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Functionality of dairy proteins and vegetable proteins in nutritional supplement powders: a review

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Abstract

Nutritional supplement powder has seen rapid growth in the past 20 years, as one of the key sub-categories in functional foods. Many powder formulations use a substantial amount of milk protein, both as a protein source and as a wall material for encapsulating sensitive nutrients, most commonly by spray drying. There has been a recent trend of replacing milk proteins with vegetable proteins, because vegetable proteins are more sustainable and cost-effective. Among vegetable proteins, soy protein has been extensively studied, and it has shown excellent functionality. However, the application of soy protein in food industry has been limited due to its allergenic nature and unpleasant flavour, and one alternative with great potential is pea protein. This review aims to summarise and compare the recent studies on different wall materials to assess their potential as key ingredients in nutritional supplement powders. In addition, common strategies to improve encapsulation efficiency are also discussed.

Introduction

Nutritional powder or nutritional supplement powder, as a key functional food, has seen rapid growth in the past 20 years due to the market demand as well as the health benefits. Despite numerous existing commercial products, concrete definition and classification of nutritional supplement powders remain unestablished. According to a recent patent (Gupta *et al.*, 2015), nutritional supplement powders are formulated with proteins, vitamins, minerals and fibre. Nutritional supplement powders can be conceptually broken down into sport supplement powders, medical nutrition, fortified foods, adult nutritional supplement powders, and other types of foods. They are developed to deliver necessary nutrients for people who need extra nutrients (Rimpiläinen *et al.*, 2015). The formula matrices of nutritional supplement powders typically contain proteins (especially milk proteins), fats, carbohydrates, as well as various micro-nutrients such as minerals, vitamins, and other functional ingredients such as lutein and curcumin, in order to meet the needs of different target consumer groups.

Microencapsulation of functional ingredients is a routinely used technique in the manufacturing of nutritional supplement powder, for the purpose of minimising degradation and oxidation of core materials induced by environmental stresses (Feng *et al.*, 2019). Due to the environmental sensitivity of typical functional ingredients in nutritional supplement powders, for instance, polyunsaturated fatty acid (PUFA)-rich oil and liposoluble vitamins, microencapsulation has become an indispensable pathway to preserve their bioactivities (Feng and Lee, 2017; Feng *et al.*, 2018; Li *et al.*, 2019). In the past several decades, several microencapsulation techniques, including spray drying, extrusion, freeze drying, and fluid bed coating, have been introduced, among which spray drying is the most commonly used technique in the industry. In addition to its excellent ability to precisely control particle size and moisture content with tuneable drying capacity, spray drying is also considered a continuous and simple

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process, fully automated with a real-time control. Other advantages of spray drying have been reported as less denaturation of whey proteins, mitigated heat coagulation, and improved stability of reconstituted milk powder (Singh and Creamer, 1991).

Although nutritional supplement powders have been commercialised and developed for a long time, a couple of critical issues and concerns still need to be addressed. The first concern is associated with the replacement of milk proteins using vegetable proteins in order to reduce cost and improve sustainability. Milk proteins contain caseins (the fraction that precipitates at pH 4.6) and whey proteins (the fraction soluble at pH 4.6) (Tavares *et al.*, 2014). Recently, several vegetable proteins, particularly soy proteins, have become a popular substitute for milk proteins. However, the unpleasant odour of soy protein limited its applications (Yada, 2004). Furthermore, the physicochemical properties of vegetable proteins differ from those of milk proteins. Therefore, conventional processing parameters need to be appropriately adapted for the processing of vegetable proteins. In addition, there have been many quality issues related with conventional spray dried products, such as the loss of nutrients especially some bioactive compounds, accelerated lipid oxidation, unstable shelf life, and poor powder physical properties. It is still unknown if these issues could be potentially improved by replacing vegetable proteins or by optimizing other factors. Recent trends indicate the prospect of vegetable proteins; hence, a systematic review is needed to discuss the potential applications of vegetable proteins in nutritional supplement powders and their limitations.

This review discusses the definition of nutritional supplement powders, their key ingredients, the significance of protecting sensitive bioactive compounds in nutritional supplement powders, and eventually the different associated approaches. This review also covers the most recent studies on encapsulation that have used different protein wall materials, with a focus on their physicochemical properties as food ingredients, as well as approaches to improve encapsulation efficiency for the purposes of producing desirable microcapsules.

Key ingredients and bioactive micronutrients

As discussed in the introduction, the definition of nutritional supplement powder is still not concrete, and therefore its key ingredients also vary substantially among each product depending on the applications and the targeted functionalities. In general, regardless of the product, nutritional supplement powders typically contain a high percentage of proteins and carbohydrates, a variable amount of fats, and a variety of micronutrients (Hamilton and Treadwell, 2003). As a major component in nutritional supplement powder, proteins provide essential amino acids for human growth and maintenance, and they also act as wall materials to prevent the loss of micronutrients. The selection of proteins is of great significance for nutritional supplemental powders. Foods vary in their protein content (Table 1), and even more so in the properties of the proteins. There are different sources of proteins, such as milk proteins (*e.g.*, casein and whey), muscle proteins, soy proteins, proteins from oil-producing plants (*e.g.*, rapeseed, sunflower, safflower), cereal proteins (*e.g.*, rice, sorghum, oats, maize), and seaweed proteins.

Table 1. Total protein contents of the edible portion of selected foods and beverages.

Type of Food	Total Protein (%)
Almond	21.1
Apple (raw, eating)	0.4
Banana	1.2
Bean (canned, baked)	5.2
Beer (bitter)	0.3
Beef (lean, raw)	20.3
Bean sprouts (raw)	2.9
Bread (white)	8.4
Cabbage (raw)	1.7
Cheese (cheddar)	25.5
Cheese (parmesan)	39.4
Chicken (lean, raw)	20.5
Chocolate (milk chocolate)	8.4
Chocolate (plain chocolate)	4.7
Cod fillet (raw)	17.4
Cornflake	7.9
Egg (whole)	12.5
Ice cream	3.6
Lentil (dried)	24.3
Milk (cow)	3.2
Milk (human)	1.3
Pasta	3.6
Potato (new)	1.7
Rice	2.6
Sweet corn (canned)	2.9
Soy milk	2.9
Tofu (steamed)	8.1
Tuna (canned)	27.5
Yogurt (plain)	5.7

Some micronutrients, such as vitamins, are critical for human metabolism, while some display bioactive functionalities, and nutritional supplemental

powders are considered important source of these micronutrients. To date, a lot of bioactive compounds have been identified and investigated for their health benefits such as anti-cancer, anti-inflammatory, anti-aging, anti-microbial, and anti-oxidation effects. Most of the bioactive compounds can be classified as bioactive lipids, lipophilic vitamins, carotenoids, flavonoids, polyphenols and phytosterols. Nevertheless, direct oral consumption of these bioactive compounds is usually associated with poor bioavailability because of their low water solubility. In addition to poor bioavailability, many compounds are sensitive to environmental stresses. For instance, the bioactivity of resveratrol diminishes upon cis-trans isomerisation triggered by light (Koga *et al.*, 2016), while Vitamin A, D, E, and K, as well as ω-3 fatty acids, are susceptible to oxidation. Therefore, incorporating bioactive compounds into food-based matrices are not feasible because foods are often exposed to detrimental scenarios such as high temperature during cooking and air exposure during serving. In nutritional supplemental powders, the high protein content helps to provide protection against environmental stresses.

Overview of the role of wall materials in nutritional supplement powder

Selection of appropriate wall materials is vital for the application of spray drying for the manufacture of nutritional supplement powder. Many nutrients such as unsaturated fatty acid, vitamin E, vitamin C, and iron are susceptible to oxygen, pressure, heat, light and water. Oxygen is a critical factor that could lead to off-flavour and rancidity. For probiotics encapsulation, the control of moisture content is a key factor to keeping high motility. Generally, the carrier materials are food-grade with high solubility in water, low viscosity, and acceptable emulsifying properties (Feng and Lee, 2016). Phase transition temperature of wall materials is another important property because it is highly relevant to the powder caking properties. Therefore, thorough criteria spanning encapsulation efficiency, storage stability, the degree of the protection and powder morphology have to be considered in the selection of wall materials (Olenskyj *et al.*, 2017; Feng and Lee, 2019a; 2019b). Extensive work has been conducted using natural gum, an animal source protein, and carbohydrate as wall materials. As the major component in nutritional supplement powder, understanding the role of milk proteins and vegetable proteins are gaining more interest. Herein, we will compare the microencapsulation feasibility of milk proteins and vegetable proteins. Furthermore, we will discuss about their current limitations as well as potential strategies, for better application in the formulation of nutritional supplement powders.

Table 2. Amino acid content of various dietary protein sources.

	Soy	Pea	Whey	Casein
Essential amino acids				
Threonine	2.3	2.5	5.4	3.5
Methionine	0.3	0.3	1.8	2.2
Phenylalanine	3.2	3.7	2.5	4.2
Histidine	1.5	1.6	1.4	2.2
Lysine	3.4	4.7	7.1	5.9
Valine	2.2	2.7	3.5	3.8
Isoleucine	1.9	2.3	3.8	3.0
Leucine	5.0	5.7	8.6	7.8
∑EAA	19.9	23.6	34.1	32.8
Non-essential amino acids				
Serine	3.4	3.6	4.0	4.2
Glycine	2.7	2.8	1.5	1.5
Glutamic acid	12.4	12.9	15.5	16.0
Proline	3.3	3.1	4.8	8.7
Cysteine	0.2	0.2	0.8	0.1
Alanine	2.8	3.2	4.2	2.6
Tyrosine	2.2	2.6	2.4	4.4
Arginine	4.8	5.9	1.7	2.9
∑NEAA	31.9	34.4	34.9	40.4

[1]Values are presented as g per 100 g raw material. Tryptophan, aspartic acid, asparagine, and glutamine were not measured.

[2]ΣEAA sum of all essential amino acids, ΣNEAA sum of all non-essential amino acids.

[3]Table is adapted from Gorissen *et al.* (2018) with modifications.

Nutrition aspect of dairy proteins and vegetable proteins

Vegetable proteins remarkably differ from dairy proteins in terms of their nutritional aspects. One of the main reasons for the differences in the two forms of protein is their amino acid profiles. Typically, plant proteins are known for their insufficient essential amino acid contents. Based on Table 2, as adapted from Gorissen *et al.* (2018), the essential amino acid contents in both soy and pea constitute much less fraction when compared to non-essential amino acids. On the other hand, casein and whey have equivalent amount of essential and non-essential amino acids. Among the essential amino acids, lysine has been recognised as one of the most bioactive and functional amino acids, which is responsible for protein folding (Yada, 2004). However, the lysine fraction is fairly low in soy and pea (~4 g/100 g), when compared with casein and whey (6 - 7 g/100 g).

In addition to the amino acid profiles, protein digestibility also plays a vital role in the bioavailability of proteins. To incorporate the impact of protein digestibility on their functionality, protein digestibility-corrected amino acid score (PDCAAS)

Table 3. PDCAAS and DIAAS for selected isolated proteins and foods.

	PDCAAS	DIAAS	Limiting AA
[1]MPC	1.00	1.18	Met + Cys
[1]WPI	1.00	1.09	Val
[1]SPI	0.98	0.90	Met + Cys
[1]PPC	0.89	0.82	Met + Cys

[1]PDCAAS: protein digestibility-corrected amino acid score; DIAAS: digestible indispensable amino acid score; MPC: milk protein concentrate; WPI: whey protein isolate; SPI: soy protein isolate; PPC: pea protein concentrate; RPC: rice protein concentrate; AA: amino acid. [2]Table is adapted from Phillips (2017).

is often used as a golden standard to evaluate and compare the quality of proteins from different sources. Similar to PDCAAS, another criterion, the digestible indispensable amino acid score (DIAAS), has been recently proposed to emphasise the importance of indispensable amino acids (Phillips, 2017). Table 3 lists the digestibility-corrected protein scores, PDCAAS and DIAAS, as well as their limiting amino acids for several common proteins. Based on the table, the pea protein concentrate (PPC) and the soy protein isolate (SPI) are slightly lower in both PDCAAS and DIAAS, when compared with the milk protein concentrate (MPC) and the whey protein isolate (WPI). Despite of their minor differences in terms of the scores, all the proteins present acceptable digestibility, which could be regarded as desirable source for amino acids. Although the assessment of the nutrient value of proteins from different sources have been performed in animal models (rats), clinical trials have not been conducted to statistically confirm the findings in human digestive system.

Dairy protein as wall material: applications and limitations in encapsulation

Whey protein

Introduction and nature

Whey protein is a by-product of cheese production, which consists mainly of three components: β-lactoglobulin (BLG, 85%), α-lactalbumin (10%), and bovine serum albumin (5%) (Çakır-Fuller 2015). As a globular protein, whey is very sensitive to heat-treatment, and typically, it forms aggregates or gels above 70°C due to denaturation (Hoffmann and van Mil, 1997). The aggregation of whey protein involves structural alteration to become a more random structure and a greater exposure of buried hydrophobic groups. This is followed by intermolecular sulfhydryl-disulphide interchange reactions, free thiol oxidation, and non-covalent interactions (Çakır-Fuller, 2015). According to the literature, BLG possesses two unique properties that make whey protein a desirable wall material for microencapsulation. With high content of rigid beta-sheet structure, BLG presents digestive resistance against pepsin, the main protease in human's stomach, and the existence of two disulfide bonds (Cys82-Cys176 and Cys122-Cys135/137) provides further protein stability (Teng *et al.*, 2015). These two distinguishing features of BLG make whey protein a supreme wall material for the controlled release of nutraceuticals in the gastrointestinal tract. In addition, BLG has several binding sites for hydrophobic ligands, which are driven by hydrophobic interaction (Tavares *et al.*, 2014).

Applications in encapsulation

Whey protein has been widely used as a wall material for microencapsulation. A variety of structural designs for encapsulation and delivery were developed in a previous study, such as emulsions, hydrogels, microbeads, nanoparticles, and films (O'Neill *et al.*, 2014). Generally, emulsification is the most common approach used in the encapsulation of bioactive compounds. In order to improve the emulsifying capacity of whey protein, heat-induced denaturation is usually conducted as pre-treatment (Mutilangi *et al.*, 1996). An oil-in-water emulsion can therefore be formed, encompassing bioactive core material in the oil phase, which is followed by spray drying in cases where powderisation is required.

Furthermore, carbohydrates have also been

Table 4. Examples of applications of whey protein as encapsulation wall material.

Protein	Co-stabiliser	Structure	Core material	Reference
Whey protein isolate	Gum Arabic	Complex coacervates	Omega-3 fatty acids and probiotic bacteria	Eratte *et al*. (2015)
Whey protein isolate	N.A.	Microbeads	Riboflavin and peptides	O'Neill *et al*. (2014)
Whey protein concentrate	Span 80	W/O/W double emulsions	Folic acid	Assadpour *et al*. (2016)
Whey protein concentrate	Pectin	W/O/W double emulsion	Saffron extract	Esfanjani *et al*. (2015)
Whey protein isolate	Casein	O/W emulsion	Lactobacillus rhamnosus GG	Burgain *et al*. (2013)

Table 5. Examples of applications of casein or caseinate as encapsulation wall material.

Protein	Co-stabiliser	Structure	Core material	Reference
Sodium caseinate	Transglutaminase	O/W emulsion and gel	Lactobacillus paracasei ssp.	Heidebach *et al*. (2009)
Sodium caseinate	N.A.	O/W/ emulsion	Beta-carotene	Cornacchia and Roos (2011)
Sodium caseinate	N.A.	O/W emulsion	Resveratrol	Hemar *et al*. (2010)
Sodium caseinate	Pectin, tween 20	Hydrogel microsphere	Refined fish oil	Matalanis *et al*. (2012)
Sodium caseinate	N.A.	Complexed nanoparticle	Curcumin	Pan *et al*. (2013)
Sodium caseinate	N.A.	Complexed nanoparticle	Thymol	Pan *et al*. (2014a)
Sodium caseinate	N.A.	Complexed nanoparticle	Curcumin	Pan *et al*. (2014b)

widely recognised for their synergistic effects on the improvement of the encapsulation efficiency of whey proteins. For instance, whey protein has been paired with gum Arabic (Eratte *et al.*, 2015), pectin (Esfanjani *et al.*, 2015), and maltodextrin (Assadpour *et al.*, 2016) to serve as hybrid wall materials. These afore-mentioned carbohydrates are typical macromolecules which could potentially alter the rheological properties, molecular interactions, and mechanical properties of the emulsion interface. Table 4 illustrates several representative applications of whey protein as microencapsulation wall materials.

Casein

Introduction and nature

Caseins are the predominant proteinaceous component of milk, accounting for approximately 80% of the total dairy protein (Pan *et al.*, 2014b). Four types of caseins (αS1-, αS2-, β-, and κ-caseins) have been identified with approximate relative ratio of 4:1:3.5:1.5, respectively. These four casein molecules are associated through protein interaction and calcium phosphate, forming casein micelles with average diameters of 150 - 200 nm (Pan *et al.*, 2014b). As a result, casein micelles also serve as natural vehicles to deliver calcium in milk (Tavares *et al.*, 2014).

In most cases, casein exists as micelles and despite numerous studies on casein micelles, their structures have remained debatable. Instead, caseinate produced upon the removal of calcium phosphate is better understood due to its less aggregated morphology and smaller size. It has been found that a higher emulsifying efficacy can be reached with the equivalent amount of sodium caseinate (Dalgleish and Corredig, 2012). The functional properties of sodium caseinate could further be improved via enzymatic hydrolysis using enzymes such as papain, pancreatin, and trypsin. It was also reported that the complexation between caseinate and pectin could help to promote its release properties (Pan *et al.*, 2014b).

Applications in encapsulation

The use of casein or caseinate for encapsulation has been studied for a long time. However, the strategies applied for encapsulation were different due to their inherently different structures. For native casein, chemical complexation is commonly used because of its binding ability, large particle size, poor solubility, and poor emulsifying capacity. Hydrophobic compounds and metal ions are suitable core materials for complexation, driven by ionic bond and hydrophobic interaction. On the contrary, caseinate is associated with greater emulsifying capacity, smaller size, and better solubility. Therefore, emulsification is more commonly used for encapsulation.

Many studies have reported that encapsulation with casein or caseinate improved the stability (Cornacchia and Roos, 2011; Matalanis *et al.*, 2012), dispersibility, and bioavailability (Pan *et al.*, 2013) of bioactive compounds. Other studies also found that the survival rates of probiotic microorganisms increased with caseinate encapsulation (Heidebach *et al.*, 2009). Table 5 lists some examples of applications of casein or caseinate as encapsulation wall material.

Limitations of milk proteins as wall materials

Although many studies have been conducted with milk proteins as wall materials for encapsulation, there are still several issues that need to be addressed. One of the issues is isoelectric sedimentation of milk proteins. The isoelectric points of whey and casein are both around pH 4 to 5, which limit their applications in many foods and beverages (Zhang *et al.*, 2015). In addition, the cost of milk proteins is relatively higher than that of vegetable proteins. Therefore, replacing milk proteins with alternatives to reduce cost as well as to improve functionality has attracted a lot of attention (Kolar *et al.*, 1979).

Vegetable protein and its application in sensitive nutrients encapsulation

The replacement of animal protein with vegetable protein in sensitive nutrients encapsulation by

spray drying technology has been studied recently (Nesterenko *et al.*, 2013). Vegetable proteins extracted from soybean, pea, sunflower, barley, and other plant sources have shown a great potential as substitutes for milk protein. However, it has also been demonstrated that different vegetable proteins display different functional properties. Hence, the properties of vegetable proteins provide the details about their behaviour in food systems.

Soy protein

Introduction and nature

The main ingredients in soy protein are β-conglycinin and glycinin. β-conglycinin is a trimer which contains three sub-units, and its molecular weight is around 150 - 200 kDa. The molecular weight of glycinin is around 300 - 380 kDa. Glycinin is a hexamer with acidic and basic polypeptides linked by a disulfide bond (Fukushima, 2011). The amino acid composition of β-conglycinin is more hydrophobic than that of glycinin, and thus, it contributes more emulsifying ability. Soy protein can form stable emulsion in the food system by decreasing interfacial tension between water and oil as well as forming a physical barrier at the interface. Soy protein isolate has a high solubility at natural pH; however, thermal treatment, homogenisation, and the presence of salts during the manufacturing process could alter its solubility. Besides, soy protein has also been reported for its good film-forming properties (Nesterenko *et al.*, 2012).

Applications in encapsulation

In previous studies, soy protein isolate was used as wall material to encapsulate flavour compounds, phospholipid, and bitter materials. Nesterenko *et al.* (2014a) found that soy protein isolate is a good wall material for the encapsulation of lipophilic vitamin (α-tocopherol) and hydrophilic vitamin (ascorbic acid) by spray drying. In addition, soy protein isolates achieved high retention efficiency in the encapsulation of α-tocopherol (Nesterenko *et al.*, 2012). de Conto *et al.* (2013) found that a wall to core ratio of 2.6:1.0 by soy protein isolate could achieve the highest yield and encapsulation efficiency in omega-3 ethyl ester. In many cases, soy protein isolate is mixed with polysaccharides as a co-stabiliser.

Pea protein

Introduction and nature

In recent years, there has been an increasing demand for pea protein in the United States and globally due to its features as a clean, gluten-free, allergen-free, non-GMO, and cost-effective protein with a neutral taste in comparison with other protein sources. Though it is still not as popular as soy protein, pea protein as a vegetable protein is gaining attention in sports nutrition and other nutritional industries, as well as in different research fields. This is due to its nutrition values (its amino acid profile, high levels of lysine, arginine, and branched chain amino acid), high digestibility, emulsifying and microencapsulation properties, potential antioxidant properties, and its other benefits such as muscle synthesis for healthy aging.

Pea protein is extracted from pea seeds which are typically not associated with allergic risk, but instead contains high nutritional content and present excellent functional properties. Globulin (salt-soluble) and albumins (water-soluble) are the two main components in pea protein isolate. Globulin counts for 65 - 80% of the total weight of pea protein (Liang and Tang, 2013), and they are composed of two main fractions - vicilin (7S) and legume (11S). The legume (11S) has a regular hexameric quaternary structure, and its molecular weight ranges from 300 kDa to 400 kDa (Liang and Tang, 2013). Surface hydrophilicity is a critical factor that can affect interfacial properties of protein. Pea protein can be used as an effective emulsifier by forming a film at appropriate pH range. However, when the pH is at the 7 - 9 range, pea protein molecules dissociate leading to a drastic increase in the surface hydrophobic properties and further reduction of interfacial tension at the oil-water interface (Liang and Tang, 2013).

When compared with other vegetable proteins, pea protein is richer in essential amino acids (de Azevedo Bittencourt *et al.*, 2013). Recently, limited enzymatic hydrolysis is becoming a commonly used technique to improve the functional properties of pea proteins, such as emulsifying properties and solubility, by means of reducing molecular weight and exposing previously folded sites (Tamm *et al.*, 2016). As a result of hydrolysis, the diffusivity and surface activity become greater, which favour enhanced emulsifying capacity. Another notable advantage of hydrolysed pea protein is associated with its antioxidant properties, mediated by its hydroxyl radical scavenging and metal chelating effects (Pownall *et al.*, 2010). The degree of hydrolysis and the selection of enzymes are both crucial treatment factors in this process. It was reported that optimal pea protein functionality could be achieved with a degree of hydrolysis ranging between 1% and 10% (O'Regan and Mulvihill, 2010).

Applications in encapsulation

Pea protein can also be used in spray drying to encapsulate heat sensitive nutrients. At the interface, pea protein is rearranged, and thus exposes its buried hydrophobic fraction to interact with the oil phase. Surface hydrophobicity of pea protein impacts its ability to migrate to the interface. Costa *et al.* (2015) demonstrated the application of pea protein isolate to encapsulate conjugated linoleic acid (CLA) and the retention rate was improved. Pierucci *et al.* (2006) made use of pea protein isolate and maltodextrin to encapsulate ascorbic acid. However, there are still limited studies on pea protein as a wall material, especially the combinations of dairy proteins and pea protein as wall materials in nutritional supplement powder. Future research can focus on this due to the multiple benefits including more complete and balanced nutrition values, improved emulsifying properties, improved microencapsulation efficiencies, potential less nutrient degradation, and better oxidative stability of nutritional powder.

Comparison between soy protein and pea protein

Overall, soy protein provides superb functionalities, and it is considered more cost-effective than milk proteins, but it can be allergenic due to the production of immunoglobulin E (IgE). Another inevitable issue of soy protein is associated with its strong off-flavours, especially in Western countries, which are usually recognised as beany or grassy flavours (Fukushima, 2011). Although soy flavour masking has been investigated in many studies, this could lead to the increase in soy products' cost.

As an alternative, pea proteins share many advantages with soy protein. Although pea protein is currently not as popular as soy protein, it does offer more benefits such as non-allergenic protein (Bajaj *et al.*, 2015), and less unpleasant flavours or odours. Pea protein have also been reported as functional ingredients that reduce the risk of cardiovascular diseases (Bajaj *et al.*, 2015). When the hydrolysis of pea protein is used as pre-treatment, the functionality and bioactivity could further be improved (Tamm *et al.*, 2016). Hence, there is a great potential for the application of pea proteins in the food industry.

Comparing the functionality of soy and pea proteins, native soy protein possesses greater solubility as well as emulsifying stability than pea protein (Schwenke, 2001). On the other hand, the surface hydrophobicity of pea protein is higher than that of soy protein, which indicates that pea protein has a greater interfacial affinity (Schwenke, 2001). Therefore, pea protein can be regarded as a prospective wall material when subjected to appropriate pre-treatment such as enzymatic hydrolysis (Gharsallaoui *et al.*, 2012) or pH shifting (Can Karaca *et al.*, 2015).

Other vegetable proteins

Other vegetable proteins such as zein protein and barley protein showed feasible emulsifying ability and fast filming properties. However, the physico-chemical properties of these vegetable proteins have not been extensively studied. Future studies could be conducted to explore new applications of the vegetable proteins in spray drying encapsulation. The emulsifying ability and the film-forming capacity are two concerns of such studies. Zein is a prolamin from corn and has been accepted as a food-grade ingredient by the Food and Drug Administration of the USA (Feng and Lee, 2017), and it has been used for the encapsulation of antimicrobial materials such as nisin in food processing (Chen and Zhong, 2014). Barley protein contains two major storage proteins - hordein (35 - 55%) and glutelin (35 - 40%), and both fractions have great hydrophobic properties. Some researchers reported barley protein as an effective emulsifier, which provides the possibility of developing barley protein encapsulation systems for hydrophilic and lipophilic bioactive compounds (Wang *et al.*, 2011). The microcapsules made from barley protein show a great ability in protecting fish oil from oxidation. Oat protein is a superior source of plant proteins due to its nutritional values, and the structure of the major composition of oat protein-globulin is compacted, such that oat protein shows poor solubility and emulsifying protein (Rasane *et al.*, 2015). Structural modification of oat proteins, before being used as wall materials in encapsulation, is a necessity. Likewise, sunflower protein, which is one of the major by-products after oil extraction, is also becoming an emerging plant protein. According to González-Pérez and Vereijken (2007), there is about 20 - 40% crude protein in the de-hulled seed. Based on sedimentation coefficient, 11S globulin (helianthinin) and 2S albumins are the two major types of proteins in sunflower. A high-molecular-weight protein fraction in sunflower protein is the minor amount. Normally, 11S (helianthinin) is a trigonal antiprism with six spherical subunits. The solubility of sunflower protein depends on the pH and ionic strength of the solution. Sunflower protein has been reported for its good emulsifying ability. During emulsification, the hydrophobic part of sunflower protein changes its orientation to the oil phase and polar charged segments stay in the aqueous phase. Under heat or low pH treatment, the flexibility of sunflower protein is improved because of protein

Table 6. Encapsulation of functional ingredients using vegetable proteins as wall material.

Protein	Co-stabiliser / pre-treatment	Structure	Core materials	Reference
Soy protein isolate	Gum Arabic	Complex coacervation	Omega-3 fatty acids	de Conto *et al*. (2013)
Soy protein isolate	Acylation	Emulsion	Vitamins	Nesterenko *et al*. (2014a)
Pea protein concentrate	Maltodextrin, carboxymethyl cellulose	Emulsion	Conjugated linoleic acid	Costa *et al*. (2015)
Pea protein concentrate	Maltodextrin	Complexation	Ascorbic acid	Pierucci *et al*. (2006)
Zein	Casein	Nano-complex	Eugenol, thymol	Chen *et al*. (2015)
Sunflower protein	N.A.	Emulsion	Beta-carotene	Cornacchia and Roos (2011)

unfolding, and thus the emulsifying ability is also increased. As a new source of vegetable protein, it has been used as wall materials in encapsulation. Nesterenko *et al.* (2013) made use of unmodified sunflower protein to encapsulate α-tocopherol and achieved a significantly higher efficiency (92.6%). It has been noted that phenolic compounds, especially chlorogenic acid and caffeic acid, need to be removed from sunflower protein because they are considered as digestion inhibitors. Table 6 lists some examples of vegetable proteins that have been used for encapsulation.

Limitations of vegetable protein in nutrients encapsulation

One limitation of vegetable proteins is their low solubility in an aqueous system. Can Karaca *et al.* (2011) reported that at pH 7.0, the solubility of isoelectric-precipitated pea protein and soy protein are 61.4% and 96.5%, respectively. Decreased solubility was also reported in sunflower proteins due to non-covalent binding with chlorogenic acid (González-Pérez and Vereijken, 2007). As compared to animal source proteins, the size of vegetable protein molecules is usually larger and their structures are less flexible. Vegetable proteins with larger size diffuse slowly into oil/water interface, which further hinders their emulsifying abilities. The large globule nature of vegetable proteins also gives rise to high emulsion viscosity, due to the formation of thicker layer and extensive interaction between adsorbed and non-adsorbed proteins.

When the water that resided in vegetable protein is removed during spray drying, protein shrinkage occurs. The shrinkage of vegetable protein produces a porous structure on the surface of powder particles, high content of surface oil, and low encapsulation efficiency. Pierucci *et al.* (2006) reported that the morphology of pea protein microcapsules is irregular and rough, and one primary reason for this could be protein shrinkage. Chemical and physical modification could be applied to improve the soft mechanical nature of vegetable proteins in the future.

Overall comparison between dairy proteins and vegetable proteins

As discussed in previous sections, vegetable proteins and dairy proteins are distinct in many aspects. Herein, we summarise their primary differences (Table 7). In general, vegetable proteins are typically large in molecular size, have inflexible geometry, and possess hydrophobic surface amino acids. These lead to low solubility, high viscosity, propensity to aggregation and precipitation, and consequently poor functionality. On the other hand, dairy proteins have smaller molecular size (10 - 20 kDa), flexible structure, and the ability to dissociate in aqueous phase. Furthermore, their natural amphiphilicity makes them effective emulsifiers and encapsulation wall materials.

Table 7. Comparison between dairy proteins and vegetable proteins.

	Vegetable protein	Dairy protein
Molecular weight	25-50 kDa	15-25 kDa
Molecular flexibility	Poor, globular	Some flexibility
Solubility	Poor, prone to aggregation	Better solubility
Emulsifying / foaming capability	Usually need pre-treatments	Naturally effective emulsifiers
Digestibility	Good	Very good
Amino acid profiles	Depending on the plant sources	Very good
Viscosity	High viscosity due to large molecular weight	Low viscosity

Methods to improve encapsulation efficiency in nutritional supplement powder

In spray dry encapsulation of nutritional supplements, some technologies have been applied to improve the encapsulation efficiency of vegetable proteins. These technologies include physical,

chemical, and enzymatic modification methods. The modification methods will alter the physicochemical properties, such as film-forming properties and emulsifying ability, which indicates the potential of expanding the applications of vegetable protein to the manufacturing of nutritional supplemental powders.

Incorporation of polysaccharides and small molecular surfactants

In polysaccharides/vegetable protein combined systems, vegetable proteins are used as emulsifying agents to stabilise the oil droplets, and carbohydrates act as matrix-forming materials. According to Zhang *et al.* (2014), the combination of maltodextrin and soy protein showed better oil retention ratio than maltodextrin or soy protein singly. During spray drying encapsulation, dehydration induces the instability of emulsions by disrupting the interfacial layer around the oil droplets and enhancing the interaction between proteins. One critical criterion for acceptable microencapsulated nutritional supplement powder is to retain emulsion structure after reconstitution.

With the addition of polysaccharides, emulsion stabilised by vegetable protein has enhanced stability against environmental stresses such as high ionic strengths, extreme pHs, and heat treatment. The enhanced stability has been attributed to the formation of network structure by polysaccharides in the continuous phase (Gharsallaoui *et al.*, 2010), as well as the thicker wall at the interface. For example, by means of layer-by-layer electrostatic deposition technique, the polysaccharide molecules can adsorb to oppositely charged protein-coated oil droplets and improve the stability of the emulsion. Many studies have reported progress recently. Aberkane *et al.* (2014) found that 0.5% pea protein isolate and 0.5% pectin combination result in better encapsulation efficiency with PUFA-rich oil than pea protein isolates alone. Costa *et al.* (2015) applied pea protein isolate and maltodextrin to encapsulate CLA by spray drying. The addition of maltodextrin reduced the particles surface roughness with excellent encapsulating ability as well as increased dispersibility, solubility, and microparticle glass transition temperature (Tg). Similar results were also reported by Pereira *et al.* (2009) in the encapsulation of ascorbic acid micro-particles with pea protein isolate and maltodextrin. In other applications, lactose was paired with vegetable proteins in hydrophobic nutrients encapsulation. When liquid is transformed to powder during spray drying, vegetable protein molecules at the interface of oil droplets are dehydrated, and the protein membrane shrinks. The encapsulation efficiency is decreased due to core material leaking. The presence of lactose in emulsion reduces the shrinkage of the vegetable protein by forming a hydrogen bond with vegetable protein and keeps it stable at the oil droplet surface. However, the addition of lactose decreased glass transition temperature and increased the risk of powder caking (Tang and Li, 2013).

Optimisation of processing conditions

Spray drying converts fluids into solid powders by atomisation, following which, the drops travel through the chamber with heated concurrent air flow. In order to achieve high efficiency, optimised processing conditions must be applied. The pre-treatment of liquid, inlet temperature, and outlet temperature are the three critical processing conditions to be optimised. According to Jakobsen and Knuthsen (2014), the larger particles size is associated with a higher viscosity of the liquid, lower atomisation pressure, higher solid content of the liquids, and slower feed rate.

In most nutrient-delivering powder products, the particles sizes are typically around 100 μm, which allows acceptable powder flowability and prevent dust generation. Therefore, it is of great significance to control the size and morphology of particles during processing. In addition, the inlet temperature and fluid flow rate are also very important because they could significantly impact the water content of the final product and other particle properties. High inlet temperature results in fast evaporation and tends to form crust powder surface. The crust surface inhibits the evaporation of internal water, which could cause expansion, followed by cracking on the membrane or subsequent premature release. However, low inlet temperature tends to form high moisture content and agglomerated particles.

Harsh processing conditions could be detrimental to products. For example, during probiotics spray drying, high inlet temperature impacts probiotic viability, whereas low outlet temperature increases the moisture of particles and reduces the probiotic viability during subsequent storage of powder. In addition, the structure of microcapsules can be tweaked to spheres, irregular shapes, multicore, multiwall sphere, and matrix by adjusting spray drying conditions (Gibbs *et al.*, 1999).

Enzymatic modification

Enzymatic hydrolysis is an effective way to improve the properties of vegetable protein. Degree of hydrolysis (DH) is commonly used to describe the extent of hydrolysis, which measures the percentage of peptide bonds cleaved. The purposes of enzymatic

treatment to proteins are to increase the solubility and functionality. Hydrolysis of soy protein changes the protein structure and surface hydrophobicity, and therefore improves the solubility and functionalities. According to Zhang *et al.* (2014), limited hydrolysis of native soy protein isolate led to a better amphiphilic property and decreased droplet size in the emulsion. In another study, limited hydrolysis of soy protein (DH, 4%) led to increased flow behaviour and decreased viscosities due to the reduction of the molecular chain length of the protein (Conde and Rodríguez Patino, 2007). Moreover, microcapsules made from limited hydrolysis of soy protein have a better oxidative stability by forming a porous and uniform surface structure. During hydrolysis, the hydrophobic groups embedded in vegetable proteins become exposed such that protein hydrolysates provide enhanced interfacial adsorption properties. Another important change is associated with the rheological properties of the emulsions, which is attributed to the reduction in the molecular size of vegetable protein such that the apparent viscosity of solution decreases as the shear resistance reduces. A similar finding was observed in rice endosperm protein by Nesterenko *et al.* (2012). However, some studies have demonstrated that hydrolysed soy protein cannot provide strong film to encapsulate core material from oxygen, leading to more surface oil (Zhang *et al.*, 2014).

Chemical modification of vegetable protein

Chemical modification of vegetable proteins is another method to improve the functional properties of vegetable protein. Acylation is one of the most common chemical methods for protein modification. Acylation is a reaction between free amino groups from protein and carboxyl group from fatty acid in activated fatty acid esters (6 - 18°C). By attaching hydrocarbon chains to vegetable protein, acylation chains increase hydrophobicity and surface functionality of the proteins (Matemu *et al.*, 2011). The introduction of hydrophobic groups into protein molecules promotes unfolding and dissociation of the quaternary structure. Nesterenko *et al.* (2014b) used dodecanoyl chloride (DDC) to induce acylation reaction with soy protein and sunflower protein. With the modified proteins, α-tocopherol achieved improved retention efficiency in encapsulation. The mechanism is that hydrophobic moieties in protein chains efficiently adsorb to oil droplet surface and improve the protection efficiency.

The selection of grafting compounds is also very important. Grafting of fatty acids with very short chain is not feasible for emulsion stability while fatty acids with very long chain could cause oil droplets flocculation. Matemu *et al.* (2011) found that the attachment of medium-chain fatty chain acids to soy protein 7S and 11S achieved desirable emulsifying properties. Improving the emulsifying ability of vegetable proteins by acylation reaction depends on the flexibility of protein, the balance of hydrophilic and hydrophobic groups, as well as surface charges.

Cationisation is another technique that has been used to improve functional properties of biopolymers (Wang *et al.*, 2011). Complex dispersion formed by protein and polysaccharides or multiple biopolymers were used to stabilise the emulsion. By such modification, protein functions such as emulsifying properties, solubility, and antioxidant activities can be improved. Hogan *et al.* (2003) found that using higher dextrose equivalent of carbohydrate to react with sodium caseinate could improve the microencapsulation efficiency of oil. As for vegetable proteins, Li *et al.* (2015) found that soy protein isolate-gum acacia conjugates showed greater emulsifying properties than soy protein isolate alone. Gan *et al.* (2008) found that the shelf life of soy protein isolate encapsulated fish oil was improved by Maillard reaction with ribose. Emulsions stabilised with pea globulin / gum Arabic also showed enhanced interfacial adsorption compared with pea globulin alone, due to the formation of protein / polysaccharides complex (Ducel *et al.*, 2004). Augustin *et al.* (2006) also reported that protein-carbohydrate conjugate is more effective in fish oil encapsulation than protein alone.

However, the degree of reaction between protein and polysaccharides should be precisely controlled to avoid the formation of brown colour and off-flavour. Interaction between polysaccharide and protein results in polymerisation reactions which could lower the emulsifying capability. For example, Li *et al.* (2015) found that the optimal reaction time between soy protein isolate and gum Arabic is six days, and afterwards, the emulsifying ability of the conjugates decreased. In addition, the health risks of such conjugates should be comprehensively studied.

Combining dairy proteins and vegetable proteins as wall material

As compared to proteins and polysaccharides, the use of dairy and vegetable proteins mixture have not been comprehensively studied. However, studies in the past few years have shown positive results, which indicate a great potential for the application of combined dairy and vegetable proteins in the food industry to reduce cost and improve product quality.

Overall, three mechanisms have been proposed to explain the potential synergistic effects between

proteins towards the improvement of encapsulation or emulsification efficacy. These mechanisms include competitive adsorption (Dickinson, 2011), surface property modification (Feng and Lee, 2016), and mechanical stability alteration (Jose *et al.*, 2016). Competitive adsorption is the mostly accepted and proven theory, though the competition is highly dependent on the protein-protein interaction and protein-interface interactions. For example, the competition of oppositely charged proteins could lead to multilayer adsorption, which could favour the encapsulation applications for the sake of thicker wall formation (Lundin *et al.*, 2010). In addition to the multilayer formation, competitive adsorption also helps to maximise the extent of surface tension reduction (Dickinson, 2011). Typically, reducing surface tension during emulsification tends to thermodynamically improve the stability against phase separation. Synergic stabilisation between milk protein and vegetable protein by surface properties modification was recently proposed using zein and sodium caseinate mixture (Feng and Lee, 2016). Sodium caseinate was found to tweak the surface hydrophobicity of zein colloidal particle surface, and hence, it increased its affinity to the oil-water interface. As a result, the emulsion droplets exhibited greater surface coverage and centrifugal stability. Another important mechanism could be attributed to the mechanical properties; Jose *et al.* (2016) found that the interaction between soy protein and whey protein provides stiffness and hardness. Although this study was done to investigate their macroscopic behaviour, the findings of the study indicate their potential mechanical enhancement as encapsulation wall materials.

There are no studies on the use of dairy and vegetable protein mixture; however, a great future is envisioned due to their synergistic interaction. In addition, vegetable protein could be regarded as a substitute for a portion of dairy protein in formulas, which is an effective approach to reducing the cost of ingredient supply in the industry. The substitution had already been applied in yogurt, coffee creamers, and whip toppings 30 years ago (Kolar *et al.*, 1979), and it is becoming a future trend as many health benefits associated with plant proteins are being revealed.

Other concerns of encapsulation efficiency

The precise control of particle size, morphology, moisture content, and surface properties is important to ensure encapsulation efficiency. In manufacturing, the control of stock feed temperature, flow rate, viscosity, inlet-outlet temperature, and the pressure in the nozzle is optimised to achieve smooth particle surface and spherical shape with a narrow size distribution. The higher concentration of feed stock solution leads to a high density of powder (Freudig *et al.*, 1999), and reconstitution property is another important parameter. The reconstitution of nutritional supplement powder involves four steps: wetting, submerging, dispersing, and dissolving. Wetting is a critical step to achieving fast dissolution, and typically, surface compositions of particles are important in the wetting process. During the formation of emulsions, hydrophilic groups are exposed to the aqueous phase; however, the presence of surface fat could reduce wettability. Therefore, reducing the surface fat is highly important in nutritional supplement powders that contain vegetable proteins.

Flowability is also an important property for powder during transportation, formulation, mixing, compression, and packaging. Particle surface composition, moisture, and size distribution are the main factors that influence flowability. According to Thalberg *et al.* (2004), flowability is associated with powder bulk density. For encapsulations using vegetable proteins, controlling the ratio between vegetable proteins and polysaccharides could alter the bulk density and surface hygroscopicity, which in turn could potentially improve encapsulation efficiency.

Conclusion

A combination of milk and vegetable proteins (especially pea protein) can be used as a robust wall material for encapsulating sensitive nutrients in nutritional supplement powders. This has gained attention as a cost-effective strategy. Competitive adsorption, surface property modification, and mechanical stability alteration have been proposed as mechanisms underlying interactions between proteins to improve their encapsulation or emulsification efficacy. Beyond this, several future technology directions have also been identified. A further reduction in particle size could be achieved using novel techniques such as ultrasound. Novel wall materials with special capability (e.g., spontaneous emulsification, self-assembly) could be studied as building blocks. At the same time, there remain many knowledge gaps, for example, the mechanism behind the digestion of nutritional powder in the human gastro-intestine tract, prediction of sensory properties of powder, and reasons for human variance and complexity regarding nutrition powder, which need to be comprehensively investigated.

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