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Characterization of a Commercial Whey Protein Hydrolysate and Its Use as a Binding Agent in the Whey Protein Isolate Agglomeration Process

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Summary

Soy lecithin is a commonly used binder in agglomerating dairy powders. Due to the increase in consumer awareness on "clean label" and also to increase the shelf-life of agglomerated whey protein isolate (WPI), the demand of lecithin-free agglomerated WPI has increased. In this work, whey protein hydrolysate (WPH) was utilized as a binder to facilitate the agglomeration of WPI. The first objective was to characterize the chemical properties of three lots of WPH obtained from a commercial manufacturer. The degree of hydrolysis (DH) of WPH was 13.82–15.35% and not significantly ($P > 0.05$) different between the lots. It was observed from the high-performance liquid chromatography (HPLC) that the major whey proteins were completely hydrolyzed indicating a consistent hydrolysis between the lots. The second objective of the study was to evaluate the effectiveness of WPH as a binder in WPI wet agglomeration. After the agglomeration was performed, agglomerated WPI samples were stored at 25°C (77°F) and analyzed for moisture, water activity, relative dissolution index (RDI), and emulsifying capacity.

Moisture content (MC) of agglomerated samples was in the range of 3–15%, whereas water activity was within the range of 0.08–0.80. There was a significant ($P < 0.05$) difference in both moisture content and water activity among the treatments. Per-wet mass, flow rate, and the WPH concentration had significant ($P < 0.05$) effects on the MC. Moreover, all interactions among the main effects also had a significant ($P < 0.05$) effect on MC. High MC and water activity were observed for the treatments with higher pre-wet volume and higher flow rate and also resulted in clumping of the powders. The treatment that had 60 g of pre-wet, 20% WPH concentration, and 5.6 mL/min flow rate combination had the highest RDI among all the samples. In conclusion, WPH can be used as a potential alternative to soy lecithin in WPI wet agglomeration.

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Introduction

Whey is a co-product obtained during the manufacturing of cheese. The major proteins in whey include β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulins (Ig). Whey proteins (WPs) are highly soluble and have unique physiochemical characteristics that influence their functionality in food applications such as gelation, emulsification, and foaming. This makes WP a beneficial ingredient in various food products. However, some approaches are performed to modify WPs' functional and physiochemical properties such as chemical, physical, and enzymatic treatments. The most recent research on protein modification includes Maillard conjugation; physical modification such as high-pressure treatment and heat-induced polymerization (thermal treatment); and enzymatic modifications. Enzymatic hydrolysis of WP is measured by the degree of hydrolysis, which is defined as the percentage of peptide bonds cleaved. A low DH (<10%) is sufficient for improving the physiochemical properties of WPs. Whereas a DH (>10%) is more suitable for improving the biological functions of the resultant peptides such as antimicrobial, antioxidant, antihypertensive, and immunomodulatory functions.

Enzymatic hydrolysis of WPs was extensively studied by the researchers. It was proven that hydrolysis improves WPs' digestibility and nutritional value and reduces allergenicity, which makes it a suitable ingredient in infant formula. Hydrolysis also improves the solubility and the emulsifying capacity at alkaline pH of the whey protein hydrolysate, which makes it a desirable ingredient in high-protein beverages and nutritional bars. Moreover, the hydrolysate can be used as an encapsulant for protection and delivery of bioactive components such as omega-3 oil and probiotic organisms. It was reported that the hydrolysate worked as an excellent binder in the WPI agglomeration process as it will be providing a "clean label" product, and it would increase the shelf-life of agglomerated whey protein isolate (WPI) as it makes it less susceptible to oxidation over the storage time.

Agglomeration improves the reconstitution properties of the powders due to the incorporation of air between powder particles, which makes the water penetration into these particles easier during subsequent rehydration. Therefore, the agglomerates readily disperse and dissolve quickly compared to non-agglomerated powders. In the present study, the first objective was to characterize the physical and chemical properties of three lots of a commercial WPH. Subsequently, optimizing and evaluating the effectiveness of WPH as a binder in WPI wet agglomeration was investigated as a second objective.

Experimental Procedures

Three lots of WPH and one lot of WPI were obtained from a commercial manufacturer (Glanbia Nutritionals, Twin Falls, ID). Initially, chemical and physical properties of WPH and WPI were analyzed in terms of peptide characterization, degree of hydrolysis, zeta potential, color, bulk, and tapped densities to evaluate the consistency of the enzymatic hydrolysis. After determining the similarities and differences among WPH lots, the effectiveness of using WPH as a binder in WPI wet agglomeration was evaluated. For this purpose, a 3 × 3 × 2 factorial design was conducted with pre-wet mass (60, 100, and 140 g), WPH concentration (15, 20, and 25%), and flow rate (4.0 and

5.6 mL/min) as independent variables. The other processing parameters such as the nozzle pressure, fluid bed pressure, and fluid bed temperature were set at 0.65 bar (9.43 psi), 0.45 bar (6.53 psi), and 60°C (140°F), respectively. WPI agglomeration was performed in a top-spray fluid bed granulator (Midi-Glatt, Binzen, Germany) as shown in Figure 2. All experiments were performed in triplicates using the three lots of WPH. Agglomeration was stopped when the temperature of the end powder reached 45°C (113°F). Agglomerated WPI samples were stored at 25°C (77°F) and analyzed for moisture, water activity, relative dissolution index (RDI), and emulsifying capacity.

Results and Discussion

The chemical compositions of WPI and WPH lots were provided by the manufacturer. Moisture content of the WPI sample was 3.61%, whereas WPH moisture content was within the range of 2.67–2.99%. The WPI protein content was 94.02% and WPH protein content was within the range of 91.57–92.28%. The major components of WPs (β-LG, α-LA, and BSA) were observed on the RP-HPLC chromatogram. In addition, two unidentified peaks were also observed. However, none of those peaks were detected on the WPH samples' chromatogram (Figure 1), which suggests the complete hydrolysis of these major whey proteins in the WPH lots.

The DH of WPH samples was 13.82–15.35% and it was not significantly ($P > 0.05$) different between the lots (Table 1). The mean particle size was within the range of 150.67–198.93 μm, and it was significantly different ($P < 0.05$) among the three WPH lots (Table 1). The mean particle size of WPH samples was bigger than the WPI due to the aggregation behavior of the hydrolysate. Zeta potential is an indicator of the surface charges of the particles and its stability to aggregation. The mean of zeta potential was within the range of -22.88 to -24.04 mV, and there was no significant difference ($P > 0.05$) among the WPH lots (Table 1). Zeta potential of the hydrolysate samples was higher than that of the intact WPI. This is due to the increase of net charge on protein hydrolysate.

According to the American Dairy Products Institute (2002; Elmhurst, IL), MC should not exceed 6% by wt in dry whey products. MC for all treatments was in the normal range (<6%), except in treatments 14 (140 g, 15% and 5.6 mL/min), 16 (140 g, 20% and 5.6 mL/min) and 18 (140 g, 25% and 5.6 mL/min) that contained an MC of 14.79, 7.56, and 7.41%, respectively (Table 2). In these treatments, clumps were formed, therefore monitoring the temperature of the end product was difficult, which resulted in a large variation of MC in these treatments. The increase of pre-wet mass had highly influenced the MC (Figure 3). Flow rate had also increased the MC in agglomerated WPI, whereas, WPH concentration had a slight effect on the MC in agglomerated WPI. High pre-wet mass and high flow rate in these treatments caused a collapse in the fluid bed, which can be explained by the plasticization of the entire particle surface due to increased humidity of the air inside the fluid bed. Large clumps were formed and settled in the bottom of the fluid bed chamber (Figure 3), because the shear forces acting on the particles in the fluid bed were no longer sufficient to destroy the numerous sinter bridges generated, which led to a rapidly progressing cake formation. High MC in the powder may influence the shelf life due to Maillard reaction, creating the lumps and leading to microbial growth.

Dissolution characteristics of agglomerated WPI samples were evaluated using Focused Beam Reflectance Measurement (FBRM). The mean RDI of the agglomerated WPI samples manufactured as per the experimental design was 63.20–95.57% (Table 2). Treatment 4 (60 g, 20%, 5.6 mL/min) had the highest RDI of 95.57%, followed by treatment 6 (60 g, 25%, 5.6 mL/min) and treatment 5 (60 g, 25%, 4.0 mL/min) that had an RDI of 86.56 and 84.12%, respectively. There was no significant difference ($P > 0.05$) among these treatments. Treatment 3 (60 g, 20%, 4 mL/min) had the lowest RDI of 63.20 among the resultant agglomerated samples, followed by treatment 15 (140 g, 20%, 4 mL/min), and treatment 1 (60 g, 15%, 4 mL/min) 67.98 and 69.32 %, respectively. There was also no significant difference ($P > 0.05$) among those treatments. Pre-wet mass and flow rate had a significant effect ($P < 0.05$) on RDI. The WPH concentration did not have a significant ($P > 0.05$) difference as a main factor. However, its interactions with other factors were significantly different ($P < 0.05$). The interactions; pre-wet × WPH concentration, pre-wet × flow rate, WPH concentration × flow rate, and pre-wet × WPH concentration × flow rate were all significantly ($P < 0.05$) different.

Conclusions

Whey protein hydrolysate samples had similar chemical and physical properties indicating a consistent manufacturing process. Agglomeration conditions, especially the pre-wet mass and the flow rate, have affected primarily the moisture content, water activity, and the RDI of agglomerated samples. Some of the agglomerated samples have failed to meet the industrial and ADPI standards of dry whey products. The WPH concentration might have more impact on the physical properties of the agglomerates than it does on the functional properties.

Table 1. The degree of hydrolysis, mean particle size, and zeta potential of commercial whey protein hydrolysate (WPH) and whey protein isolate (WPI) samples in the present study

Samples	Degree of hydrolysis, %	Mean particle size (μm)	Zeta potential (mV)
WPH lot 1	14.80±0.35	181.23±0.60[b]	-23.86±2.03
WPH lot 2	15.35±0.64	150.67±3.65[c]	-24.04±1.40
WPH lot 3	13.82±0.39	198.93±9.77[a]	-22.88±1.16
WPI	-	112.17±14.40	-19.59±1.58

[a-c] Means within a column with different superscripts are significantly different ($P > 0.05$).
All values are expressed as mean ± SD (n = 3).

Table 2. Moisture content (%), emulsifying capacity (g of oil/mg of protein), and relative dissolution index (%) of all agglomerated WPI treatments as per experimental design

Treatment	Pre-wet mass (g)	WPH concentration (%)	Flow rate (mL/min)	Moisture content (%)	Emulsifying capacity (g of oil/mg of protein)	Relative dissolution index (%)
1	60	15	4.0	3.36±0.15^{c}	4.66±0.29	69.324±3.79^{def}
2	60	15	5.6	5.37±1.60^{bc}	4.58±0.34	73.37±6.48^{bcdef}
3	60	20	4.0	3.35±0.52^{c}	4.59±0.29	63.20±0.94^{f}
4	60	20	5.6	4.15±0.16^{c}	4.40±0.42	95.57±4.32^{a}
5	60	25	4.0	3.48±0.52^{c}	4.40±0.52	84.12±5.02^{abc}
6	60	25	5.6	4.25±0.07^{c}	4.33±0.42	86.56±3.03^{ab}
7	100	15	4.0	4.48±0.73^{c}	4.93±0.20	81.83±3.24^{abcd}
8	100	15	5.6	5.39±0.37^{bc}	4.75±0.11	78.05±0.67^{bcdef}
9	100	20	4.0	4.26±0.41^{c}	4.80±0.13	82.83±5.19^{abcd}
10	100	20	5.6	3.77±0.25^{c}	4.82±0.20	75.25±5.55^{bcdef}
11	100	25	4.0	3.73±0.45^{c}	4.73±0.27	79.13±1.56^{bcde}
12	100	25	5.6	3.66±0.16^{c}	4.47±0.55	76.50±6.62^{bcdef}
13	140	15	4.0	3.30±0.13^{c}	4.63±0.11	74.19±4.91^{bcdef}
14	140	15	5.6	14.79±4.5^{a}	4.52±0.30	76.64±6.17^{bcdef}
15	140	20	4.0	3.51±0.06^{c}	4.75±0.29	67.98±4.58^{ef}
16	140	20	5.6	7.56±1.32^{b}	4.82±0.20	75.30±4.48^{bcdef}
17	140	25	4.0	3.56±0.1^{c}	4.76±0.25	70.64±4.18^{cdef}
18	140	25	5.6	7.41±3.46^{b}	4.71±0.39	75.37±10.42^{bcdef}

WPH = whey protein hydrolysate. WPI = whey protein isolate.
$^{a\text{-}f}$ Means within a column with different superscript are different ($P < 0.05$).
All values are expressed as mean ± SD (n = 3).

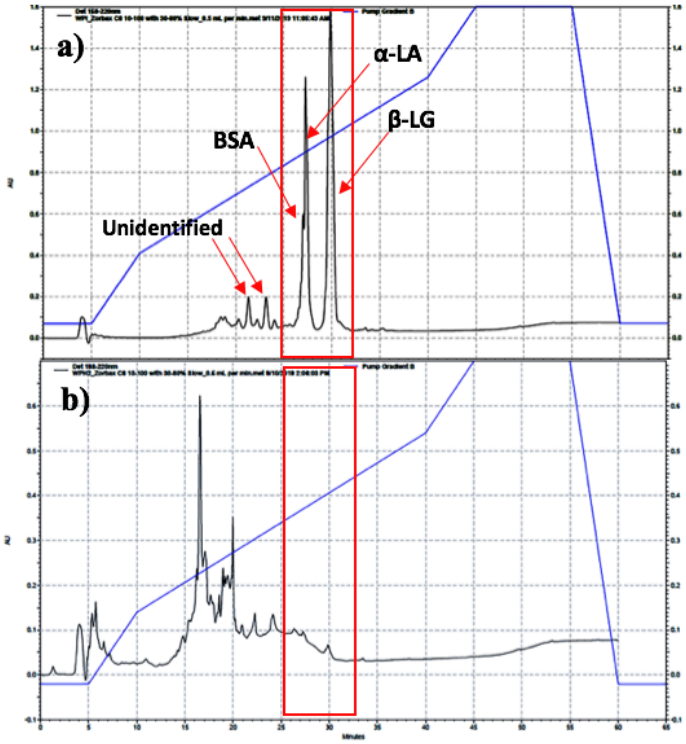

Figure 1. a) The Reversed-phase High-performance liquid chromatography (RP-HPLC) chromatogram of whey protein isolate, shows the major whey proteins before hydrolysis. b) The RP-HPLC chromatogram of whey protein hydrolysate (WPH). No peaks were observed in the times of 26.3, 27.2, and 29.9 min indicating a complete hydrolysis of the major whey proteins in WPH samples. β-LG = β-lactoglobulin. α-LA = α-lactalbumin. BSA = bovine serum albumin.

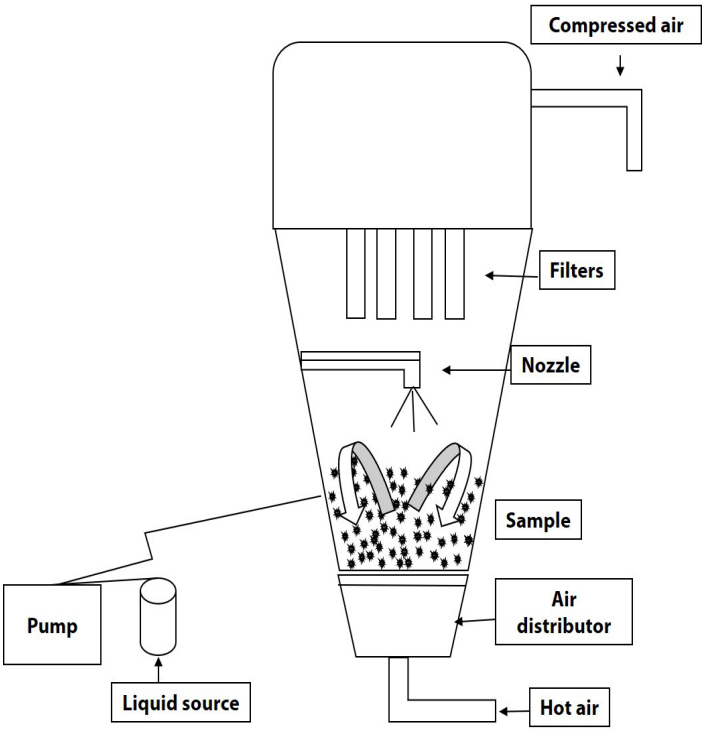

Figure 2. The top-spray fluid bed granulator (Midi-Glatt, Benzin, Germany) that was used in agglomerating whey protein isolate powder.

Figure 3. A representative image of agglomerated powder produced in treatment that had the combination of 140 g of pre-wet mass and 5.6 mL/min flow rate. The resultant powder did not meet the industrial specification of whey protein isolate.