2.20 ± 0.19a	2.44 ± 0.28a	24.3 ± 14.9a	33.1 ± 18.8a	86.5 ± 17.9b	83.8 ± 20.4b	4.93 ± 0.12a	7.15 ± 0.95a
D[4,3]	1.36 ± 0.14a	1.26 ± 0.16a	9.87 ± 2.35b	9.85 ± 2.66b	18.5 ± 4.25c	18.2 ± 4.54c	4.32 ± 0.81ab	4.10 ± 1.00ab
D[3,2]	0.61 ± 0.01a	0.61 ± 0.02a	0.67 ± 0.03ab	0.69 ± 0.02b	0.63 ± 0.01ab	0.64 ± 0.03ab	0.69 ± 0.00b	0.72 ± 0.03b
Protein samples	100Q:0L$_P$	60Q:40L$_P$	100Q:0L$_P$	60Q:40L$_P$	100Q:0L$_P$	60Q:40L$_P$	100Q:0L$_P$	60Q:40L$_P$
Dv(10)	0.30 ± 0.00a	0.32 ± 0.03a	0.31 ± 0.00a	0.35 ± 0.01a	0.30 ± 0.00a	0.30 ± 0.00a	0.29 ± 0.00a	0.33 ± 0.01a
Dv(50)	0.87 ± 0.00ab	0.79 ± 0.06a	1.13 ± 0.01cd	1.40 ± 0.03e	1.11 ± 0.02cd	1.26 ± 0.05de	1.02 ± 0.01bc	1.24 ± 0.05de
Dv(90)	2.45 ± 0.03a	1.73 ± 0.05a	87.5 ± 16.7b	99.8 ± 19.6b	167 ± 15.2c	238 ± 47.0d	32.9 ± 15.7ab	74.1 ± 8.76b
D[4,3]	1.36 ± 0.13a	0.92 ± 0.05a	19.6 ± 4.07b	23.2 ± 5.34b	37.4 ± 3.98c	56.6 ± 12.9d	9.61 ± 1.60ab	16.8 ± 2.18b
D[3,2]	0.64 ± 0.00ab	0.61 ± 0.06a	0.74 ± 0.00bcd	0.88 ± 0.01e	0.72 ± 0.01acd	0.77 ± 0.01ce	0.68 ± 0.01ac	0.80 ± 0.02de

n.d., not determined due to emulsion destabilization and presence of large flocs of protein.
Values within a column, for individual treatments, not sharing a common letter differ significantly ($P < 0.05$).
D[4,3] = volume-weighted mean particle diameter.
D[3,2] = surface-weighted mean particle diameter.
Dv(10) = particle size below which 10% of sample volume is found.
Dv(50) = particle size below which 50% of sample volume is found.
Dv(90) = particle size below which 90% of sample volume is found.

Physical stability of emulsions

Separation rates of the different samples before and after heat treatment, with or without treatment with α-amylase or 0.2% SDS were analysed using an analytical centrifuge (LUMiSizer®; LUM GmbH, Berlin, Germany). Samples were subjected to centrifugal force (145 × *g*, for 8 h at 22 °C), while near-infrared light illuminated the entire sample cell to measure the intensity of transmitted light as a function of time and position over the entire sample length. Transmission profiles were collected every 3 min during accelerated testing of emulsions and protein-only samples to provide information on changes in the light transmission through the measurement cell as a function of the specific position in the cell and, effectively, indicating progressive migration of emulsion components (i.e. creaming and/or sedimentation). The data were presented as integral transmission as a function of time.

Statistical data analysis

All analyses were conducted in triplicate. The data generated were subject to one-way analysis of variance (ANOVA) using R i386 version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Tukey's paired comparison test was used to determine statistically significant differences ($P < 0.05$) between mean values for different samples. For the amino acid data, a Student

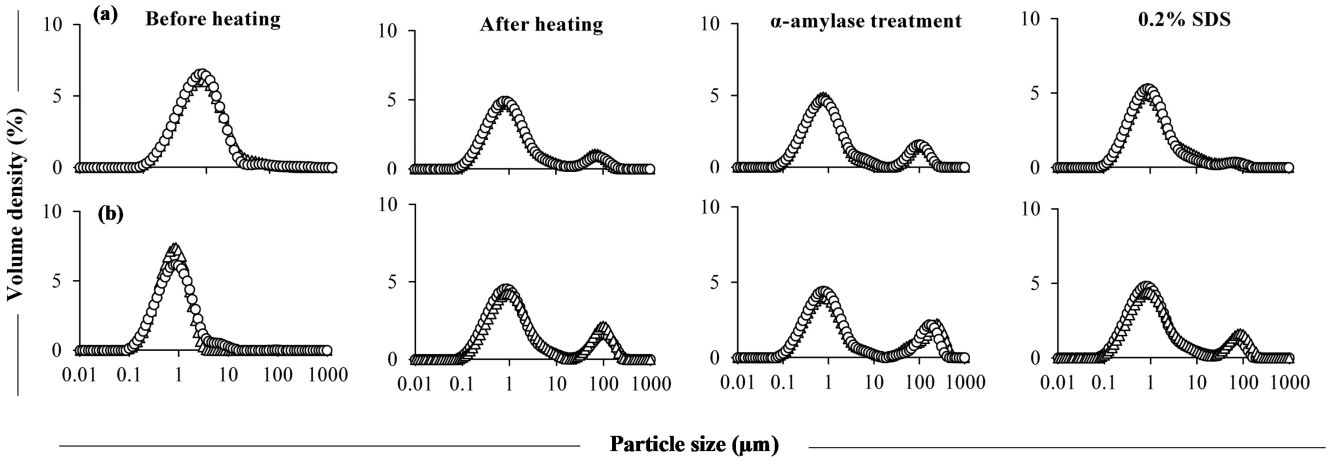

Figure 4. Particle size distribution of emulsions (a) and protein-only solutions (b) stabilized using different ratios of quinoa and lentil protein of 100:0 (—○—) and 60:40 (—△—), before and after heating at 95 °C for 30 s with or without the addition of α-amylase or 0.2% sodium dodecyl sulfate (SDS) as dissociating agent.

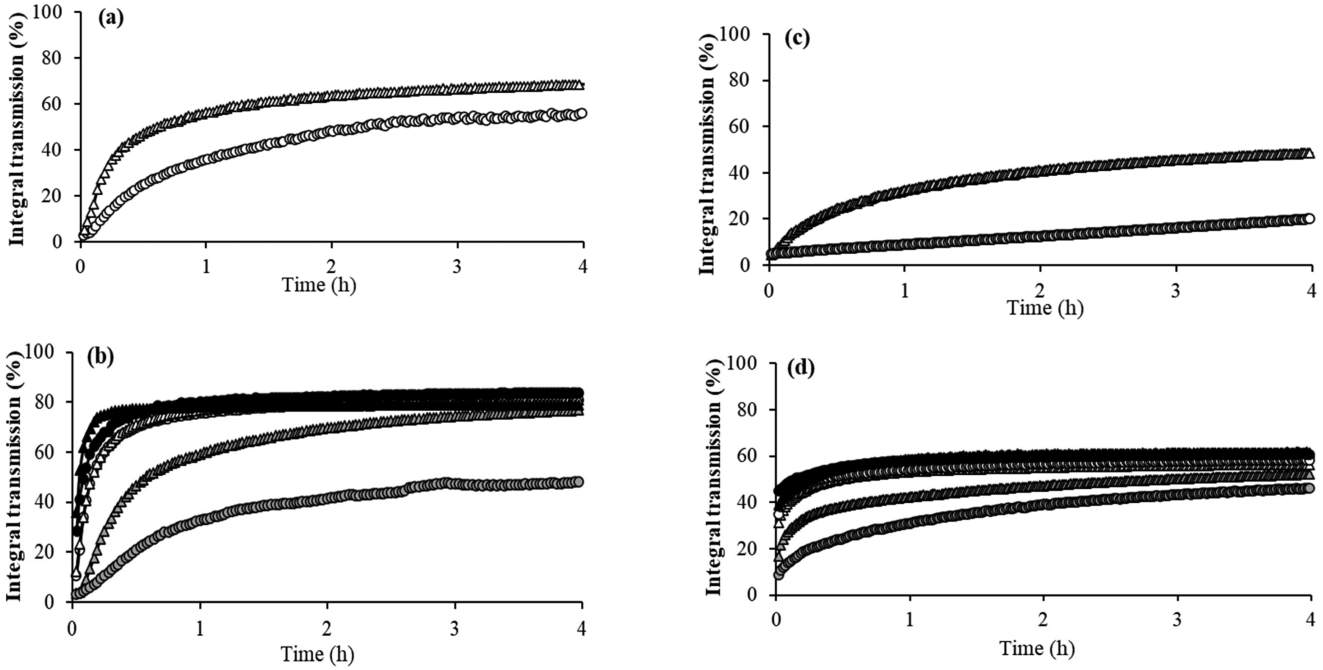

Figure 5. Separation profiles expressed as integral transmission as a function of time for emulsion (a, b) and corresponding protein-only (c, d) samples before (a, c) and after (b, d) heating (95 °C, 30 s) with quinoa:lentil ratios of 100:0 (—△—) and 60:40 (—○—) with addition of α-amylase (black fill) or SDS (grey fill).

t-test for independent samples was carried out with the same statistical program to understand the significant differences between samples.

RESULTS AND DISCUSSION

Amino acid composition

The amino acid composition of both LPI and QPRF protein ingredients is provided in Fig. 1. QPRF had a higher content of histidine, methionine, threonine and tryptophan, whereas LPI had a higher content of isoleucine, leucine, lysine, phenylalanine and valine. Vilcacundo and Hernández-Ledesma[29] reported that all essential amino acids are present in quinoa protein with relatively high proportions of lysine, methionine and threonine, with these usually being the limiting amino acids in conventional cereals, such as wheat and maize. Legume storage proteins are relatively low in sulfur-containing amino acids (i.e. methionine and cysteine), whereas the amount of other essential amino acids, such as lysine, are higher than in cereal grains.[30] Therefore, with respect to lysine and sulfur-containing amino acids, legume and cereal proteins are nutritionally complementary. Elsohaimy *et al.*[31] studied the amino acid composition of quinoa protein isolate, reporting high levels of glutamic and aspartic acid, with low levels of proline and arginine. However, in the present study, the amount of arginine present in QPRF was high, being the second most abundant amino acid, which may be due to differences in amino acid composition of different varieties of the cultivar.[9] Mäkinen *et al.*[32] reported high levels of arginine, being the fifth most abundant amino acid, in a novel quinoa protein isolate ingredient. According to these results, the combination of lentil and quinoa protein can represent a suitable option to best balance essential amino acid levels in nutritional samples, for example, meeting the essential amino acid content in infant formula according to the European legislation.[28]

Rheological properties

Starch pasting cell

The apparent viscosity of the 100Q:0L_E sample (34.3 mPa s) before heating (i.e. at 15 °C) was significantly higher than that of the 60Q:40L_E sample (22.7 mPa s). On increasing the temperature to 95 °C, a decrease in viscosity was observed for both samples, until a specific temperature (~65 °C) was reached where the viscosity of the samples began to increase until a peak viscosity was reached (Fig. 2). The 100Q:0L_E sample had significantly ($P < 0.05$) higher peak viscosity (102 mPa s) than the 60Q:40L_E sample (61.7 mPa s) and, accordingly, higher final viscosity on reaching and holding at 15 °C (80 and 61.6 mPa s, respectively) (Table 2). Addition of α-amylase to the samples before heat treatment led to a reduction in viscosity during heat treatment, with 100Q:0L_E and 60Q:40L_E having very similar final viscosity values of 41.8 and 46.0 mPa s, respectively. The same general behaviour and trends were shown by the control protein samples (100Q:0L_P and 60Q:40L_P) as for the emulsions during the heating regime; however, the viscosity values (e.g. initial, peak and final viscosity) were lower for the protein-only than the emulsion samples, due mainly to the lower total solids content. All the α-amylase-treated samples, both emulsions and protein samples, showed a considerably lower viscosity in comparison with the non-enzymatically treated samples. Starch degrading enzymes, such as α-amylases, hydrolyse the 1–4 glucosidic bonds in starch in a random endo-action at any 1–4 linkage, reducing the molecular size and the viscosity of the starch solution during the pasting treatment.[33]

The pasting profiles observed both in the emulsion (100Q:0L_E and 60Q:40L_E) and protein-only samples (100Q:0L_P and 60Q:40L_P) are characteristic of starch: when starch granules suspended in water are subjected to heat treatment, amylose leaches from them, with the resulting swelling of the starch granules leading to an increase in viscosity.[34] Generally, the maximum viscosity is achieved when the starch granule reaches its maximum swelling and is not yet disrupted.[35] After the holding period at the

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maximum temperature (i.e. 95 °C), the starch granules are disrupted, resulting in the leaching out and alignment of amylose molecules; while during cooling, reassociation between starch molecules, especially amylose chains, will result in the formation of a gel structure and, therefore, viscosity will increase. In this study, reductions in peak and final viscosity were observed in 60Q:40L_E, which had less starch and more lentil protein in comparison with 100Q:0L_E. This behaviour can be attributed to (a) lower starch content and (b) starch–protein interactions, as proteins can form crosslinks with starch, affecting its functionality.[36]

Although having the same carbohydrate content, the starch content of 100Q:0L_E was higher than 60Q:40L_E, which would be expected to influence its pasting properties. In relation to this, Joshi *et al.*[37] reported that the strength of a lentil protein gel increased exponentially with the increase in volume fraction of lentil starch, whereas the paste viscosity decreased with increasing proportion of lentil protein. In addition, it has been reported by several authors that the inclusion of protein isolates can affect the pasting behaviour of starch suspensions. In particular, Bravo-Núñez *et al.*[38] studied the pasting properties of different mixtures of maize starch and plant protein (i.e. pea and rice) and reported that the replacement of 50% of the starch by either pea or rice protein resulted in an overall decrease in the apparent viscosity, which the authors ascribed to the reduction of starch content and the role of starch–protein interactions. The authors related the starch–protein interactions to the association of proteins with starch granules limiting their swelling and, thereby, changes associated with gelatinization and gel formation. Debet and Gidley[35] observed that the proteins inherently linked to starch granules (i.e. maize and wheat starch) restrict the swelling of starch granules, leading to lower peak viscosity. Similarly, Noisuwan *et al.*[39] observed a decrease in the apparent viscosity of a starch suspension during heat treatment when combining it with dairy proteins, relating this to the delay in diffusion of water into the starch granules, due mainly to protein adsorption by the starch granules. Narciso and Brennan[40] studied different starch and protein (i.e. pea and whey protein) suspensions and observed a reduction in peak, breakdown and final viscosity during heat treatment when the ratio of protein to starch was increased. The authors associated these results with the plasticizing effect of the proteins, preventing molecular rearrangement of amylose basmati starch gels, leading to lower final viscosity. In addition, the authors related the lower final viscosity achieved when combining pea protein and starch to the high molecular weight of the former, therefore containing larger surface areas, and thus greater propensity to contain active areas for adhesion, compared to low-molecular-weight proteins.

Viscosity

The apparent viscosity obtained at 100 s^{-1} confirmed the trends in viscosity observed with the starch pasting cell. The results showed that both 100Q:0L_E and 60Q:40L_E samples had similar initial viscosity (7.56 and 5.56 mPa s, respectively; Fig. 3). After heat treatment, the viscosity of 100Q:0L_E (41.3 ± 2.05 mPa s) was significantly higher than 60Q:40L_E (25.1 ± 5.76 mPa s). Enzymatic treatment with α-amylase led to a reduction in final viscosity in both samples, which is expected as the starch is hydrolysed into lower-molecular-weight carbohydrates. The development of viscosity in the samples could be controlled by addition of α-amylase, reaching lower final viscosity values, with no significant differences ($P < 0.05$) between samples for final viscosity.

Particle size distribution

The particle size distribution of the different emulsion and protein samples was analysed before and after heat treatment, and after treatment with α-amylase and addition of 0.20% SDS. 100Q:0L_E and 60Q:40L_E samples displayed monomodal particle size distributions after homogenization, with volume-weighted mean particle diameter (D[4,3]) values of 1.26 and 1.36 μm, respectively, with no significant differences between the two emulsions (Table 3). However, after heat treatment at 95 °C, a second population of larger particles (~100 μm) was identified (Fig. 4). In a similar manner, the protein samples showed the same trends, with the appearance of larger particles after heat and α-amylase treatment. Interestingly a different behaviour was observed in the protein-only samples, where 60Q:40L_P had higher D[4,3] values than 100Q:0L_P. In the measurement of viscosity, higher final viscosity was also measured for the sample containing a mixture of the two proteins (i.e. 60Q:40L_P).

Ruiz *et al.*[14] reported average particle size for quinoa protein isolate suspension (1% protein: w/w) ranging from 50 to 3761 nm. In addition, it has been reported that quinoa starch granules have sizes ~1 μm.[24] On the application of heat treatment (100 °C for 2.5 min, pH 6.5) to quinoa protein suspensions, the formation of large aggregates was observed, being attributed, at least partially, to disulfide protein–protein linkages.[41] Similarly, Opazo-Navarrete *et al.*[23] heat-treated suspensions of quinoa protein, with and without the addition of fibre or starch, for 30 min at 60 or 120 °C, and observed formation of aggregates at both temperatures, with the aggregates formed having higher molecular weight on heat treatment at 120 °C. With regard to lentil protein, Jeske *et al.*[19] reported particle sizes of 0.22 μm for lentil protein after homogenization under the same pressure conditions as the present study (i.e. 180 bar). In that study, the protein solutions where heated at 65 or 85 °C for 30 min and 2 min, respectively, with no measured changes in particle size. Previous work[42] has also shown that an emulsion sample formulated with lentil protein alone remained stable, even after heating at 140 °C for 2 min at pH 6.8. From these results, and comparing with the available literature, it appears that quinoa protein seems less heat stable than lentil protein, in spite of the fact that denaturation temperatures for quinoa protein have been reported to be high (in the range 97–98 °C).[14] After heat treatment in the presence of α-amylase, a decrease in viscosity was observed; however, an increase in particle size distribution was observed from 1.36 and 1.26 to 18.5 and 18.2 μm for 100Q:0L_E and 60Q:40L_E, respectively, with the same trend for the respective protein-only samples (i.e. 100Q:0L_P and 60Q:40L_P). The greater increase in particle size (larger particles) when α-amylase was added to all the samples can be attributed to the positive influence of interactions between starch and protein on their colloidal stability, which causes a decrease in protein aggregation during heating.[36] In this way, some authors, such as Schmitt *et al.*,[43] have reported that the potential formation of complexes between proteins and polysaccharides may offer a practical approach of controlling the thermal aggregation of globular proteins.

Stability analysis

As all of the unheated emulsions had relatively small mean particle size, the physical stability of the emulsions was studied using analytical centrifugation under accelerated conditions of 1000 rpm over 8 h. The 60Q:40L_E sample showed higher physical stability than the 100Q:0L_E sample before heat treatment, with the former showing lower transmission over time (Fig. 5). A similar

trend was observed for the protein-only samples, with 60Q:40L_P being more stable. As the protein profile of the interfacial layer of protein-stabilized oil-in-water emulsions is known to influence thermal stability of such systems, the samples were heated at 95 °C, during which the transmission of all samples increased significantly. This change in transmission was attributed to the samples becoming more unstable due to the formation of larger particles on heat treatment, as evident from particle size analysis (Fig. 4). To determine what factors contributed to these heat-induced changes in emulsion stability, addition of 0.20% SDS to the protein-only and emulsion samples demonstrated that the original increase in particle size was, at least partially, due to protein aggregation-mediated flocculation (i.e. reversible with the use of a dissociating agent) of oil droplets in the emulsions.[44] To investigate the contribution of starch from the quinoa protein ingredient to the measured differences in heat stability of the emulsions, the application of α-amylase during heat treatment led to a reduction in viscosity, while the particle size increased significantly, leading to reduced physical stability of both protein-only and emulsion samples. Similarly, Jeske *et al.*[45] reported higher separation rates in protein and emulsion samples with quinoa when applying α-amylase treatment; furthermore, the authors reported a higher sediment and cream height when applying an α-amylase treatment.

CONCLUSION

This study demonstrated for the first time how the combination of quinoa and lentil proteins presents a good option to achieve an optimized amino acid profile for the formulation of plant-based liquid emulsions with enhanced colloidal and thermal stability. Inclusion of lentil protein with quinoa protein in a protein-stabilized oil-in-water emulsion system conferred enhanced physical stability on the unheated emulsions and also contributed to lower viscosity development during thermal treatment. These differences in emulsion stability were due to modulation of protein–protein interactions, and also to the contribution of starch from the quinoa protein ingredient. The results of this study provide new scientific knowledge on the influence of mixtures of plant proteins from different botanical sources on the physicochemical quality of emulsion-based food systems.

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