



Impact of storage conditions on solubility, heat stability and emulsifying properties of selected spray dried whey protein concentrates

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ABSTRACT

Storage conditions and composition of whey protein concentrates (WPCs) may destabilize the whey proteins (WPs) thus affect their functionality. Functional characteristics of native, sweet, acid and salty-WPC powders were analyzed after storage at 25 °C and RH (22, 33%) for 90 days. Native-, sweet- and acid-WPCs exhibited high solubility (82–97%), which was largely retained during storage. Solubility of salty-WPC was 52–55% initially, which gradually increased by ~5% during storage. Ionic sodium in salty-WPC interlinked WPs through salt bridges and charge screening, exposing reactive sites for intensive aggregation. Heat stability of salty-WPC was highest (64s), while lowest was recorded for native-WPC (16s). In presence of ionic sodium in salty-WPC, WPs denature due to salt bridging, charge screening and osmotic effects leading to intensive aggregation. High emulsion activity was recorded for salty-WPC, while those for other WPCs were similar. Emulsion stability varied as native- > acid- > salty- > sweet-WPC. High number of hydrophobic segments was likely exposed on the protein surface in salty-WPCs due to sodium-induced WP denaturation, thus increasing the number of proteins absorbed to emulsion interface, enhancing emulsion activity. Functionality of different WPCs during storage predominantly depended on the inherited composition.

1. Introduction

Whey proteins are widely used as ingredients in many food systems due to their unique nutritional qualities and functional characteristics (De Wit, 1990). Whey protein concentrates (WPC) and isolates (WPI) are mainly manufactured from sweet whey streams, released during the production of rennet type hard cheeses. These protein rich products are a popular choice of an ingredient in many products, such as energy supplements, nutritional drinks, protein bars, desserts, confectionaries and infant formula (Písecký, 2005). High protein and low lactose contents and the presence of fat and minerals at defined ratios provide a unique functional behavior, which can also be varied based on the solution characteristics such as temperature, ionic strength, pH and other compositional characteristics. Similar to sweet whey, acid and salty whey streams are generated in high volumes mainly due to increasing demand for strained yoghurts, cream and salted cheeses. Our previous study has shown that the WPCs produced out of these two whey streams could be excellent food ingredients due to their distinct protein characteristics and surface composition (Nishanthi, Chandrapala, & Vasiljevic, 2017b). Therefore, it would be desirable to study their functional characteristics to assess their applicability in food systems.

Solubility of WPCs is primarily important as it affects the applicability of whey proteins in foam, emulsion and gel systems. Whey proteins are globular in their native form with protein surfaces covered with hydrophilic residues thus are highly soluble over a broad range of pH (Zhu & Damodaran, 1994). However, their denaturation under varying environmental conditions including pH (Pelegrine & Gasparetto, 2005), temperature (Dissanayake & Vasiljevic, 2009), ionic strength, protein concentration (Dissanayake, Ramchandran, Donkor, & Vasiljevic, 2013a) and other compositional constituents including lactose, fat and organic acids (Dissanayake, Ramchandran, Piyadasa, & Vasiljevic, 2013b) expose hydrophobic and thiol groups, which were previously buried in the interior of the molecules, allowing for hydrophobic interactions, disulphide bonds and thiol/disulphide interchange reactions. This in turn results in poor rehydration but excellent heat stability (Dissanayake & Vasiljevic, 2009).

Whey proteins are also effective surface-active agents since they can lower interfacial tensions between hydrophobic and hydrophilic components in foods. Thus, WPC are widely used in the formation of oil-in-water and water-in-oil emulsions (McClements, 2009), due to stabilizing effect resulting from the formation of a protective barrier around the fat droplets, preventing emulsion coalescence. Emulsifying

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properties of whey proteins highly depend on the pH of the solution, as it determines the protein charge, solubility, flexibility and aggregation behavior (Das & Kinsella, 1989). At pH above isoelectric point (pH > 5), β -lactoglobulin (β -LG), the major whey protein, has high flexibility thus provides high stability against flocculation. It was suggested that for whey protein stabilized emulsions, conformational flexibility is more important than increased surface hydrophobicity (Shimizu, Kamiya, & Yamauchi, 1981). Heating (Demetriades, Coupland, & McClements, 1997b), ionic strength (Demetriades et al., 1997b), presence of sucrose (Kulmyrzaev, Bryant, & McClements, 2000), sodium ions (Demetriades, Coupland, & McClements, 1997a) or calcium ions (Ye & Singh, 2000) have been reported as factors that influence flocculation, thus stability of emulsions.

In addition to the environmental factors, storage of WPCs also alters the structure and interactions of whey proteins (Nishanthi, Chandrapala, & Vasiljevic, 2017c), thus affecting their functionality. For instance, storage of two WPCs with different protein concentrations (WPC34 and WPC80) revealed that change of solubility during 18 months of storage at 25, 30 and 35 °C and under 70 and 90% relative humidity (RH) was influenced by their protein concentration (Tunick et al., 2016). In the absence of compositional variations, storage time, temperature and RH influence the emulsification properties of WPC powders. For instance, Hsu and Fennema (1989) found that the storage temperature and humidity should not exceed 20 °C and 22%, respectively in order to largely retain the emulsification properties of WPC after 6 months of storage. Thus, storage conditions and composition of WPCs are important parameters that can influence combined functionality of whey proteins during storage (Norwood et al., 2017; Tunick et al., 2016).

Our previous studies on salty and acid whey streams revealed that their unique compositional features such as high contents of lactic acid and calcium (Ca) salts in acid whey and high content of sodium (Na) salts in salty whey impact whey proteins present in these whey streams and their concentrates by altering their structural characteristics and types of interactions, which are otherwise absent in native or sweet whey (Nishanthi, Vasiljevic, & Chandrapala, 2017a; Nishanthi et al., 2017b). In combination with compositional characteristics, storage conditions affected the protein conformation, their reactivity (Nishanthi et al., 2017c) and physical characteristics of the stored WPCs (Nishanthi, Chandrapala, & Vasiljevic, 2018). Storage-induced structural changes in proteins and other compositional constituents may alter the functionality of these WPCs. Thus, it is important to study the functional characteristics of these WPCs and their variation under different storage conditions. Therefore, the aim of the current study was to evaluate the solubility, heat stability and emulsifying property of sweet-, acid- and salty-WPC powders subjected to storage at 25 °C and two different relative humidities (RH) (22 and 33%) over a storage period of 90 days. Native-WPC was used as the reference.

2. Materials and methods

2.1. WPC production and storage

Four WPCs (native-WPC, sweet-WPC, acid-WPC and salty-WPC) were produced as described previously (Nishanthi et al., 2017b). The average composition of four WPCs on dry matter basis was: native-WPC – 841 g/kg protein and 40 g/kg lactose; sweet-WPC – 618 g/kg protein and 29 g/kg lactose; acid-WPC – 670 g/kg protein, 240 g/kg lactose, 70 g/kg lactic acid and 50 g/kg calcium (Ca); salty-WPC – 109 g/kg protein, 270 g/kg lactose and 120 g/kg sodium (Na). WPCs were dehydrated initially by placing them in vacuum desiccators over phosphorous pentoxide at room temperature (25 °C) for 4 weeks.

WPCs were stored at 25 °C and under two equilibrium relative humidities (RH; 22 and 33%) using saturated salt slurries containing potassium acetate (22 ± 2%) or magnesium chloride (33 ± 1%) respectively as described by Nishanthi et al. (2017c). Sampling was

conducted at 3 time points representing 0, 30 and 90 days of storage. The first sampling (0 day) was conducted after allowing for a 2-week equilibration period from the initial date of the storage trial after pre-testing for the water activity. After opening desiccators at a required time point, the analysis was carried out within 3 days.

2.2. Preparation of 50 g/kg protein dispersions from WPCs

Protein dispersions containing 50 g/kg of proteins were prepared by mixing the required amount of WPC powder in Milli-Q water at room temperature. The dispersions were stirred for about 2 h using a magnetic stirrer and stored in the refrigerator for overnight to ensure complete hydration. The pH of the whey protein dispersions did not change substantially throughout the storage trial. Thus, the average pH values for native-WPC, sweet-WPC, acid-WPC and salty-WPC were 6.5, 6.4, 4.2 and 5.5, respectively.

2.3. Solubility

Protein solubility was determined using a method developed by Morr et al. (1985). Ten mL of the prepared protein dispersion (50 g/kg of protein) was centrifuged (Avanti J-26XP centrifuge, Beckman Instrument Australia Pty Ltd., Gladesville, NSW, Australia) at 20,000 × g for 20 min at 20 °C. The supernatant was then filtered using a Whatman No. 1 filter paper. The protein content of the supernatant and the original dispersion was estimated using the Kjeldhal method (AOAC, 2000) and the nitrogen conversion factor of 6.38. Solubility was given by the protein content of the supernatant expressed as a percentage of the total protein content in the original dispersion.

2.4. Heat coagulation time (HCT)

Exactly 3 mL aliquots of 50 g/kg protein dispersions were placed in glass tubes (10 mm diameter and 75 mm length), sealed and heated at 140 °C in a shaking oil bath (Ratek, Boronia, Australia). The heat exposure time until the first appearance of visible protein aggregates was considered as the HCT (Dissanayake, Liyanaarachchi, & Vasiljevic, 2012).

2.5. Emulsification

The method described by Dissanayake et al. (2012) based on Pearce and Kinsella (1978) was used to determine the emulsifying activity index (EAI) and emulsion stability index (ESI) to estimate the emulsification capacity and the stability of the emulsions formed of the dispersions prepared from WPCs, respectively. Approximately 8 mL of canola oil and 24 mL of 50 g/kg protein dispersions were mixed and held in a water bath heated to 40 °C for 20 min. The mixture was then homogenized using a B25 lab high shear mixer (Xingsheng Light Industry Machinery, Wenzhou, China) at 10,000 rpm for 1–2 min. Aliquots were obtained from the center of the emulsion samples for further analysis.

2.5.1. Emulsion activity index (EAI)

EAI was calculated as

$$EAI = \frac{2T}{\phi C} \quad (1)$$

Where,

T – turbidity of the emulsion

ϕ – oil volume fraction

C – weight of proteins per unit volume of aqueous phase prior to the formation of the emulsion

Oil volume fraction (ϕ) was calculated using equation (2), based on

the method described by Pearce and Kinsella (1978). Exactly 3 mL aliquots of the emulsions were dried in an oven at 120 °C overnight till a constant weight was obtained.

$$\varphi = \frac{C - A - E(B - C)}{C - A + (B - C) \left[\frac{(1 + E) \cdot D_0}{D_s - E} \right]} \quad (2)$$

Where.

A – weight of empty pan

B – weight of the pan with the emulsion

C – weight of the pan with the dried emulsion

E – concentration of proteins in the dispersion (mass per unit mass of solvent)

D_0 – Density of the oil

D_s – Density of the protein dispersions

Turbidity (T) of the emulsion was determined using equation (3) (Pearce & Kinsella, 1978).

$$\text{Turbidity } (T) = 2.303 \frac{A}{l} \quad (3)$$

Where.

A – absorbance of the diluted emulsion

l – path length of the cuvette

2.5.2. Emulsion stability index (ESI)

Five mL of emulsion was refrigerated at 4 °C for 24 h. Absorbance of the diluted emulsions with 1 g/kg SDS was measured at 500 nm (Pearce & Kinsella, 1978). Turbidity prior (T) and after refrigeration was calculated using equation (2) and the change of turbidity (ΔT) was calculated with in the time interval (Δt) of 24 h.

$$ESI = \frac{T \times \Delta t}{\Delta T} \quad (4)$$

2.5.3. Absorbed protein on the emulsion interface

The method described by Dissanayake et al. (2012) was used with minor modifications to estimate the content of adsorbed protein. The emulsions were centrifuged at 20 °C for 30 min at 12,000 × g (Avanti J-26XP centrifuge, Beckman Instrument Australia Pty Ltd., Gladesville, NSW, Australia). The Kjeldahl method with the nitrogen conversion factor of 6.38 was used to estimate the protein content of the liquid layer. Adsorbed protein was calculated as follows,

$$\text{Absorbed protein} \left(\frac{\text{mg}}{\text{ml}} \right) = \text{Protein in dispersion} \left(\frac{\text{mg}}{\text{ml}} \right) - \text{Protein in aqueous layer} \left(\frac{\text{mg}}{\text{ml}} \right) \quad (5)$$

2.6. Statistical analysis

The experiment was organized in a randomized split plot blocked design with 2 main factors - a type of WPC as the main plot and RH as the subplot. The replications served as blocks. Replicated samples were sub sampled and analyzed. The results were statistically analyzed using the General Linear Model (GLM) and ANOVA procedure. The level of significance was preset at $p < 0.05$.

3. Results

3.1. Solubility of WPCs

Solubility of WPCs was significantly ($p < 0.05$) affected by the type of WPC and the humidity during storage (Table 1), while storage time

Table 1

Solubility (%) and heat coagulation time (HCT) (s) of whey protein concentrates (WPCs) stored at 25 °C and relative humidity of 22 or 33% for a storage period of 90-days.

Sample	Humidity (%)	Storage period (days)	Solubility (%)	HCT (s)
Acid-WPC	22	0	91.2 ^a	69 ^a
		30	90.1 ^{ah}	53 ^b
		90	88.9 ^{ahl}	37 ^c
	33	0	94.0 ^b	81 ^d
		30	90.2 ^{ah}	52 ^b
		90	89.6 ^{ahl}	51 ^b
Native-WPC	22	0	97.2 ^c	55 ^b
		30	93.2 ^a	20 ^{ef}
		90	90.2 ^{ah}	16 ^e
	33	0	92.6 ^a	43 ^c
		30	92.0 ^a	22 ^f
		90	90.2 ^{ah}	16 ^e
Salty-WPC	22	0	55.7 ^d	98 ^g
		30	58.6 ^e	72 ^a
		90	61.5 ^f	54 ^b
	33	0	52.3 ^g	92 ^h
		30	55.2 ^d	88 ^h
		90	57.7 ^{de}	73 ^a
Sweet-WPC	22	0	91.2 ^a	53 ^b
		30	87.9 ^{hk}	32 ^{ci}
		90	85.0 ⁱ	28 ⁱ
	33	0	81.7 ^j	54 ^b
		30	85.4 ^{ik}	27 ^{fi}
		90	86.8 ^{ikl}	27 ^{fi}
SEM			0.9	2

^{a-l} Different lowercase superscripts in the same column depicts the significant differences between means of solubility and heat coagulation time (HCT). Results are expressed as means of two independent replications. SEM = Pooled standard error of the mean.

had no major impact. At the beginning of the storage, native-WPC showed the highest solubility among all four WPCs, while salty-WPC had a significantly ($p < 0.05$) low protein solubility under both RH levels. During 90 days of storage, solubility of native-, sweet- and acid-WPCs did not change significantly. Solubility of native-, sweet- and salty-WPCs was high under 22%, as compared to 33% RH. On the contrary, acid-WPC had a greater solubility under 33 than that at 22% RH.

3.2. Heat stability of WPCs

Heat stability of WPCs was measured as the heat coagulation time (HCT) and presented in Table 1. Storage time, RH and the composition of WPC significantly ($p < 0.05$) influenced the HCT. At all times, under any RH level, HCT varied in the order of salty- > acid- > sweet- > native-WPC. In general, HCT declined throughout the storage irrespective of RH. Heat stability of native-WPC declined greatly (71 and 62% under 22 and 33%, respectively), while that of salty-WPC declined to a lesser extent (45 and 20% under 22 and 33%, respectively).

3.3. Emulsification properties of WPCs

The type of WPC and the storage time significantly ($p < 0.05$) influenced the emulsion activity index (EAI), while relative humidity had only a minor influence (Table 1). Salty-WPC showed significant effect on EAI with storage time, while other WPCs had no significant effect. EAI of native-, sweet- and acid-WPCs were approximately similar at the start of the storage. Under all storage conditions, EAI decreased over 90 days with averages of 1241, 783, 886 and 7671 m²/g for native-, sweet-, acid- and salty-WPCs, respectively.

Emulsion stability index (ESI) of WPCs stored at 25 °C and RH levels of 22 and 33% for 90 days are shown in Table 1. ESI of WPC was significantly ($p < 0.05$) influenced by all experimental factors including storage time, humidity and the type of WPC. In all WPCs, ESI observed at the beginning of the storage was significantly ($p < 0.05$) greater

Table 2

Emulsion activity index (EAI), emulsion stability index (ESI) and adsorbed proteins (mg/mL) of emulsions prepared from whey protein concentrates (WPCs) stored at 25 °C and relative humidity of 22 or 33% for a storage period of 90-days.

Sample	Relative humidity (%)	Storage period (days)	EAI (m ² /g)	ESI (h)	Absorbed protein (mg/mL)
Native-WPC	22	0	2336 ^a	98 ^d	4 ^a
		30	1709 ^a	44 ^{bf}	5 ^a
		90	1185 ^a	35 ^b	10 ^b
	33	0	2325 ^a	233 ^c	4 ^a
		30	1179 ^a	67 ^{af}	4 ^a
		90	994 ^a	37 ^{bi}	5 ^a
Sweet-WPC	22	0	2523 ^a	55 ^{fijm}	5 ^a
		30	2332 ^a	43 ^{bj}	6 ^a
		90	1742 ^a	40 ^{bj}	17 ^e
	33	0	2983 ^a	140 ^k	5 ^a
		30	2867 ^a	50 ^{bjl}	6 ^a
		90	2198 ^a	46 ^{bm}	16 ^{ef}
Acid-WPC	22	0	2483 ^a	76 ^a	3 ^a
		30	1713 ^a	50 ^{bf}	4 ^a
		90	1469 ^a	41 ^{bf}	11 ^b
	33	0	2278 ^a	213 ^c	4 ^a
		30	1707 ^a	45 ^{bf}	5 ^a
		90	1519 ^a	39 ^{bf}	11 ^{bf}
Salty-WPC	22	0	18789 ^b	75 ^{ai}	3 ^a
		30	15815 ^{cf}	65 ^{ahilm}	3 ^a
		90	11787 ^d	44 ^{bj}	42 ^c
	33	0	16545 ^{ec}	172 ^g	2 ^a
		30	14412 ^f	81 ^{da}	2 ^a
		90	8204 ^g	46 ^{bhj}	34 ^d
SEM			678	7	2

^{a-m} Different lowercase superscripts in the same column depicts the significant differences between means of emulsion activity index, emulsion stability index and adsorbed proteins. Results are expressed as means of two independent replications. SEM = Pooled standard error of the mean.

under 33% RH, than that under 22% RH. ESI varied as native- > acid- > salty- > sweet-WPC at the beginning of storage, irrespective of the RH conditions. ESI declined with increase in storage time under both RH levels.

Amount of adsorbed proteins (mg/mL) at the interface in the emulsions prepared from each WPC stored under 22% and 33% RH at 25 °C is shown in Table 2. Storage time and the type of WPC significantly ($p < 0.05$) influenced the variation of the amount of adsorbed proteins. At the beginning of the storage, the amount of adsorbed proteins was approximately similar for all WPCs. However, it increased under all storage conditions by 5.8, 11.8, 7.6 and 35.4 mg/mL in native, sweet, acid and salty-WPC, respectively, during 90 days. By the end of the storage, salty-WPC showed significantly high levels of adsorbed proteins, followed by sweet- and acid-WPC, while native-WPC showed the least.

4. Discussion

4.1. Solubility

Results of the current study highlight how compositional variations of WPCs influenced their solubility during storage. In this case, the relative humidity of storage exhibited a significant impact on the solubility while storage time showed very minor influence. In addition, native-, sweet- and acid-WPCs exhibited a good solubility immediately after manufacturing, which was largely retained for the 90 days storage period at 25 °C. On contrary, salty-WPC was the least soluble. Greater coverage of particle surface with the native-form of proteins (Nishanthi et al., 2017c) allow greater extent of protein-water interactions, thus resulting in a high solubility of native-WPC. Proteins in salty-WPC are subjected to charge screening in the presence of high concentration of positively charged sodium ions (Zhu & Damodaran, 1994). In addition,

the high osmotic pressure surrounding the proteins exerted in presence of high concentrations of sodium ions unfolds the tertiary structures, exposing reactive sites, such as free thiol groups and hydrophobic segments for protein-protein interactions (Nishanthi et al., 2017b). Aggregated proteins have unevenly distributed charge on the protein surface with comparatively less hydrophilic segments, thus limiting the number of protein-water interactions. Furthermore, the surface of the salty-WPCs is primarily covered with fat (~40% of surface area) rendering the particle surface more hydrophobic (Nishanthi et al., 2017c), hence promoting particle agglomeration and reducing solubility. Opposing effect can be expected in presence of high amount of minerals on the surface of salty-WPCs (Nishanthi et al., 2017c), which would enhance hydrophilic interactions with water, and thereby increase solubility. However, the results of the current study suggest that protein denaturation, subsequent aggregation and high surface fat coverage govern the solubility of salty-WPC. Unlike the other WPCs, solubility of salty-WPC increased over the storage period. The increased amounts of lactose and minerals on the surface and decreased surface fat of salty-WPCs (Nishanthi et al., 2017c) increased the solubility during 90 days of storage, irrespective of RH. The exposure of more hydrophilic segments on the particle surface forms a saturated monolayer surrounding the particle surface with the increased absorption of water (Kelly, O'Mahony, Kelly, & O'Callaghan, 2016). The solidified salts on the particle surface dissolve, which limits their osmotic impact on the proteins. This in turn lowers the extent of protein aggregation and thereby increases the protein solubility. The relative humidity influenced the solubility of WPCs in a complex manner, as indicated by increased solubility for native, sweet and salty WPCs and a decline in solubility for acid-WPC under 22% RH as compared to 33%. Compositionally, acid-WPC consists of high concentrations of calcium based lactate salts and lactic acid. Due to presence of lactic acid, acid WPC had a pH of 4.2. Due to the low dissociation constant of lactic acid, deprotonation takes place readily. This in turn lowers the negative surface charge density of whey proteins, thus inducing protein aggregation through non-covalent interactions such as van der Waals and electrostatic attractions (Dissanayake et al., 2013b). Ca also promotes interlinking of proteins through formation of ionic bridges and shielding of repulsion (Zhu & Damodaran, 1994). In addition, Ca binds to strong binding sites of whey proteins and induces conformational changes. These conformational changes expose the hydrophobic segments on the protein surface, facilitating the protein-protein interactions and aggregation (Zhu & Damodaran, 1994). Thus, low relative humidity increases the impact of Ca and lactic acid resulting in a decreased solubility.

4.2. Heat stability

Heat stability is an important functional property, which demonstrates the ability of whey proteins to endure commercial heat processing, without subjecting them to detrimental physical changes such as precipitation, turbidity, gelation, increments in viscosity and phase separation. Heat stability of WPC depends on its composition, mainly whey protein profile and non-protein components such as sugars, salts and fat. Among two main whey proteins, thermal stability of α -lactalbumin (α -LA) is greater than that of β -LG, mainly due to lack of free thiol groups and abundance of holo-form of α -LA, in which Ca is bound to its binding site (McSweeney & Fox, 2013). Throughout the storage, HCT varied in the following order: salty- > acid- > sweet- > native-WPC. High heat stability of salty-WPC could be mainly attributed to the presence of whey proteins in an intensively aggregated form, thus with no available reactive sites such as free thiol groups for further aggregation. Electrostatic charge screening and salt bridge formation induced by the presence of high concentrations of Na reduce the intermolecular distance between proteins allowing for covalent bonding. Similar to salty-WPC, heat destabilization of acid-WPC is due to the covalent interlinking of proteins in presence of high Ca concentration

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(Crowley et al., 2014). Furthermore, high lactic acid concentration, thus low pH, leads to a high degree of protein aggregation due to prevalent positive charge, thus creation of more non-covalent bonds such as hydrophobic, van der Waals and electrostatic interactions. Acid-WPC is comparatively high in casein, thus high number of denatured whey proteins are in association with the caseins, providing a stability against heat (Singh, 2004). In contrast, in sweet-WPC, concentration of ionic salts is low comparative to salty- and acid-WPC, thus preventing protein interlinking through covalent and non-covalent interactions. Therefore, most of the proteins are in non-aggregated, reactive state, thus less heat stable. The lowest heat stability of native-WPC is attributed to the presence of large amounts of native whey proteins due to the minimal thermal treatment during its manufacturing process (Nishanthi et al., 2017b). Undenatured whey proteins are easily susceptible to unfolding and aggregation at 140 °C. Native whey proteins present in this WPC are more prone to conformational changes exposing reactive thiol and hydrophobic sites enabling various reactions in comparison to aggregated whey proteins present in salty-WPC (Nishanthi et al., 2017b).

4.3. Emulsification properties of WPCs

Emulsion activity index (EAI), as one of two important emulsification parameters, is governed by capacity of a protein to unfold and adsorbed over the interface, stabilizing newly created area (Pearce & Kinsella, 1978). Consequently, molecular flexibility and conformational changes of proteins are extremely important, as these govern other properties such as surface hydrophobicity, protein flexibility, solubility, a degree of disulphide bonds, affinity of proteins for the oil–water interface, a level of hydrogen interactions and other stabilizing forces (Monahan, McClements, & Kinsella, 1993). Slight denaturation improves the emulsifying properties by exposing hidden hydrophobic groups, thus balancing the hydrophilic and hydrophobic interactions to minimize the surface tension (Dissanayake et al., 2012). Emulsion stability index (ESI) reflects the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing, and flocculation. Main contributory factor is the consistency of the interface, which does not change with time (Dissanayake & Vasiljevic, 2009). Emulsions with increased net negative charge exert adequate repulsion that enables dispersion of droplets and retards coalescence (Klemaszewski & Kinsella, 1991). Salty-WPC had the highest EAI, which could be attributed to the presence of high concentrations of Na ions. Sodium ions enhance charge screening, exposing the interiorly located hydrophobic segments of β -LG such as Cys₁₂₁-Val₁₂₃, Ala₈₁-Phe₈₃, Ile₇₁-Ala₇₃ and Leu₁₀₃-Phe₁₀₅. Due to greater exposure of hydrophobic amino acids, whey proteins have a considerable potential to engage in hydrophobic interactions with the oil phase and stabilize emulsions. Such exposure of previously buried hydrophobic sites is further favored by enhanced osmolality in the presence of elevated Na concentration. In addition to proteins, fat present on the surface of salty-WPC plays an important role in emulsion activity. Coverage of free fat is high on the particle surface of salty-WPC (Nishanthi et al., 2017c), which contains a hydrophobic hydrocarbon chain and hydrophilic carboxyl group, thus a high affinity to oil-water interface. Furthermore, the amount of adsorbed proteins to the oil-water interface was highest in the emulsion produced with salty-WPC at the end of storage, implying a high EAI. Similar to Na in salty-WPC, Ca in acid-WPC interacts with whey proteins through two possible mechanisms. First, Ca screens the negative charge repulsion of proteins to a greater extent, reducing the intermolecular distance, thus allowing protein aggregation via intermolecular non-covalent interactions. Second, Ca crosslinks proteins via salt bridges (Zhu & Damodaran, 1994), thus exposing reactive sites to the protein surface, consequently imparting a thermodynamically more favorable condition for a stable emulsion. Furthermore, inherent acidity of acid-WPC with lactic acid imparts a prevailing positive charge on whey protein surface, misbalancing the inter-protein electrostatic environment. This in turn

exposes more hydrophobic sites to the surface, resulting in a comparatively high EAI (Dissanayake et al., 2012). With the influence of Ca and lactic acid, comparatively high EAI is expected to occur in acid-WPC, compared to native- and sweet-WPCs, which was not apparent in the current study. Perhaps, the intensive heat treatment applied in the manufacturing of Greek yoghurt, from which this acid whey was collected for the current study, caused an excessive denaturation and aggregation, consequently decreasing the affinity between proteins and the dispersed phase (de la Fuente, Singh, & Hemar, 2002). This is confirmed by a very low amount of adsorbed proteins onto the oil-water interface in the emulsion prepared from acid-WPC. In contrast, emulsion stability of acid- or salty-WPCs was lower compared to that of native-WPC, mainly due to inherently high concentrations of Na in salty-WPC and lactic acid or Ca in acid-WPC. High concentration of Ca in acid-WPC promotes the formation of insoluble aggregates between denatured whey proteins. Denatured and aggregated whey proteins have a localized and unevenly distributed negative charge, thus have low capacity to maintain the repulsion between emulsion droplets. Similar effect can be seen in salty-WPC due to the Na-induced protein aggregation. Furthermore, Ca and Na in the dispersed medium of emulsions formed by acid- and salty-WPCs form bridges between droplets and promote flocculation, consequently destabilizing the emulsion. The salts present in the dispersed medium partially shield the electrostatic repulsive forces, thus allow droplet flocculation through hydrophobic interactions and thiol-disulphide interchange reactions of proteins present in different droplets. This explains why emulsion stability of salty-WPC was low despite of its emulsion activity being high. In contrast to salty- and acid-WPC, native-WPC formed the most stable emulsions, mainly attributed to a minimal protein denaturation occurring during its manufacturing process. As shown by the high kinetic energy and low reaction order of β -LG reactivity (Nishanthi et al., 2017c), whey proteins in native-WPCs are in their native form, with a high overall negative charge, consequently preventing close approach of droplets and stabilizing the emulsion. In native-WPC, high solubility supports formation of a stable emulsion through rapid adsorption at the interface to form a strong cohesive film. In the current study, the emulsion prepared using sweet-WPC was the least stable, most likely due to presence of high concentration of Maillard reaction products (Norwood et al., 2016). Sweet-WPC contained 29 g/kg of lactose, which is adequate enough to conjugate with β -LG and α -LA (Nishanthi et al., 2017c), consequently reducing their surface activity. When surface active proteins are insufficient to fully cover the newly formed oil-water interface, flocculation may occur gradually, disturbing the stability of the emulsion. In all WPCs, EAI and ESI declined as storage progressed, mainly due to gradual progression of whey protein aggregation during storage. As a result of denaturation and aggregation related structural changes, molecular flexibility is minimized decreasing the affinity of whey proteins for the oil-water interface, thus affecting the emulsion activity. In addition, the negative charge distribution becomes localized on the protein surface, thus affecting the emulsion stability. Exposed hydrophobic and thiol segments in all types of emulsions lead to a greater amount of proteins to be adsorbed onto the emulsion interface, thus increasing the proteins accumulation in oil-water interface.

5. Conclusion

Functionality of different WPCs varied based on their inherent composition and storage conditions. Protein conformational changes occur due to the combined effect of storage and compositional variation governing functional properties among different WPCs. Mainly the high concentration of sodium salts in salty-WPC caused whey protein denaturation and aggregation, thus leading to low solubility and high heat stability. Denatured whey proteins and high fat coverage on particle surface of salty-WPC led to high emulsification activity, but low emulsion stability, compared to the reference native-WPC. Therefore, salty-WPC could potentially be used in heat-processed emulsions such

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as beverages, processed cheese spreads and salad dressings with minor modifications to mediate its salty flavor. Furthermore, presence of lactic acid in high concentration led acid-WPC to be equally soluble to native-WPC. The protein interlinking in presence of calcium in acid-WPC, subsequently resulted in intensive aggregation, thus provide a comparatively high heat stability. For these reasons, the acid-WPC may have potential applications in infant formula, desserts and bakery products.

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