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The effect of direct and indirect heat treatment on the attributes of whey protein beverages

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ABSTRACT

Thermal processing of ready-to-drink high protein beverages can have a substantial impact on the physical and sensory properties of the final product for long-life milks such as extended shelf life and ultra high temperature processed products. Direct and indirect heat treatment technologies were applied to whey protein isolate (WPI) -based beverages containing 4, 6 or 8% (w/w) protein. Lower levels of protein denaturation (66–94%) were observed using direct heating compared with indirect heating (95–99%) across protein levels and heating temperatures (121 and 135 °C final heat). Direct heat treatment resulted in significantly lower viscosity and less extensive changes to the volatile profile, compared with indirect heat treatment. Overall, the application of direct and indirect heat treatment to WPI solutions resulted in significantly different final products in terms of appearance, physical characteristics and volatile profile, with direct heating resulting in many enhanced properties compared with conventional indirect heat treatment.

1. Introduction

Nutritional beverages are a rapidly growing market segment, with sales increasing by an average of approximately 5% annually (Chen & O'Mahony, 2016; Cochrane et al., 2012). These products can be formulated to cater for a variety of consumer needs such as functional sports foods for high performance athletes and body-builders, meal replacement drinks for dietetic nutrition, and low-sugar drinks for diabetic patients (Beecher, Drake, Luck, & Foegeding, 2008; Jelen, 2009; Shiby, Radhakrishna, & Singh Bawa, 2013).

When developing protein beverages, whey proteins are commonly used as a protein source due to their excellent nutritional qualities, bland flavour, ease of digestibility and functionality in beverage systems (Rittmanic, 2006). Formerly considered a waste by-product of cheese and casein production, whey protein has become highly valued for its nutritional and functional properties (Boland, 2011; Evans & Gordon, 1980; Fitzsimons, Mulvihill, & Morris, 2007; Mulvihill & Ennis, 2003; Smithers, 2008). However, technological processes used in dairy-based beverage manufacture may impair the high nutritional value of whey proteins, whereby protein denaturation and aggregation and loss of solubility decrease protein digestibility and the bioavailability for enzymatic digestion (Pellegrino, 2013). As a result, selection of thermal processing technology is an important factor affecting the level of protein denaturation and nutritional value of products, in addition to reducing aggregate-related storage stability issues in long-life products, such as increases in viscosity, turbidity and sedimentation (Le et al., 2016; Villumsen et al., 2015a,b).

Typical heat treatment processes used during manufacture of whey protein beverages are in the extended shelf life (ESL) heat treatment range (120–135 °C for 2–4 s) or ultra high temperature (UHT) range (135–145 °C for 2–4 s) (Britz & Robinson, 2008; Deeth & Lewis, 2016; Rysstad & Kolstad, 2006). There are two classical modes of high temperature short

time (HTST) heating, i.e., indirect and direct heating, used for the commercial sterilisation of milk and milk products (Deeth & Lewis, 2016; Roux et al., 2016).

Indirect systems, using systems like tubular and plate heat exchangers, promote heat transfer across an interface while, for direct systems, like injection and infusion, the heating medium, steam, is in direct contact with the product and subsequently removed through flash cooling (Burton, 1994; Hsu, 1970; Lewis & Heppell, 2000; Schroyer, 1997). The heat transfer interface of indirect heating systems reduces the heat transfer rate and localised heating at the interface can result in higher levels of protein denaturation and fouling compared with direct systems (Akkerman et al., 2016; Karayannakidis, Apostolidis, & Lee, 2014; Murphy, Tobin, Roos, & Fenelon, 2011).

In direct heating systems, almost instantaneous heating is achieved due to the mixing of the heating medium and product. This method involves a more efficient and rapid rate of heat transfer than indirect heating, as it makes use of the latent heat of evaporation as the steam condenses, resulting in reduced residence time and a lower thermal load imparted on the product (Britz & Robinson, 2008; Datta, Elliott, Perkins, & Deeth, 2002; Dickow, Nielsen, & Hammershøj, 2012b; Karayannakidis et al., 2014; Lee, Barbano, & Drake, 2017).

In a number of studies direct heat treatment technology led to a reduced level of whey protein denaturation compared with indirect heating for skim milk (Akkerman et al., 2016; Lee et al., 2017; Lyster, Wyeth, Perkin, & Burton, 1971) and whey protein concentrate (Dickow, Kaufmann, Wiking, & Hammershøj, 2012a). However, direct treatments are also reported to result in a greater average particle size and sediment formation compared with indirect systems, due to the reduced area of thermal transfer surfaces in direct systems for deposition of aggregates (Burton, 1968; Datta et al., 2002; Malmgren et al., 2017). These studies imply that aggregates that would generally adhere to hot surfaces and be found in fouling material during traditional indirect processing are still present in the final product.

The rapid cooling in direct heating can remove volatiles in milk such as dissolved oxygen, heat-induced sulphur volatiles and other volatiles, in addition to removing excess water, resulting in less heat-induced flavour changes (Deeth & Lewis, 2016; Lee et al., 2017). Previous studies have identified direct heating processes as the best technological option to limit thermally-induced changes in milks (Roux et al., 2016; Van Asselt, Sweere, Rollema, & de Jong, 2008).

The heat treatment technology employed in dairy beverage production can have a significant impact on the taste, physical stability, and shelf life of the product. Little has been published in relation to the heat treatment of high protein whey solutions using direct heat treatment technology (Dickow et al., 2012a) or the comparison of direct and indirect technologies. The aim of this study was to investigate the impact of direct and indirect heat treatment technology at high temperatures (70 ^{o}C/121 ^{o}C and 80 ^{o}C/135 ^{o}C with preheat and final holding time of 30 s and 2 s, respectively) on selected physicochemical characteristics of high protein ready-to-drink whey protein beverages and to determine if either technology produced significantly enhanced product quality.

2. Materials and methods

2.1. Materials and formulation

Model whey-protein beverages were formulated at protein concentrations of 4, 6 and 8% (w/w), reflective of current market product protein concentrations, using whey protein isolate (BiPro®), supplied by Davisco Foods International (Le Sueur, MN, USA), which had a composition of 91.8% protein, 0.21% fat, 2.03% ash, and <0.2% lactose. The WPI powder were reconstituted in 150 L batches using reverse-osmosis water heated to 45 °C, to aid

solubilisation of the ingredients. A YTRON ZC powder induction unit (YTRON Process Technology GmbH, Bad Endorf, Germany), consisting of a high-shear, rotor-stator mixer connected to a recirculation pump, was used for ingredient induction with a 20 min recirculation time. The dispersion was stored in a tank equipped with an impeller and stirred at a low speed overnight at 4 °C. The pH was adjusted to pH 6.8 using 0.1 M HCl or KOH, as required, before and after overnight storage.

2.2. Heat treatment

Two pilot-scale thermal processing plants were used to carry out direct and indirect heat treatment of the WPI dispersions. Direct heating was applied using a UHT steam infusion pilot plant 422463 (APV, Silkeborg, Denmark), which consists of a plate heat exchanger for preheating followed by steam infusion and flash cooling vessel, and a plate heat exchanger for final cooling (Fig. 1a). Indirect heating was applied using a MicroThermics tubular UHT pilot plant (MicroThermics, NC, USA), consisting of two tubular heat exchangers for preheating and final heating operations and two tubular heat exchangers for initial and final cooling operations (Fig. 1b). Both the direct and indirect pilot plants were used with a preheat holding time of 30 s and a final heat holding time of 2 s (Fig. 1c). Two types of heating conditions were applied to the WPI dispersions using the direct and indirect pilot plants; 70 °C preheat with 121 °C final heat, and 80 °C preheat with 135 °C final heat. These temperature combinations are commonly used for extended-shelf-life (ESL) and ultra-heat-treatment (UHT) processes, respectively (Burton, 1994; Bylund, 1995; Rysstad & Kolstad, 2006). The temperature combinations used will be referred to as ESL (70/121 °C) and UHT (80/135 °C) to ease description.

2.3. Particle size analysis and molecular weight distribution

Particle size distribution data of whey protein dispersions was determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK). Samples were dispersed in ultra-pure water for analysis in polystyrene disposable cuvettes. A refractive index of 1.45 was used for protein samples, while a refractive index of 1.330 was used for the dispersant. All samples were analysed at a temperature of 25 °C.

Size-exclusion high-performance liquid chromatography (SE-HPLC) was used to monitor the formation of heat-induced aggregates by determining the molecular weight (M_W) profile of the samples as described by Buggy, McManus, Brodkorb, McCarthy, and Fenelon (2016). The HPLC system used consisted of a Waters 2695 separation module with a Waters 2487 dual-wavelength detector at 280 nm, controlled using Waters Empower® software (Waters, Milford, Massachusetts, USA) using two columns in series (TSKgel G2000SWXL and G3000SWXL, 7.8 mm ID, 30 cm length, 5 μm particle size, Tosoh Biosciences LLC, USA) with a guard column (TSKgel SWXL, 6 mm ID × 4 cm length, 7 μm particle size).

2.4. Colour analysis

To investigate potential heat-induced changes in colour due to aggregation of heat labile proteins colour measurements were carried out before and after heat treatment. The colour of each dispersion was measured and expressed as L*, a* and b* values using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The L* value indicates lightness, a* values indicate redness-greenness, b* values indicate

yellowness-blueness. Samples were loaded into a disposable cuvette and placed in front of a white calibration plate (L*, a*, b*) before measurement in triplicate.

2.5. Viscosity

Viscosity can impact final product acceptability for consumers, and was measured using an ARG2 controlled-stress rheometer (TA Instruments, Crawley, UK) equipped with concentric cylinder geometry at 25 °C. The procedure involved the samples being pre-sheared at 500 s^{-1} for 1 min followed by equilibration at 0 s^{-1} for 1 min, to neutralise the short-term rheological history of the formulations. The shear rate was then increased from 5 to 500 s^{-1} over 2 min, held at 500 s^{-1} for 1 min then decreased from 500 to 5 s^{-1} over 2 min (Murphy et al., 2013).

2.6. Protein analysis and total solids measurement

The total solids content of the dispersions was measured using a Smart System 5, Smart Trac (CEM Corporation, Matthews, NC, USA).

Determination of total protein content of samples was carried out using the Kjeldahl method of analysis (IDF, 2001), using a nitrogen to protein conversion factor of 6.38.

For soluble protein analysis, denatured and aggregated protein material was removed by adjusting the sample to the isoelectric point at pH 4.6 using a 0.1 M acetate buffer to a final protein concentration of 2.5 g L^{-1} protein, centrifuging at 20,000 × *g* for 20 min at 4 °C and filtering through 0.2 µm low-protein binding PES filters (Agilent Technologies, CA, United States). The prepared samples were evaluated using high-performance liquid chromatography (HPLC) using a Waters 2695 separation module, a Waters 2487 dual wavelength absorbance

detector running on Waters Empower® software (Milford, MA, USA). Reversed-phase (RP) HPLC was completed using a PolymerX 5 µm RP-1, 150 × 4.6 mm column (Phenomenex, Cheshire, UK) as described by Kehoe, Wang, Morris, and Brodkorb (2011). α-Lactalbumin, β-lactoglobulin A and β-lactoglobulin B standards (Sigma Aldrich, Ireland) were used to calibrate the method.

2.7. Volatile analysis

Volatile compounds were identified using head-space solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS), described by Stefanovic, Kilcawley, Rea, Fitzgerald, and McAuliffe (2017), with some modifications. The sample volume was 4 mL and all samples were run in triplicate. Samples were processed using Shimadzu GCMS solutions software using the flavour and fragrance library (FFNSC 2) in combination with in house libraries and NIST 2011 Mass Spectral Library, AMDIS (www.amdis.net) software and linear retention indices were carried out using the method of Van den Dool and Kratz (1963). Batch processing was carried out with metaMS (Wehrens, Weingart, & Mattivi, 2014) (www.rdocumentation.org). The unheated and heat-treated dispersions were frozen, immediately after thermal processing, until required for volatile analysis.

2.8. Statistical analysis

All heat treatment trials were carried out in triplicate, and the subsequent data sets were subjected to analysis using the MINITAB® 15 (Minitab Ltd., Coventry, UK) statistical analysis package. The statistical significance of treatment effects on physical characteristics

investigated was evaluated by means of one-way analysis of variance (ANOVA) with Tukey and Dunnetts' post hoc analysis. Three-way ANOVA was completed using the factors: protein content, heat treatment technology, and temperature of heat treatment. A paired *t*-test was carried out on particle size data to further investigate the effect of heat treatment. Principal component analysis (PCA) of protein beverage volatiles was performed using The Unscrambler X multivariate analysis programme, v10.3 (CAMO ASA, Trondheim, Norway).

3. Results

3.1. Particle size and molecular weight distribution

3.1.1. Particle size distribution

In general, the particle size (z-average) of the protein dispersions increased as a result of heat treatment (Tables 1 and 2; $p < 0.001$). This was particularly the case in directly heated dispersions, with statistically significant increases found for directly ESL and UHT treated dispersions at 4 and 6% (w/w) protein, and for directly ESL treated at 8% (w/w) protein, according to Dunnett's post hoc analysis data (not shown). A paired t-test revealed that indirect ESL heat treatments gave a higher particle size than their indirect UHT-treated counterparts at 4%, 6%, and 8% (w/w) protein concentrations ($p < 0.05$, 0.01 and 0.001, respectively), with the distinction between ESL and UHT treatments becoming stronger with increasing protein concentration. Directly heat-treated samples showed no significant difference in particle size between ESL and UHT treatments.

3.1.2. Molecular weight distribution

The M_W profiles of the aggregates formed in the soluble fraction of the beverage dispersions was determined using size-exclusion chromatography. The M_W distributions were similar for the unheated dispersion at all protein concentrations, with high proportions of low M_W proteins relative to native proteins (Fig. 2). For all heat-treated dispersions, the proportion of low M_W aggregates decreased, while the presence of medium- and high-M_W aggregates increased with increasing thermal load and protein concentration.

For all protein concentrations, direct ESL treatment produced the lowest proportion of high M_W aggregates (≥ 300 kDa) compared with all other heat treatments. In general, direct UHT, indirect ESL and indirect UHT treatments resulted in statistically similar M_W profiles for the soluble phase. The difference in the proportion of particles with a M_W greater than 300 kDa between direct and indirect UHT treatments increased with increasing protein concentration, resulting in a significantly greater proportion of high M_W aggregates in the soluble fraction following indirect UHT treatment for 8% (w/w) protein concentration compared with those which were directly treated.

The proportion of total protein material with a M_W of 8–15 kDa decreased significantly for all heat treatments except for the direct ESL treatment at 4% protein. The proportion of protein material with a M_W of 8–15 kDa were not significantly different between direct UHT, indirect ESL and indirect UHT in most cases, although the proportion could be seen to decrease as the thermal load increased, i.e., direct UHT > indirect ESL > indirect UHT.

3.2. Colour analysis

All heat treatments resulted in a significant change in L* value or lightness, from the unheated dispersion, with the exception of ESL and UHT indirectly treated 8% (w/w) dispersion (Table 3). The protein content of dispersions, heating technology and heating temperature each had a significant effect on L* ($p < 0.001$; Table 3 and Fig. 3). For 4% protein dispersions, the lightness was similar for direct and indirect UHT heat treatments, while the corresponding direct and indirect ESL-treated dispersions were statistically different from each other. Direct ESL heat treatment at 6% (w/w) protein resulted in a significantly higher L* value than all other heat treatments for 6% (w/w) protein. Indirect UHT treatment resulted in a significantly lower L* value compared with that of all other heat treatments at 6% protein. For 8% protein dispersions, the L* of both direct heat treatments was significantly greater than after indirect heat treatments. A paired *t*-test showed that dispersions treated by indirect ESL had a higher L* value than their indirectly UHT-treated counterparts ($p < 0.01$). Similar to the L* value, the a* value was significantly reduced by heat treatment, implying a reduction in redness, with the exception of indirect heat treatments at 8% (w/w) protein concentration. Heat treatment significantly reduced the b* value of all protein concentrations, implying a reduction in measured yellowness (Table 3). These changes in colour identified are visually observable and may have an impact on consumer perception.

3.3. Viscosity

Protein concentration, choice of heating technology and severity of heat treatment all had a significant effect on the viscosity of protein dispersions as determined by three-way ANOVA ($p < 0.001$; Table 2). The extent of increase in viscosity upon heating increased with increasing protein concentration of the dispersions, where the 8% (w/w) protein dispersions

were the most affected by heat treatment (Table 1). Overall, direct heat treatment resulted in a lower final viscosity than indirect heat treatment, although this difference was not statistically significant in some cases below 8% protein level (Table 1).

While 4% (w/w) protein dispersions showed no significant viscosity increase on heating, the viscosity of indirectly-treated 6% (w/w) protein dispersions increased significantly with ESL treatment. At 8% (w/w) protein, heat-treated dispersions showed a significant increase in viscosity during heat treatment, with direct ESL and UHT treatments resulting in similar viscosities, which were lower than that achieved by indirect heating. Similar to the trends for 6% (w/w) protein dispersions, indirect ESL treatment of 8% (w/w) protein dispersions resulted in a significantly higher viscosity (9.02 mPa s) compared with indirect UHT treatment (4.61 mPa s), despite the higher final heating temperature in the latter. For indirect heating, there was a statistically significant interaction determined between the heating technology and heat treatment temperature ($p < 0.001$).

3.4. Protein content, profile and level of soluble protein

3.4.1. Total solids and protein content of WPI dispersions

Direct heating was associated with significantly decreased total solids contents of dispersions, in some cases with reductions of 4.95–8.58%, and the effect was particularly significant around 8% protein level (Table 1), while the total solids content was unaffected by indirect heat treatment for all protein concentrations. Three-way ANOVA analysis confirmed that heating technology had a significant effect reducing the total solids level ($p < 0.001$), while the severity of heat treatment (i.e., ESL or UHT) did not affect total solids content (Table 2).

The total protein content of unheated and heated dispersions followed similar trends to that of total solids due to the high protein content of the WPI powder used in dispersions (Tables 1 and 2). While reductions in total protein content were observed for all directly heated dispersions, this reduction was only statistically significant for dispersions containing 6 and 8% (w/w) total protein. The reduction in total solids and total protein observed in directly heat-treated dispersions (i.e., steam injection and infusion) is likely the result of dilution, with condensed steam not being completely removed by flash cooling during direct processing. Product dilution, or concentration, during direct heating is common, and has been reported in numerous studies (Dickow et al., 2012a; Dumpler, Wohlschläger, & Kulozik, 2017; Lewis & Heppell, 2000; Murphy et al., 2011; Murphy, Tobin, Roos, & Fenelon, 2013). Net dilution or concentration within the system can be reduced by maintaining equal temperatures at preheat and flash cooling stages, and implementing finer instrument control.

3.4.2. Soluble protein

RP-HPLC showed that direct and indirect heat treatment resulted in significant levels of whey protein denaturation compared with the unheated dispersions (Fig. 4). Three-way ANOVA analysis of RP-HPLC data revealed that all protein fractions investigated were significantly affected by heating technology ($p < 0.001$) and the temperature of heat treatment ($p < 0.001$). Direct heating resulted in lower levels of protein denaturation (i.e., more native protein) for direct ESL thermal treatment in particular. Direct ESL heat treatments resulted in the retention of significantly high levels of native α-lactalbumin (α-la) compared with indirect heating, for all dispersions tested ($p < 0.05$). The lowest level of native α-la was obtained using indirect UHT treatment, to a significant degree for the 4 and 6% (w/w) protein dispersions ($p < 0.05$). Although directly UHT-treated dispersions had a higher level of native α-la after heat treatment than indirect ESL treatment, the difference was not statistically

significant in most cases (Table 1). For both the β-lactoglobulin A (β-lg A) and B (β-lg B), direct ESL treatment resulted in the lowest levels of denaturation, with the exception of the level of β-lg A in the 6% protein dispersion which, while lower, was not statistically different from that of the other heat treatments.

3.5. Volatile analysis

A range of 62 volatile aromatic organic compounds were identified in the beverage dispersions, including ketones, aldehydes, alcohols, esters, furans, sulphur- and benzene-containing compounds (results not shown). Differences between directly and indirectly treated dispersions were identified for many compounds. Indirect treatment increased levels of aldehyde compounds were observed ($p < 0.05$), such as pentanal, hexanal, heptanal, octanal and 2-methylpropanal, which is known to promote the 'stale' flavour in high-temperature-treated milks (Zabbia, Buys, & De Kock, 2012). A significant increase in the levels of dimethyl trisulphide and other sulphur compounds was found for indirectly heat-treated dispersions ($p < 0.05$). Such sulphur compounds are related to strong 'cooked' flavours in high temperature treated milks as a result of β-lactoglobulin denaturation (Al-Attabi, D'arcy, & Deeth, 2008). The generation of furan compounds was also noted, although the increased levels of 2-pentylfuran and 2-butylfuran with indirect heating were not significantly higher than those following direct heating.

The PCA plot shows that the volatiles profile of heat treated dispersions can be discriminated on the basis of the heating technology and severity of thermal treatment applied, particularly for indirect heat treatment (Fig. 5). The volatile profile of directly-heated dispersions related more closely to unheated dispersions than to those which were indirectly-heated. Although some differences between unheated and direct ESL dispersions could be

observed, particularly for the 8% (w/w) protein dispersion, as protein concentration increased, a strong PCA grouping was not obtained with regards to ESL heat treatment applied with direct heating technology. More distinctive grouping was observed for the direct UHT treated dispersions. However, indirect heat treatment of dispersions resulted in clear differences between the unheated, ESL and UHT dispersions, which increased as the heating temperature increased. The PCA plot also showed differences based on protein content, which may have been due to a higher level of *d*-limonene found in 4% (w/w) protein dispersions than in higher protein content dispersions, although the difference levels was not statistically significant. *d*-Limonene is a terpene derived from animal feed and commonly found in milk; levels will vary dependent upon diet and metabolism in the rumen (Hansen & Heinis, 1992).

4. Discussion

The application of direct and indirect heating technologies resulted in significant differences in the physical characteristics of the high protein dispersions. These differences have the potential to impact consumer perception and acceptability, as they relate to protein bioavailability, appearance and volatile profile of the final product.

A significantly higher level of soluble protein was recorded following direct heat treatment compared with indirect heat treatment. This reduced level of protein denaturation can be attributed to the lower overall thermal load imparted due to rapid heating and cooling (Fig. 1c) (Burton, 1994; Lewis & Heppell, 2000; Murphy et al., 2013). Pellegrino, Masotti, Cattaneo, Hogenboom, and de Noni (2013) reported that the retention of a higher level of native whey proteins preserves the nutritional quality and digestibility of proteins in dispersions which may be of interest to health-conscious consumers of high protein

beverages. Direct ESL treatment resulted in less protein denaturation for all dispersions, and the level of protein denaturation increased (albeit not to a significant degree in all cases) as the thermal load increased, i.e., direct ESL < direct UHT < indirect ESL < indirect UHT. These ranges are consistent with those reported in previous studies (Burton, 1994; Elliott, Dhakal, Datta, & Deeth, 2003; Lewis & Heppell, 2000).

The appearance of directly and indirectly treated dispersions was noticeably different. While directly-treated dispersions were equally opaque at each of the protein concentrations, indirectly-treated dispersions were seen to have reduced opacity as the protein concentration increased, as measured by a reduction in L* value (Fig. 3; Table 3). The significant changes in L* were consistent with the some general trends in particle size. For indirectly-treated dispersions, ESL-treated dispersions had a greater particle size and L* value than their UHT-treated counterparts, as predicted by Rayleigh's Law, which relates particle size to colour change (Chung, Degner, & McClements, 2014; Desobry-Banon, Richard, & Hardy, 1994; McClements, 2002). This increased level of whiteness in whey protein dispersions obtained from direct heating systems may have a knock-on impact on customer perception.

Some directly-treated dispersions were found to have a larger particle size compared with indirectly-treated dispersions, despite having a lower degree of whey protein denaturation. These findings may seem counterintuitive; however, this is in agreement with the findings of previous studies (Burton, 1968; Datta et al., 2002; Malmgren et al., 2017) that proposed that the presence of some larger aggregates was related to reduced levels of deposition and fouling in direct heating systems. As the larger aggregates are not retained on heat transfer interfaces within the heating system during direct steam infusion, they remain in the product stream, contributing to increased whiteness and particle size. The difference in particle size may also be related to differences in denaturation and aggregation mechanisms due to the thermal profiles of the direct and indirect systems (Fig. 1c). Denaturation and

aggregation occur in two distinct stages; the first consists of the unfolding of β-lg, and the second involves the association of these unfolded molecules to form aggregates (Joyce, Brodkorb, Kelly, & O'Mahony, 2017; Mulvihill & Donovan, 1987). Anema and McKenna (1996) found that aggregation of unfolded proteins was the rate-determining step during high-temperature processing of directly heat-treated reconstituted whole milk. The different thermal profile of the two thermal processing technologies could lead to the formation of different types of aggregates after denaturation as a result of these mechanisms.

As the average particle size of indirectly treated dispersions decreased, the viscosity of the dispersions increased, due to an increase in particle-particle interactions between a larger number of smaller particles (Table 1). Indirect ESL treatment resulted in a large increase in viscosity, from 3.42 to 9.02 mPa s, compared with both direct heat treatments and to the indirect UHT treatment, despite the higher final heating temperature. This may be due to the effect of preheating temperature, which has been shown to impact the heat stability of protein dispersions, stabilising against heat-induced physical changes during high temperature processing (Drapala, Auty, Mulvihill, & O'Mahony, 2016; Dumpler & Kulozik, 2016; Srichantra, Newstead, McCarthy, & Paterson, 2006). In this study, no such effect was seen when direct heat treatment was applied, suggesting that preheat treatment may have a less significant effect during direct heating compared with indirect.

Jansson et al. (2014) reported that the severity of heat treatments related to the development of off-flavours in milk. The results of the present study are consistent with this, as direct heat treatment, with its lower thermal load, produced a volatile profile which was closer to that of the unheated dispersion than its indirect counterpart. In addition to the reduced severity of heating during direct heat treatment, studies have shown that the rapid vacuum flash cooling step in this process can also aid in the removal of volatiles, improving the flavour of heat-treated dispersions (Deeth & Lewis, 2016; Lee et al., 2017).

5. Conclusion

The application of direct or indirect heating technology had a significant impact on the end-product functionality, appearance and sensory properties of whey protein dispersions. Direct heating resulted in many favourable product properties and significantly less thermal damage across all protein concentrations compared with indirect heating. This direct heating technology enabled the retention of higher levels of native whey protein, as determined by RP- and SE-HPLC, lower viscosity and minimal change in volatile profile. However, the products produced were more opaque than indirectly heat-treated dispersions, particularly at higher protein concentrations. Direct heat treatment can be used to process challenging whey protein beverages with a high-protein content, achieving final product properties that are unattainable with traditional indirect heat treatment methods. The application of this technology to the growing high-protein beverage market would result in products with greater nutritional value and flavour.

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Figure legends

Fig 1. Process flow diagram of (a) direct and (b) indirect heat treatment plants and (c) time-temperature heating and cooling profiles of indirect (tubular heat exchanger) (—◇—) and direct (steam infusion or injection) (—⊟—) heat treatment technologies.

Fig 2. Molecular weight distribution of the soluble fraction of unheated and heat-treated whey protein dispersions with molecular weights of 8–15 kDa (■), 15–30 kDa (■), 30–80 kDa (■), 80–300 kDa (■), >300 kDa (■).

Fig 3. Images of whey protein dispersions at 4, 6 and 8% (w/w) protein after direct and indirect with (a) ESL (70 °C preheat and 121 °C) and (b) UHT (80 °C preheat and 135 °C) heat-treated formulations.

Fig 4. Levels of native whey protein in the pH 4.6-soluble fraction measured by RP-HPLC; α-lactalbumin (■), β-lactogloblin B (■), and β-lactoglobulin A (■) expressed as a percentage of total native whey protein for whey protein beverage dispersions at 4%, 6%, and 8% (w/w) total protein.

Fig 5. Principal component analysis plot of the volatile profiles of unheated, directly and indirectly heated whey protein dispersions with 4%, 6%, or 8% total protein.

Table 1

Physicochemical properties of protein beverages containing 4, 6, or 8% total protein, before and after direct steam infusion and indirect tubular heat treatment. [a]

Beverage solutions	Heat treatment	pH	Total solids (%, w/w)	Total protein (%, w/w)	Soluble protein (%, w/w)	Viscosity (mPa s)	Particle diameter (nm)
4% Protein	Unheated	$6.81^{a} \pm 0.03$	$4.13^{a} \pm 0.05$	$4.10^{a} \pm 0.08$	$3.57^{a} \pm 0.10$	$3.29^{ab} \pm 0.05$	$98.2^{c} \pm 0.76$
	Direct ESL	$6.84^{a} \pm 0.04$	$3.78^{b} \pm 0.06$	$3.82^{a} \pm 0.17$	$1.72^{b} \pm 0.29$	$3.33^{b} \pm 0.04$	$278^{a} \pm 2.42$
	Direct UHT	$6.91^{a} \pm 0.03$	$3.92^{ab} \pm 0.08$	$3.96^{a} \pm 0.01$	$1.20^{c} \pm 0.11$	$3.41^{ab} \pm 0.03$	$243^{ab} \pm 38.0$
	Indirect ESL	$6.89^{a} \pm 0.02$	$4.10^{a} \pm 0.08$	$4.08^{a} \pm 0.07$	$0.75^{c} \pm 0.14$	$3.49^{ab} \pm 0.02$	$218^{b} \pm 4.60$
	Indirect UHT	$6.92^{a} \pm 0.04$	$4.06^{a} \pm 0.07$	$4.08^{a} \pm 0.06$	$0.94^{c} \pm 0.06$	$3.53^{a} \pm 0.04$	$195^{b} \pm 17.2$
6% Protein	Unheated	$6.82^{ab} \pm 0.03$	$6.37^{a} \pm 0.08$	$6.18^{ab} \pm 0.05$	$5.85^{a} \pm 0.09$	$3.37^{b} \pm 0.03$	$121^{c} \pm 4.21$
	Direct ESL	$6.77^{b} \pm 0.02$	$5.96^{a} \pm 0.08$	$5.82^{bc} \pm 0.04$	$2.19^{b} \pm 0.18$	$3.42^{b} \pm 0.02$	$192^{ab} \pm 7.77$
	Direct UHT	$6.90^{a} \pm 0.07$	$5.82^{a} \pm 0.33$	$5.61^{c} \pm 0.04$	$1.36^{c} \pm 0.14$	$3.50^{b} \pm 0.07$	$168^{b} \pm 10.9$
	Indirect ESL	$6.85^{ab} \pm 0.02$	$6.29^{a} \pm 0.10$	$6.20^{a} \pm 0.13$	$0.75^{d} \pm 0.12$	$3.91^{a} \pm 0.02$	$216^{a} \pm 0.86$
	Indirect UHT	$6.87^{ab} \pm 0.02$	$6.25^{a} \pm 0.07$	$6.22^{a} \pm 0.14$	0.96 ± 0.08^{d}	$3.69^{ab} \pm 0.02$	$136^{c} \pm 12.5$
8% Protein	Unheated	$6.81^{a} \pm 0.04$	$8.44^{a} \pm 0.06$	$8.22^{a} \pm 0.07$	$7.71^{a} \pm 0.11$	$3.42^{d} \pm 0.04$	$97.4^{ab} \pm 1.48$
	Direct ESL	$6.81^{a} \pm 0.06$	$7.83^{c} \pm 0.16$	$7.56^{b} \pm 0.19$	$3.59^{b} \pm 1.22$	$4.10^{cd} \pm 0.06$	$244^{a} \pm 11.6$
	Direct UHT	$6.82^{a} \pm 0.07$	$8.02^{bc} \pm 0.12$	$7.86^{ab} \pm 0.08$	$1.30^{a} \pm 0.09$	$4.18^{bc} \pm 0.07$	$187^{ab} \pm 83.7$
	Indirect ESL	$6.83^{a} \pm 0.05$	$8.28^{ab} \pm 0.03$	$8.13^{a} \pm 0.03$	$0.67^{c} \pm 0.02$	$9.02^{a} \pm 0.05$	$211^{ab} \pm 4.57$
	Indirect UHT	$6.86^{a} \pm 0.01$	$8.39^{a} \pm 0.03$	$8.12^{a} \pm 0.06$	$1.00^{c} \pm 0.06$	$4.61^{a} \pm 0.01$	$114^{b} \pm 1.67$

[a] For each beverage solution (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$).

ESL relates to a 70 °C preheat temperature and 121 °C final heat temperature. UHT relates to a 80 °C preheat temperature and 135 °C final heat temperature.

Table 2

Statistical significance of the effects of target protein level, heating technology, severity of heat treatment and interactions of these factors on the physicochemical characteristics of heat treated solutions, assessed by three-way ANOVA. [a]

Characteristic		Protein level	Technology	Heat treatment	Protein level* technology	Technology* heat treatment	Protein level* heat treatment
pH		**	NS	**	NS	NS	NS
Total solids content		***	***	NS	NS	NS	NS
Total protein content		***	***	NS	**	NS	NS
Total soluble protein content		*	***	**	**	***	NS
Native protein	α-la	NS	***	***	NS	***	NS
	β-lg A	*	***	***	NS	***	NS
	β-lg B	NS	***	***	NS	*	NS
Colour coordinates	L*	***	***	***	***	*	***
	a*	***	***	***	***	*	*
	b*	*	***	NS	***	NS	*
Colour difference, ΔE		***	***	***	***	***	***
Viscosity		***	***	***	***	***	***
Particle size		***	***	***	NS	NS	NS
Molecular weight distribution	≥ 300 kDa	***	***	***	**	***	NS
	80–300 kDa	***	NS	NS	***	NS	NS
	30–80 kDa	***	NS	*	**	NS	NS
	15–30 kDa	***	***	***	NS	***	NS
	8–15 kDa	***	***	***	NS	***	NS

[a] Protein level refers to the target protein content to which the solutions are formulated; *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$, NS indicates no significant difference.

Table 3

Whey protein beverage colour, expressed as L*, a*, b* values for protein beverages containing 4%, 6%, or 8% total protein, before and after direct steam infusion and indirect tubular heat treatment. [a]

Solutions	Heat treatment	L*	a*	b*
4% Protein	Unheated	$39.3^{c} \pm 1.21$	$-0.65^{a} \pm 0.09$	$2.38^{a} \pm 0.35$
	Direct ESL	$64.2^{b} \pm 1.35$	$-1.46^{b} \pm 0.29$	$-5.14^{b} \pm 0.85$
	Direct UHT	$66.3^{ab} \pm 1.92$	$-1.85^{b} \pm 0.12$	$-5.27^{b} \pm 0.45$
	Indirect ESL	$68.8^{a} \pm 0.92$	$-2.30^{c} \pm 0.01$	$-6.60^{b} \pm 0.23$
	Indirect UHT	$66.5^{ab} \pm 0.80$	$-2.34^{c} \pm 0.02$	$-8.33^{c} \pm 0.47$
6% Protein	Unheated	$32.6^{d} \pm 0.82$	$-0.13^{a} \pm 0.03$	$0.76^{a} \pm 0.42$
	Direct ESL	$67.8^{a} \pm 1.30$	$-1.82^{cd} \pm 0.18$	$-5.15^{b} \pm 1.09$
	Direct UHT	$63.7^{b} \pm 2.02$	$-1.47^{c} \pm 0.23$	$-4.27^{b} \pm 0.70$
	Indirect ESL	$60.2^{b} \pm 0.77$	$-2.02^{d} \pm 0.02$	$-8.45^{c} \pm 0.21$
	Indirect UHT	$46.7^{c} \pm 0.22$	$-0.73^{b} \pm 0.04$	$-10.9^{d} \pm 0.09$
8% Protein	Unheated	$36.6^{b} \pm 0.41$	$-0.23^{a} \pm 0.07$	$2.81^{a} \pm 0.24$
	Direct ESL	$60.2^{a} \pm 1.86$	$-1.79^{b} \pm 0.11$	$-6.83^{c} \pm 0.74$
	Direct UHT	$63.6^{a} \pm 3.85$	$-1.69^{b} \pm 0.45$	$-3.09^{b} \pm 1.57$
	Indirect ESL	$41.5^{b} \pm 0.71$	$-0.32^{a} \pm 0.19$	$-7.21^{c} \pm 0.49$
	Indirect UHT	$38.1^{b} \pm 0.37$	$0.35^{a} \pm 0.08$	$-6.20^{c} \pm 0.26$

[a] For each beverage solution (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$). ESL relates to a 70 °C preheat temperature and 121 °C final heat temperature; UHT relates to a 80 °C preheat temperature and 135 °C final heat temperature.

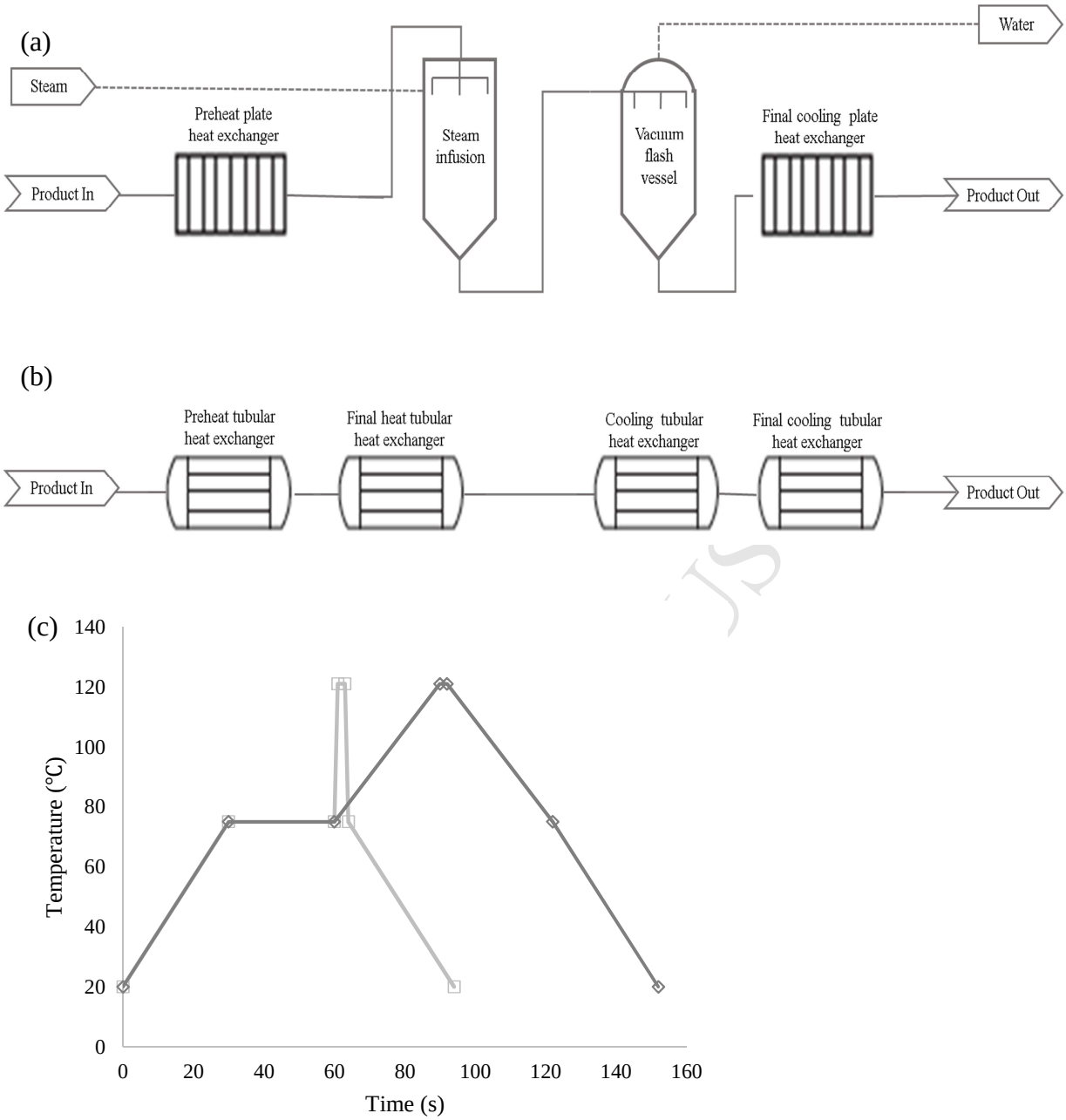
(a)
Steam
Water
Preheat plate heat exchanger
Steam infusion
Vacuum flash vessel
Final cooling plate heat exchanger
Product In
Product Out
(b)
Preheat tubular heat exchanger
Final heat tubular heat exchanger
Cooling tubular heat exchanger
Final cooling tubular heat exchanger
Product In
Product Out
(c)
Temperature (°C)
0
20
40
60
80
100
120
140
Time (s)
0
20
40
60
80
100
120
140
160

Fig 1.

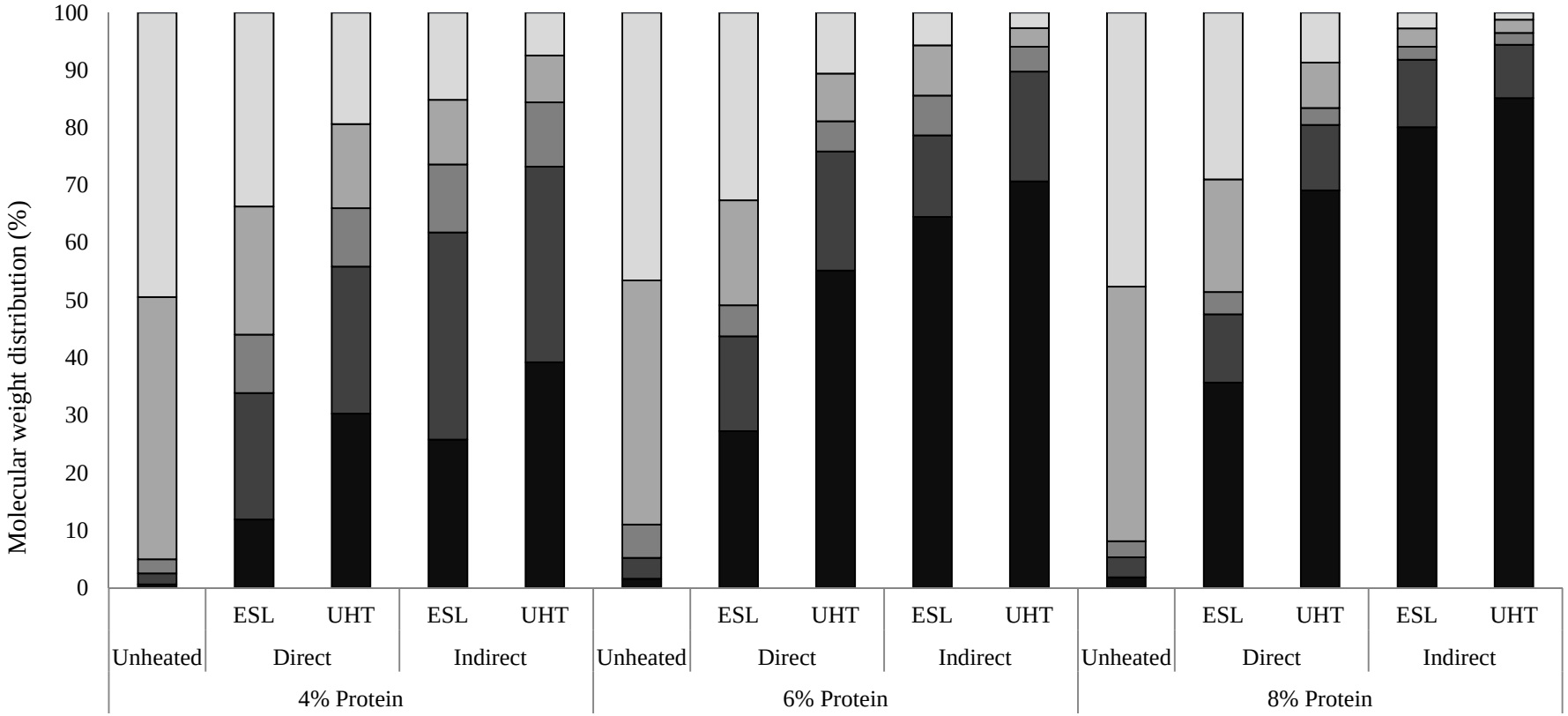

Fig 2.

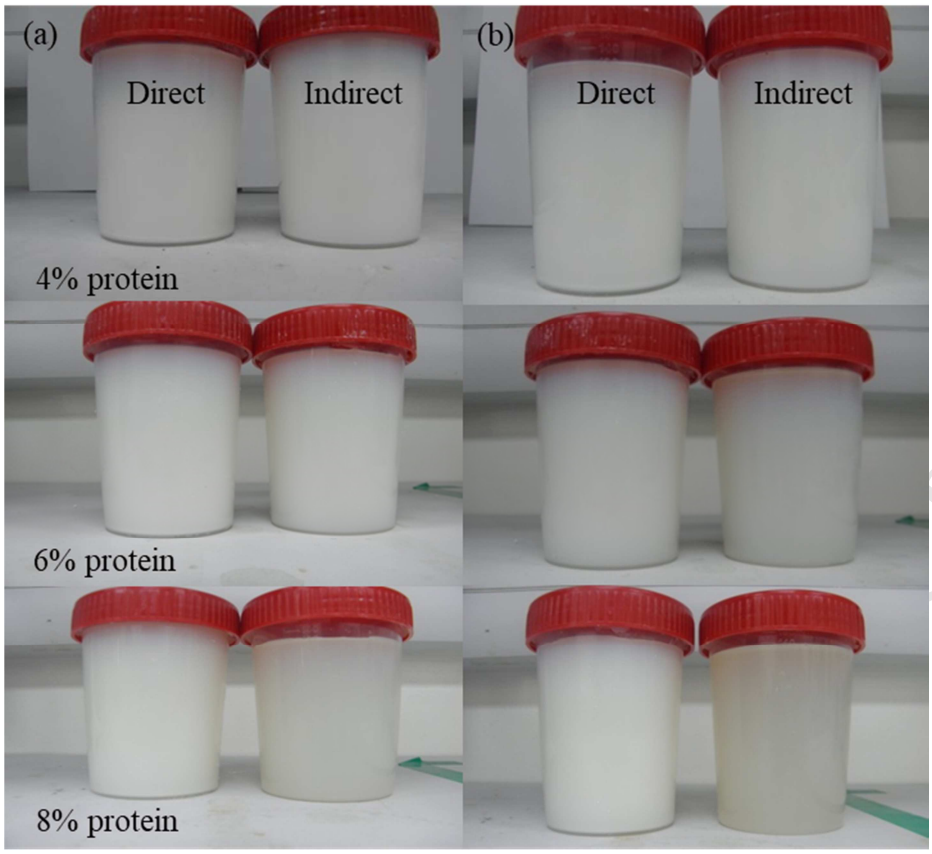

Fig 3.

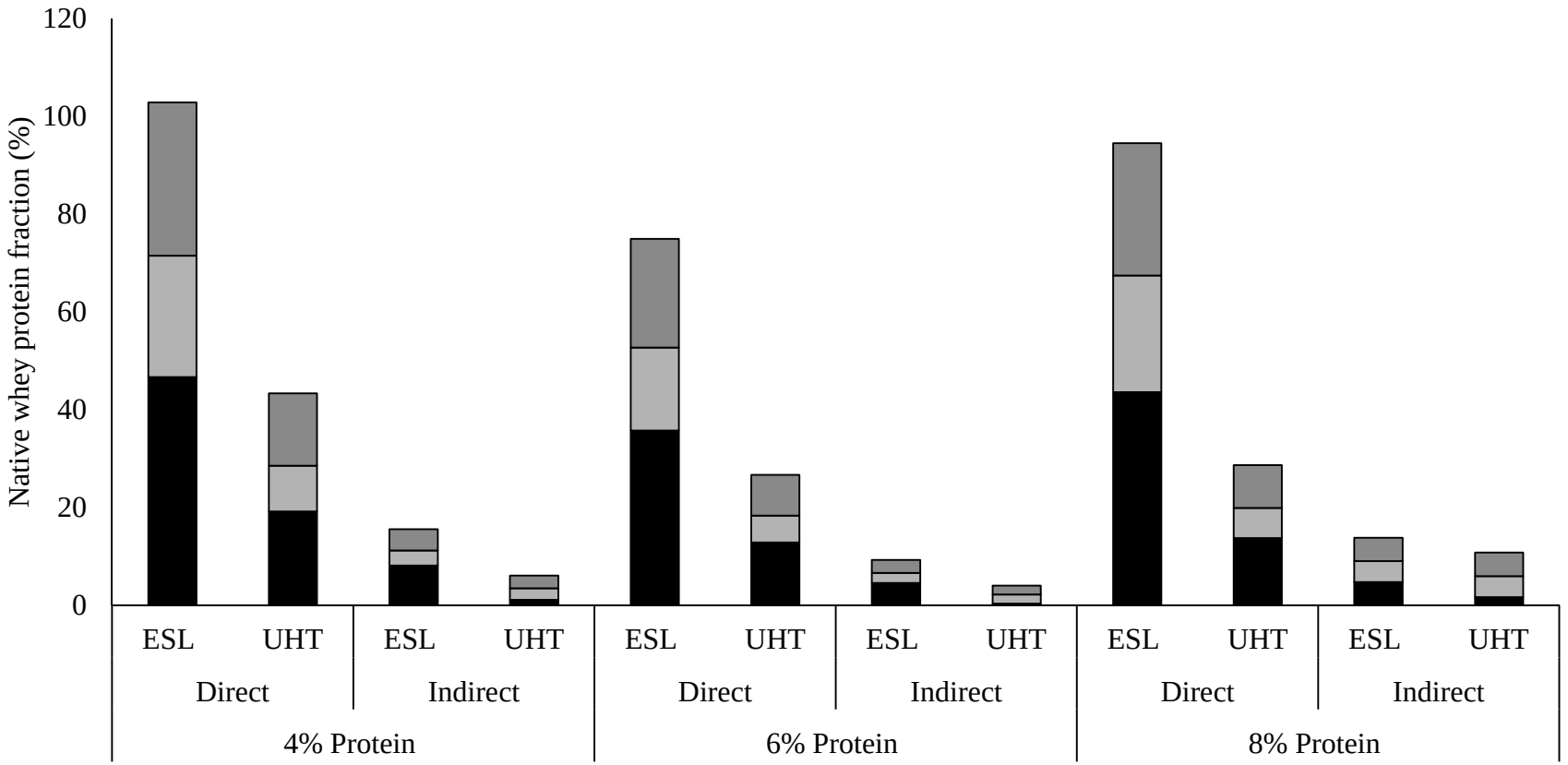

Fig 4.

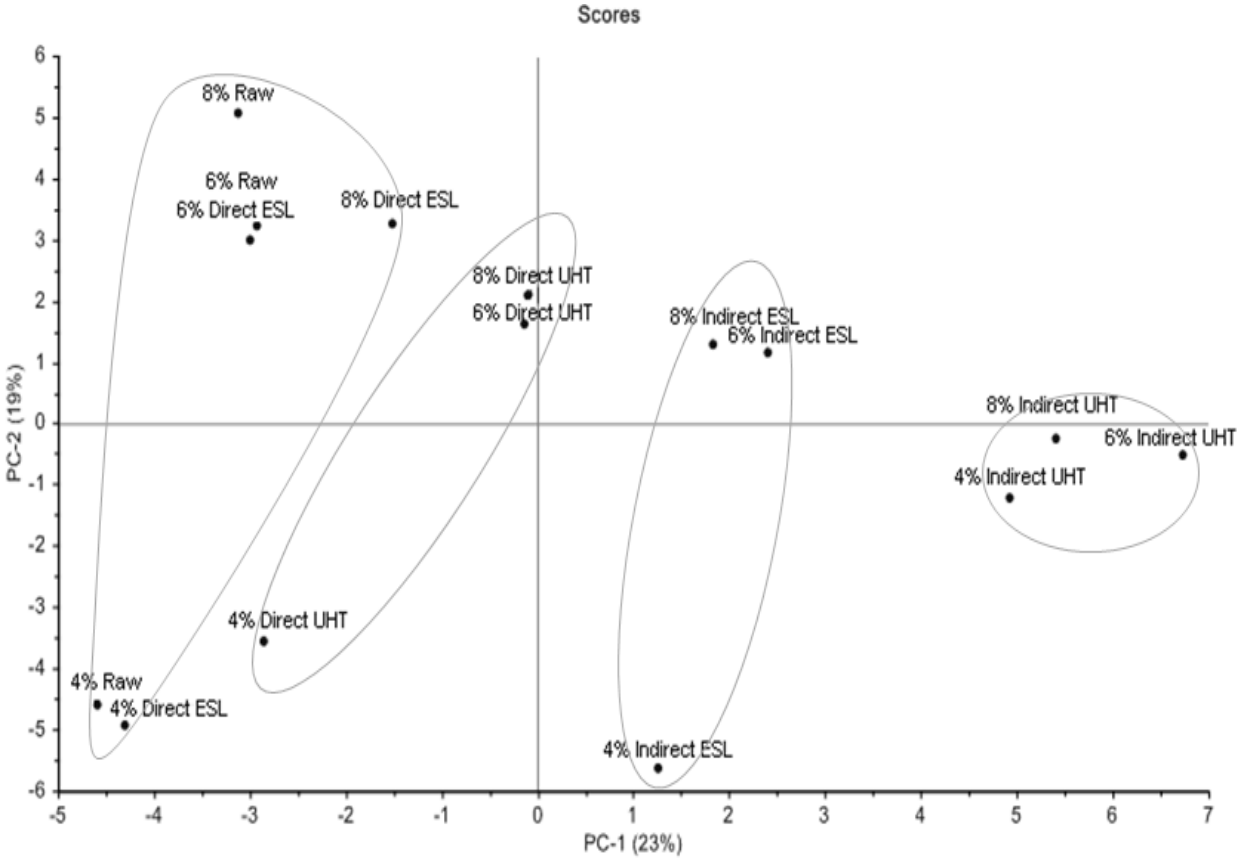

Fig 5.