Development of a spray-dried conjugated whey protein hydrolysate powder with entrapped probiotics

Shayanti Minj^{1,2} and Sanjeev Anand^{1,2*}

¹Midwest Dairy Foods Research Center, St. Paul, MN 55108-6074

²Dairy and Food Science Department, South Dakota State University, Brookings 57007

ABSTRACT

Bifidobacterium animalis ssp. *lactis* ATCC27536 and *Lactobacillus acidophilus* ATCC4356 were encapsulated in a conjugated whey protein hydrolysate (WPH10) through spray drying. Probiotic cultures were added at the ratio of 1:1 into the conjugated WPH10 solution at a spiking level of about 10 log₁₀ cfu/mL. The mixture was spray dried in a Niro drier with inlet and outlet temperatures of 200°C and 90°C, respectively. The final dried product was determined for cell viability and further stored for 16 wk at 25°, 4°, and –18°C to monitor viability and functionality. Micro images showed the presence of link bridges in non-conjugated WPH10, whereas, in the case of conjugated WPH10, round particles with pores were observed. The mean probiotic counts before and after spray drying were 10.59 log₁₀ cfu/mL and 8.98 log₁₀ cfu/g, respectively, indicating good retention of viability after spray drying. The solubility and wetting time of the WPH10-maltodextrin (MD) encapsulated probiotic powder were 91.03% and 47 min, whereas for WPH10, the solubility and wetting time were 82.03% and 53 min, respectively. At the end of storage period, the counts were 7.18 log₁₀ cfu/g at 4°C and 7.87 log₁₀ cfu/g at –18°C, whereas at 25°C the counts were significantly reduced, to 3.97 log₁₀ cfu/g. The solubility of WPH-MD powder was 82.36%, 83.1%, and 81.19% at –18°C, 4°C, and 25°C, respectively, and wetting times were 61 min, 60 min, and 63 min at –18°C, 4°C, and 25°C, respectively. By contrast, for WPH10 powder, the solubility significantly reduced to 69.41%, 69.97%, and 68.99% at –18°C, 4°C, and 25°C, and wetting times increased to 71 min, 70 min, and 72 min at –18°C, 4°C, and 25°C, respectively. The conjugated WPH10 is thus demonstrated as a promising carrier for probiotics and can be further used as an ingredient for developing functional foods, to har-

ness their enhanced functionality and health benefits derived from both WPH and probiotics.

Key words: probiotics, conjugated, whey protein hydrolysate, encapsulated, functionality

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Culligan et al., 2009). The 2 most common genera of probiotic microorganisms are *Bifidobacterium* spp. and *Lactobacillus* spp. (de Araújo Etchepare et al., 2020). To acquire beneficial effects, it is recommended that, at the time of consumption, the foods containing probiotics should contain 10⁶ to 10⁷ live cells per milliliter or gram of the product (Tripathi and Giri, 2014). However, several technological factors, including temperature, pH, moisture, nutrient depletion, and osmotic and oxidative stress, pose considerable challenges in incorporation of these bacteria in different food matrices (Wilkinson, 2018). In addition, resistance to gastrointestinal conditions has always been a major objection (de Araújo Etchepare et al., 2016).

Microencapsulation technology has been widely known to increase the stability of probiotics with controlled release characteristics (Zanjani et al., 2014). Spray drying is one of most used techniques in the microencapsulation process, due to its short time, low cost, and good stability and quality of end products (Mis Solval, 2011; Felix et al., 2017). Compared with other conventional microencapsulation techniques, the spray drying process can be easily scaled up to produce microcapsules in a continuous processing operation (Ré, 1998; McHugh 2018). A variety of encapsulants, including alginate, milk protein concentrates and isolates, and lipids, have been studied (Ragavan and Das, 2018; Yasmin et al., 2019). However, large-scale production of gel beads, their large size diameters, dispersion, and requirement of transfer from organic solvents present serious difficulties in food applications

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*Corresponding author: Sanjeev.Anand@sdsu.edu

(Picot and Lacroix, 2003). In the case of milk whey proteins, although they have been a versatile nutritionally beneficial system, enriched with bioactive properties, major challenges related to their low thermal stability are faced. At high processing temperatures, whey proteins tend to destabilize, leading to denaturation, gelation, foaming, and decreased solubility (Adjonu et al., 2013). At high ionic strength, they are inclined to aggregation, which causes sedimentation during storage (Mulcahy et al., 2016).

The thermal stability of whey proteins has been addressed by conjugating them with reducing carbohydrates (Yang et al., 2012). Proteins conjugated with a reducing carbohydrate through a Maillard reaction process have shown to exhibit improved physiological and functional properties, including heat stability, solubility, emulsification capacity, water binding and antioxidant activity (Zhang and Zhong, 2010). During the early stages of heating, conjugation occurs, where a covalent bridge is formed between protein and carbohydrate molecules. This interlinkage of the carbohydrate side chains with protein limits the aggregation between proteins and peptides, which in turn increases protein hydration and steric repulsion between proteins (Sosa et al., 2016). In view of the potential benefits of probiotics and conjugation, the intent of the present work was to study the effect of microencapsulation of probiotics in a conjugated whey protein on the viability and functionality of the end product. In our previous studies, a whey protein hydrolysate (WPH10) conjugate was developed by conjugating the protein with maltodextrin. The conjugate exhibited higher bioactive attributes (Minj and Anand, 2019) and, hence, was used to encapsulate probiotic cultures *Bifidobacterium* and *Lactobacillus* through a spray drying process. The product was stored at different temperature conditions to evaluate the viability of probiotic organisms and functional properties.

MATERIALS AND METHODS

Ingredients

The WPH10, with a 10% degree of hydrolysis and 89.98% protein, was procured from a commercial dairy plant. Maltodextrin with a dextrose equivalent of 10 was purchased from Sigma-Aldrich.

Preparation of WPH10-Maltodextrin Conjugate

The WPH10-maltodextrin (MD) conjugate solution was prepared according to the protocol described by Mulcahy et al. (2016). The WPH10 (5% wt/vol, protein) and maltodextrin (dextrose equivalent = 10;

5% wt/vol) were dispersed in distilled water at room temperature and mixed under magnetic stirring for at least 2 h to ensure complete dissolution. The pH of the mixture was then adjusted to 8.2 with 0.5 N KOH and allowed to hydrate for 18 h at 4°C. After hydration, the pH was readjusted to 8.2 with 0.5 N KOH. Further, the solution was transferred to a conical flask and heated at 90°C for 24 h in a shaking water bath. At the end of the heating period, the conjugated solution was cooled to room temperature for microencapsulation.

Probiotic Cultures and Inoculum Preparation

Pure freeze-dried probiotic cultures (*Bifidobacterium animalis* ssp. *lactis* ATCC27536 and *Lactobacillus acidophilus* ATCC4356) were obtained from ATCC. Fresh cultures were obtained after 2 respective transfers in de Man, Rogosa, and Sharpe broth supplemented with 0.05% (wt/vol) L-cysteine 37°C for 72 h, under anaerobic conditions. Propagation of the cultures were continued, and the cells were harvested in their late log phase by centrifugation at $7,000 \times g$ for 10 min at 4°C. The cell pellets were washed twice in PBS (0.9%) and suspended to achieve cell suspensions of 10 log cfu/mL. These cell suspensions were mixed in a ratio of 1:1 into the conjugated solution before microencapsulation.

Probiotic Cell Microencapsulation

The earlier inoculated whey protein hydrolysate-maltodextrin conjugate solution was spray dried in a pilot spray dryer (Atomizer Versatile Utility Spray Dryer, Niro). The solution was fed into the dryer by a peristaltic pump at a flow rate of 110 mL/min and a compressed air pressure of 55 psi. The inlet and outlet air temperatures were maintained at 200°C and $90 \pm 5^\circ\text{C}$, respectively. The obtained spray-dried probiotic powder was collected and analyzed for viability of probiotic. The WPH10 without maltodextrin was used as a control to represent the non-conjugated sample. Mixing of maltodextrin with WPH10 without heating (or conjugation) and maltodextrin alone led to coagulation and a sticky product during spray drying conditions and, hence, could not be used as controls for the encapsulation process.

Viability of Probiotics and Encapsulation Yield

Viability of the probiotic organisms was evaluated by standard pour plate technique. One gram of each powder sample was drawn out, and serial dilutions were made using PBS. The diluted samples were plated on de Man, Rogosa, and Sharpe agar supplemented with 0.05% (wt/vol) L-cysteine. The plates were incubated

at 37°C for 72 h under anaerobic conditions. Total count was taken in log cfu per gram. Viable counts were taken before and after spray drying to determine the survival rate (i.e., encapsulation yield, EY), calculated as follows:

$$EY = \frac{\log N}{\log N_0} \times 100,$$

where N_0 is the viable count (in log cfu/g) of dry powder before spray drying, and N represents the viable count (in log cfu/g) of dry powder after spray drying.

Morphological Analysis by Scanning Electron Microscopy

The surface morphology of the WPH-MD conjugate entrapped probiotic powder was evaluated by scanning electron microscopy according to Yang et al. (2012) and Rosenberg et al. (1985). Spray-dried powder samples were coated with 10 nm of gold under vacuum by fine-coat ion sputter. Scanning electron microscopy was carried out at an accelerating voltage of 5 kV, and the images were captured with magnifications of 5,000 $\times$ and 10,000 $\times$.

Moisture Determination

The moisture content of the spray-dried probiotic powder was determined according to IDF (1993) standard 26A. First, 1 g of dry powder sample (A) was weighed into a porcelain dish. The dish was placed in a hot-air oven at 103°C for 4 to 5 h. At the end, the sample was drawn out and cooled, and the weight was measured (B). The moisture content was calculated as follows:

$$\left(\frac{A - B}{A} \right) \times 100,$$

where A = initial weight of the sample, and B = final weight of the sample.

Storage Studies

The collected spray-dried WPH10 conjugate encapsulated probiotic powder was packaged in airtight containers and stored at temperatures of -18 , 4 , and 25°C . During storage, the samples were pulled out after 2, 4, 8, 12, and 16 wk to monitor cell viability, moisture content, and functionality. The probiotic cell viability during storage was analyzed according to the standard plate technique method described previously.

Functional Properties

Solubility. The protein solubility of the WPH10-MD conjugate encapsulated probiotic powder was analyzed according to the method of Westergaard (2004). In a beaker, 100 mL of distilled water was taken at room temperature, and 5 g of sample (p) was added. The suspension was stirred for 30 min using a magnetic plate stirrer. The pH of the solution was 6.77 ± 0.05 . Next, 40 mL of the solution was drawn out in a tube, weighed, and centrifuged at $7,000 \times g$ for 10 min at room temperature. The supernatant was transferred carefully in a pre-dried (1 h at $103 \pm 2^\circ\text{C}$, cooled in desiccators) and pre-weighed aluminum bowl. It was dried for 4 to 5 h in an oven at $103 \pm 2^\circ\text{C}$, cooled, and weighed (y). Solubility (%) was calculated as follows:

$$\% \text{ Solubility} = 100 - \left(\frac{y}{p} \times 100 \right),$$

where y = final weight after drying, and p = initial weight of the sample.

Wettability. The method used to calculate wettability was an extension of the International Dairy Federation method (IDF, 1979). The method essentially measures the time for a given mass of powder to sink beneath the water surface, referred to as the wetting time. First, 250 mL of distilled water was weighed in a 600-mL dry beaker and heated to 50°C (ensuring that the inner side of the beaker, just above the water level, remains dry). Next, 10 g of sample was taken and slowly added with the help of a dry cone and aluminum foil (held with a stand) over the water. The time was recorded immediately after all the powder was added, until all the powder particles had sunk below the surface of the water.

Statistical Analysis

Experiments were performed in triplicate, and one-way ANOVA was applied to differentiate between mean values. All figures with error bars were made using Sigmaplot software version 13 (SPSS Inc.) for Windows 10 (Microsoft Corp.).

RESULTS AND DISCUSSION

Viability of Probiotics and Encapsulation Yield

The achievement of high viability of probiotic organisms throughout the drying and storage period is challenging and is classified as one of the major problems in producing a commercial probiotic food (Mounsey and O'Riordan, 2008). For efficacy, the recommended

Table 1. Viability of the probiotic microorganisms in the conjugated whey protein hydrolysate (WPH10-MD) after spray drying, and its encapsulation efficiency (%)¹

Sample	Before drying (log cfu/mL)	After drying (log cfu/g)	Encapsulation yield (%)
WPH10 encapsulated probiotic powder (control)	10.62 ± 0.01 ^a	4.03 ± 0.02 ^c	37.94 ± 0.17 ^d
WPH10-MD encapsulated probiotic powder	10.58 ± 0.08 ^a	8.98 ± 0.02 ^b	84.87 ± 0.02 ^e

^{a-e}Values followed by different superscripts are significantly different ($P < 0.05$).

¹Mean ± SD (n = 3).

viable probiotic counts in a probiotic food are 10^6 to 10^7 cfu/g (Tripathi and Giri, 2014). Table 1 and Figure 1 show the viability of probiotic organisms in the conjugated WPH10 matrix through spray drying and 16-wk storage of the microcapsules at -18 , 4 , and 25°C . After spray drying, the probiotic cell counts obtained in the conjugated WPH10 matrix were $8.98 \pm 0.02 \log_{10}$ cfu/g, with an encapsulation yield of $84.87 \pm 0.02\%$. However, when probiotic cells were encapsulated in non-conjugated WPH10 (control), under similar spray drying conditions, viable counts of $4.03 \pm 0.02 \log_{10}$ cfu/g were achieved, with an encapsulation yield of $37.94 \pm 0.17\%$. These results imply good retention of the viability of probiotics in the conjugate carrier, with a higher survival rate compared with non-conjugated WPH10. Higher survival in the WPH10 conjugate is likely due to improved thermal resistance of the WPH10 and modification of the particle to a matrix type following conjugation with maltodextrin. The use of mixtures of whey proteins and carbohydrates or fats are currently being studied, to enhance the stability of whey proteins as carriers. It has also been reported that use of proteins and polysaccharide mixtures can help link proteins to protect lactic acid bacteria (Nale et al., 2018) and can also be used as a dietary fiber under gastrointestinal conditions (Salavati Schmitz and Allenspach, 2017). Picot and Lacroix (2004) reported that entrapment of *Bifidobacterium* spp. through spray drying in a heat-denatured whey protein isolate (WPI) carrier containing milk fat can be suitable in terms of cell viability compared with WPI alone. Guérin et al. (2003) investigated the viability of probiotics entrapped in gel beads coated with WPI conjugated with pectin, under gastrointestinal conditions. They observed that the resistance of the cells to acidic and bile conditions was enhanced with a reduction of less than 2 log in the case of coated gel beads. Comparatively, the survival of the cells in free form and in the uncoated gel beads was lower, with 4 to 7 $\log_{10}$ reduction. In a study conducted by Oliveira et al. (2007), *Bifidobacterium* spp. and *Lactobacillus* spp. were encapsulated in a casein and pectin matrix by coacervation, followed by spray drying; those authors found that the wall material was efficient in protecting the microorganisms during dry-

ing and in gastrointestinal conditions. In another study, *Lactocaseibacillus rhamnosus* GG was encapsulated at 10^9 cfu/mL in a formulation prepared using WPI and maltodextrin through spray drying, and the results showed only 1 log reduction of the bacteria, with improved survival during storage conditions (Ying et al., 2012).

Morphology of the WPH Conjugate Encapsulated Probiotic Microcapsules

Following spray drying, the morphology of WPH10 before and after conjugation and after entrapment of the probiotic microorganisms in the conjugated WPH10 were analyzed through scanning electron microscopy. The images from scanning electron microscopy are presented in Figure 2. In the images of non-conjugated WPH10, the powder particles were non-porous smooth spheres. However, some link bridges between the particles and disruption of the structures could be observed after drying. This explains the high hygroscopicity of the whey protein and low thermal stability of the particles. However, in the case of conjugated WPH10, a matrix-type structure could be observed, where the particles presented a round, porous surface. This confirms a modification of the whey protein particle structure after conjugation. In this study, greater viability of probiotic organisms was observed in the matrix-type particles (conjugated whey protein) compared with the non-porous spherical particles (non-conjugated whey protein), making the conjugated whey protein suitable for encapsulation. Further, the addition of maltodextrin decreased the hygroscopicity of the particles, which restricted the formation of link bridges. In Sidlagatta et al. (2020), addition of maltodextrin to sweet orange juice was shown to inhibit sugar crystallization and increase the glass transition temperature. Similar observations were reported by Yang et al. (2012), whose morphological analysis of a spray-dried whey protein hydrolysate through scanning electron microscopy before conjugation showed a smooth surface with link bridges between the particles, whereas after conjugation with β -maltodextrin, the particles presented a matrix-type surface with fewer link bridges and many

pores. In Rocha et al. (2009), after conjugation of a casein protein hydrolysate with maltodextrin, similar matrix-type microspheres were produced.

After entrapment of probiotic cells in the conjugated WPH10, varying sizes of microcapsules were produced, in the range of 10 to 20 μm in diameter. The images are displayed in Figure 3. Scanning electron micro images of the microencapsulated probiotic powder showed no free or non-encapsulated cells on the outside or on the surface of the microcapsules, which indicates successful entrapment of the probiotics with a high encapsulation efficiency. To confirm this, the powder particles were dispersed in distilled water (1 g in 9 mL of water) and observed under light microscope (Figure 2). The images clearly show the probiotic cells around the broken con-

jugate particles and some inside the pores. This verifies that the matrix structure of the conjugated WPH10 is maintained even after the microencapsulation and shows that the cells remained entrapped within the carrier material. These observations are comparable to *Bifidobacterium infantis* entrapped microcapsules produced using a caseinate-fructooligosaccharide-oil dried glucose syrup emulsion (Crittenden et al., 2006). However, the microcapsules produced in our work provide advantages because of their smaller size compared with beads produced using alginates, gelatin, or xanthan gums (typically $>100 \mu\text{m}$ in diameter), which affects the mouthfeel and texture when added into food matrices (Sun and Griffiths, 2000; Krasaekoopt et al., 2004; Crittenden et al., 2006).

Moisture Determination

Moisture content is one of the important factors in determining the shelf stability of food products. It also affects the physical and chemical characteristics of a food, which influence the freshness and storage stability of the food for a long period of time (Isengard, 2001). Both the non-conjugated and conjugated entrapped probiotic powders showed moisture contents of $0.91 \pm 0.08\%$ and $0.64 \pm 0.14\%$ (i.e., below 5%), which is recommended in spray-dried powders for preventing cell loss during storage of dried cultures (Peighambardoust et al., 2011). The moisture content in the microcapsules was greatly influenced by the outlet temperature and the cubic effect of the outlet temperature, suggesting that temperature plays an important role in the drying process (Felix et al., 2017). Hence, with a higher outlet temperature of $90 \pm 5^\circ\text{C}$ and an inlet temperature of 200°C , a powder with low moisture content was produced.

Viability of Probiotics During Storage

Viability of probiotic microorganisms during storage conditions is an important criterion for foods containing probiotics. Therefore, the WPH10 conjugate-entrapped probiotic microcapsules were stored at 25°C , 4°C , and -18°C for 16 wk, to monitor cell viability and compared with non-conjugated WPH10. The results are shown in Figure 1. Throughout storage at 4 and -18°C , the probiotic counts were maintained, and at the end of the storage period, a reduction of approximately 1 $\log_{10}$ cfu/g was observed, with final counts of 7.18 ± 0.008 and $7.87 \pm 0.008 \log_{10}$ cfu/g, respectively. At 25°C , the counts significantly ($P < 0.05$) dropped to $3.97 \pm 0.008 \log_{10}$ cfu/g. In the case of non-conjugated WPH10 (Figure 3), the probiotic counts sharply declined to 2.07 ± 0.008 and $2.59 \pm 0.069 \log_{10}$ cfu/g at 4 and -18°C ,

Figure 1. Viability of probiotic organisms in (a) conjugated (WPH10-MD) encapsulated probiotic powder, compared with (b) non-conjugated whey protein hydrolysate (WPH10) entrapped probiotic powder, during storage at -18 , 4 , and 25°C for 16 wk. Error bars represent SEM from 3 independent experiments.

respectively, whereas at 25°C storage, the counts declined to $2.39 \pm 0.061 \log_{10}$ cfu/g at the end of 4 wk, and no counts were observed after 8 wk of storage. Overall, the survival of the organisms was better at 4°C and at −18°C at the end of 16 wk than at ambient

temperature (25°C). From these data, it was observed that the stability of the conjugated WPH10 was higher compared with non-conjugated WPH10 under storage conditions, and survival of the cells was higher at 4 and −18°C compared with 25°C. The final concentration of

Figure 2. Scanning electron micro images of powder particles, with magnifications from 900 to 5,000. Panels a1 and a2: non-conjugated whey protein hydrolysate (WPH10); b1 and b2: maltodextrin-conjugated whey protein hydrolysate (WPH10-MD); c1: non-conjugated WPH10 entrapped probiotic powder; c2: conjugated (WPH10-MD) entrapped probiotic powder particles; c3: light microscope image of conjugated (WPH10-MD) entrapped probiotic powder (178- μ m field of view at 1,000 $\times$ magnification). Black arrow indicates the link bridges between WPH10 powder particles. Red arrow indicates the pores on the conjugated WPH10. Black circle indicates probiotic cells surrounding the broken conjugate powder particles.

7 log₁₀ cfu/g of viable cells is within the recommended levels for probiotics in foods (i.e., 10⁶–10⁷ cfu/g; Anekella and Orsat, 2013).

In one study, Fung et al. (2011) encapsulated *L. acidophilus* in dietary fiber through electrospinning and obtained good survival when stored at 4°C for 21 d. In addition, similarly, the thermal resistance of encapsulated probiotics indicates possible protection of cells when incorporated in heat-processed foods. Yasmin et al. (2019) encapsulated *Bifidobacterium longum* BL-05 and studied its survival at 4°C for 28 d. Storage at refrigeration temperatures exhibited a log reduction of 1.72 and 1.99 for all encapsulated treatments. This can be correlated with the present work, in which multilayer microencapsulation using protein and carbohydrate

mixtures significantly improved the survival of probiotics at 4°C during long-term storage.

The results obtained can also be related to a study by Xu et al. (2016), where a *Lactobacillus* spp. was encapsulated in a pea protein isolate-alginate matrix and stored at −15°C. After 84 d of storage, the encapsulated bacteria showed the highest survival rate compared with all other samples. It was reported by Conrad et al. (2000) that at freezing temperatures cell death can occur due to formation of ice crystals that can cause structural damage to the cell membranes and result in changes in the physiological state of the cells. However, in our studies, minimal log reduction at −18°C indicates the ability of the conjugated WPH10, as a carrier, to protect cells at freezing temperatures. This makes it suitable to be added to frozen food or to food that requires freezing.

In storage at 25°C, the stability of the cells in conjugated WPH10 was maintained, and counts were within the recommended levels up to 4 wk of storage, providing better protection compared with non-conjugated WPH10, which presented a sharp decline within 15 d of storage. This tells us that, at the analyzed temperature (25°C), it is difficult to maintain viable counts, and the loss of viability at ambient temperatures may be attributed to changes in the moisture content of the powder, which can lead to cell membrane disruption and deactivation (Ilango et al., 2016). The increase in moisture content with storage time could be associated with moisture absorption from the storage environment. Crystallization of lactose sugar could be another reason, as some amount of water is released during the phase transition from the amorphous state to the crystalline state (Palacha and Sitkiewicz, 2008). Moreover, the viability of bacteria during storage also depends on the sensitivity of the bacteria to oxygen and the ability of encapsulating material as a protector. Following spray drying, lipid oxidation during storage alters the composition of the lipid cell membrane. Other factors that influence the survival of organisms during storage are the composition of the encapsulated powder, oxygen content, and glass transition temperature. Hence, the viability of organisms during ambient storage conditions could be enhanced by using effective desiccants or deoxidants, suitable packaging material, and vacuum packaging.

Figure 3. Solubility (%) of (a) conjugated (WPH10-MD) encapsulated probiotic powder, compared with (b) non-conjugated whey protein hydrolysate (WPH10) entrapped probiotic powder, during storage at −18, 4, and 25°C for 16 wk. Error bars represent SEM from 3 independent experiments.

Solubility During Storage

The ability of a powder to completely dissolve in water is termed solubility (Gaiani et al., 2011). The protein solubility of the WPH10-MD encapsulated probiotic powder was analyzed according to the method of Westergaard (2004), with slight modifications, and was

compared with the control non-conjugated WPH10. The change in solubility from the initial day until 16 wk of storage is displayed in Figure 3. Following spray drying, the solubility of the WPH10-MD encapsulated probiotic powder at the initial day of storage was $91.03 \pm 0.98\%$, whereas the solubility of WPH10 was $82.03 \pm 0.88\%$. During hydrolysis, the release of specific peptides and exhibition of hydrophobic residues could promote peptide or protein-peptide aggregation, which could be related to the lower solubility of WPH10 powder (Creusot and Gruppen, 2007). In addition, peptides released during hydrolysis possess low molecular masses and reduced secondary structure, which can restrict the changes induced by heat (Chobert et al., 1988). In the case of conjugated WPH10 encapsulated probiotic powder, conjugation offers an advantage. During the process of conjugation, the bulky dextran molecules become attached to the protein molecules, which in turn increases the steric hindrance between the molecules. This thereby increases hydration of the protein (Mulcahy et al., 2016).

Further, both the powder samples were stored at 3 different temperatures (-18 , 4 , and 25°C). The samples were withdrawn after 2, 4, 6, 8, and 16 wk, and solubility were analyzed. After 2 wk of storage the solubility of WPH-MD powder was $89.68 \pm 0.27\%$ for -18°C , $90.23 \pm 0.99\%$ for 4°C , and $89.05 \pm 0.52\%$ for 25°C ; whereas for WPH10 powder, the solubility was $78.85 \pm 0.17\%$ for -18°C , $79.3 \pm 0.2\%$ for 4°C , and $78.29 \pm 0.7\%$ for 25°C . A slow but continuous decrease in solubility was observed in both the samples. For 8 wk of storage, WPH-MD powder showed relatively good solubility at all the storage temperatures ($>85\%$). At the end of wk 10, the solubility of WPH-MD powder declined to $82.36 \pm 0.62\%$ for -18°C , $83.1 \pm 0.99\%$ for 4°C , and $81.19 \pm 0.7\%$ for 25°C ; and for WPH10 powder the solubility declined to $69.41 \pm 0.24\%$ for -18°C , $69.97 \pm 0.92\%$ for 4°C , and $68.99 \pm 0.89\%$ for 25°C . The decrease in solubility of the powder samples with storage time is possibly due to the formation of a complex matrix of cross-linked proteins at the surface of the powder, which eventually restricts water transport and subsequently impedes hydration of the powder particles (Anema et al., 2006). However, studies have also shown that solubility will not decrease until a significant level of cross-linking of the protein molecules at the powder surface has occurred, and this may explain why the solubility did not immediately decrease on storage. With storage time and temperature, the degree of cross-linking between proteins increases (Sharma et al., 2012). Hence, the solubility of the powder samples gradually decreased with time, and the samples at room temperature exhibited lower solubility compared

with the samples stored at 4 and -18°C . Also, Singh et al. (1992) addressed that increase in the moisture level of the milk powder samples during prolonged storage contributes to several changes in the milk protein structure, which leads to decrease in solubility.

Wettability During Storage

Wettability of food powders is a result of a molecular interaction between solid and liquid phase, and is generally explained as the ability of the powder particles to overcome the surface tension caused by the water. The surface composition of powders plays an important role in the wetting process (Fang et al., 2008).

The wettability of the WPH10-MD encapsulated probiotic powder was analyzed according to IDF, 1979, with slight modifications, and was compared with the reference sample, WPH10. The change in the wetting time from the initial day to 16 wk of storage is displayed in Figure 4. Following spray drying, the wetting time of the WPH10-MD encapsulated probiotic powder at the initial day of storage was 47 ± 2 min, whereas the wetting time of WPH10 was 53 ± 2 min. Both the powder samples showed higher wetting times compared with a commercial whole milk powder and skim milk powder (>1 min), which can be explained by their high protein content as compared with whole milk powder. In both the cases, the powder particles became wet very quickly and turned instantly into lumps surrounded by the gelatinous layer, thereby reducing the wetting of the powder. One possible reason could be the high presence of protein on the surface of the powders. Similar observations have been reported by Silva and O'Mahony (2017) in milk protein concentrate. The WPH10-MD encapsulated probiotic powder showed less wetting time compared with the WPH10 powder, which can be related to the effects of conjugation, as linking of carbohydrate side chains with protein decreases the exposure of protein molecules on the particle surface (Mulcahy et al., 2016). Another possible explanation could be decrease in the hygroscopicity of the particles after addition of maltodextrin. Sharma et al. (2012) explained that, if the particle surface is more hygroscopic, it dissolves too quickly, which thickens the liquid and restricts further penetration of liquid into the powder mass.

Further, both the powder samples were stored at 3 different temperatures (-18 , 4 , and 25°C). The samples were withdrawn after 2, 4, 6, 8, and 16 wk, and wetting time were recorded. After 2 wk of storage, the wetting time of WPH-MD powder was 49 ± 1 min for -18°C , 48 ± 1 min for 4°C , and 50 ± 1 min for 25°C ; whereas for WPH10 powder, the wetting time was 56 ± 1 min

Figure 4. Wetting time (min) of (a) conjugated (WPH10-MD) encapsulated probiotic powder, compared with (b) non-conjugated whey protein hydrolysate (WPH10) entrapped probiotic powder, during storage at -18 , 4 , and 25°C for 16 wk. Error bars represent SEM from 3 independent experiments.

for -18°C , 56 ± 1 min for 4°C , and 59 ± 1 min for 25°C . A continuous increase in the wetting time was observed in both the samples, and by the end of wk 16 the wetting time of WPH-MD powder increased to 61 ± 1 min for -18°C , 60 min for 4°C , and 63 min for 25°C ; and for WPH10 powder, the wetting time increased to 71 ± 1 min for -18°C , 70 ± 1 min for 4°C , and 72 ± 1 min for 25°C . The increase in the wetting time with storage time can be attributed to the formation of a complex matrix of cross-linked proteins at the surface of the powder, which eventually restricts water transport and subsequently impedes hydration of the powder particles (Anema, 2009). Similarly, Kim et al. (2002) observed a decrease in the rate of wetting with an increase in the swelling of the whey protein concentrate particles.

CONCLUSIONS

The morphology of the conjugated WPH10 presented a matrix-type microsphere and, hence, was used for encapsulation of probiotic microorganisms. After microencapsulation through spray drying, the conjugated WPH10 demonstrated as a carrier for probiotic microorganisms with viable counts of $8.98 \log_{10}$ cfu/g with an encapsulation yield of 84.87%. Hence, the survival rate was significantly higher in the conjugated WPH10 matrix compared with non-conjugated WPH10. Additionally, conjugation of whey protein hydrolysate positively affected the viability of the probiotic cells under different storage temperatures (4°C , 25°C , and -18°C). The microspheres were able to maintain and preserve the viability of the cells throughout the storage period at freezing (-18°C) and refrigeration temperatures (4°C). Conjugation with maltodextrin was effective in enhancing the functionality of the final spray-dried product. With the improved solubility and wettability of conjugated whey protein hydrolysate (WPH10-MD) compared with non-conjugated WPH10, WPH10-MD possesses great potential to be incorporated as a food ingredient. Overall, such probiotic powder formulation, with improved functionality and having value-added benefits from both WPH10 and probiotics beyond utilizing their individual benefits, can offer several food applications.

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ORCIDS

Sanjeev Anand <https://orcid.org/0000-0002-7479-9965>