Food Hydrocolloids 57 (2016) 229–235

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Potato protein based nanovehicles for health promoting hydrophobic bioactives in clear beverages

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ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form
14 January 2016
Accepted 27 January 2016
Available online 1 February 2016

Keywords:
Potato protein
Vitamin D
Nanoparticles
Nutraceutical delivery
Clear beverages
Fortification

ABSTRACT

Vitamin D is a fat soluble nutraceutical of great importance for multi-system function: calcium and bone metabolism, insulin reactivity, cell differentiation, the immune system and more. However its deficiency is a pandemic problem, which calls for fortification of staple foods and popular beverages with this vital micronutrient. Fortifying beverages with vitamin D poses tough challenges especially due to its low aqueous solubility, and acid-sensitivity. We studied the possibility of using potato proteins as protective nanovehicle for delivery of vitamin D in clear beverage solutions. Potato proteins are produced from a widely available and inexpensive raw material. They are considered GRAS and non-allergenic. Moreover, Potato proteins are natural and applicable in vegetarian, vegan and KOSHER PARVE products. Vitamin D3 (VD) – potato protein nanoparticles were formed in phosphate buffer at pH 2.5 and the solutions obtained were transparent. The VD – potato protein co-assemblies were much smaller than the VD aggregates without potato protein. The nanocomplexation provided significant protection and reduced VD losses during pasteurization, and during simulated shelf life tests under several different sets of storage conditions. Hence potato protein shows promise as a good protective carrier for VD, and possibly other hydrophobic bioactives, for enrichment of clear beverages and other food & drink products, to promote human health.

1. Introduction

Potato protein has conventionally been regarded as a waste of starch manufacture. Nevertheless, its nutritional value has been shown to be greater than that of other vegetable and cereal proteins (Friedman, 1996; Kaldy, 1972; Kapoor, Desborough, & Li, 1975), higher than that of casein (Jackman & Yada, 1988) and comparable to that of whole egg (Friedman, 1996; Jackman & Yada, 1988; Kaldy, 1972; Kapoor et al., 1975).

Potato proteins are commonly divided into three fractions: Patatin, protease inhibitors and other high molecular weight proteins representing 40%, 50% and 10% of total soluble protein, respectively (van Koningsveld et al., 2006; Waglay, Karboune, & Alli, 2014). According to some sources (Racusen & Weller, 1984; Waglay et al., 2014), patatin is an 88 KDa dimer glycoprotein (each monomer is ~40 KDa), while another source, based on X-ray crystallography, suggests it is a trimer (Rydel et al., 2003). It appears to serve as a storage protein, though it has a few enzymatic activities. It was shown that patatin exhibited both lipid acyl hydrolase and acyltransferase activities with many lipid substrates (Bárta, Bártová, Zdráhal, & Šedo, 2012; Liu, Han, Lee, Hsu, & Hou, 2003; Ralet & Guéguen, 2000). Patatin has been shown to possess antioxidant ability and excellent foaming and emulsifying properties (Liu et al., 2003; van Koningsveld et al., 2006; Waglay et al., 2014). The second fraction comprises protease inhibitors, ranging from 5 to 25 KDa, which have beneficial properties such as anti-carcinogenic (Blanco-Aparicio et al., 1998) and anti-microbial (Kim et al., 2005) properties. It was reported that they have good emulsification and emulsion stabilization properties over very wide ranges of pH and ionic strength (Ralet & Guéguen, 2000). Hydrolyzed potato protein has been found to possess both antioxidant and emulsifying properties (Nieto et al., 2009).

Nevertheless, the potential of potato proteins for nanoencapsulation of hydrophobic nutraceuticals has apparently not yet been studied.

Nanoencapsulation is a rapidly developing field of technology which has great potential to overcome solubility limitations, protect sensitive compounds from degradation during production and shelf-life (Semo, Kesselman, Danino, & Livney, 2007; Zimet &

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http://dx.doi.org/10.1016/j.foodhyd.2016.01.027
0268-005X/

Livney, 2009), mask undesired off-flavors (Shpigelman, Cohen, & Livney, 2012), and promote bioavailability of encapsulated nutraceuticals (Chen, Remondetto, & Subirade, 2005; Haham et al., 2012).

The solubilization of hydrophobic health-promoting bioactives in clear drinks is highly sought by beverage producers to provide added value for the consumer, but it still poses tough challenges, particularly in shelf stable drinks. Most food grade surfactants, which may be used for the task are synthetic e.g. the Tween (polysorbate) series, and thus preclude an "all-natural ingredients" labeling. Other ways to enrich beverages with hydrophobic nutraceuticals, like gum Arabic, milk proteins, soybean proteins and Maillard reaction conjugates (Danino, Livney, Ramon, Portnoy, & Cogan, 2009; Ron, Zimet, Bargarum, & Livney, 2010; Shpigelman, Israeli, & Livney, 2010), are either expensive, not always available, or are using allergenic components. Saponins, which are natural low molecular weight surfactants, have shown limited efficacy at low pH (Yang, Leser, Sher, & McClements, 2013).

Potato proteins are produced from a widely available and inexpensive raw material. They are considered GRAS and non-allergenic (Eldred, 2001; Giuseppin, 2012) and hence are not included in the list of known food allergens, which must be declared on the label-a crucial safety requirement for the well-being of consumers and a major advantage for the manufacturers. Moreover, Potato proteins are natural and applicable in vegetarian, vegan and KOSHER PARVE products. Furthermore, because potatoes have a high water content and an acidic pH (5.4–5.9 (US-FDA)), their proteins are considerably water-soluble at low pH. The relatively high solubility of potato protein isolate at the low pH range makes it particularly useful for the enrichment of beverages with an acidic pH, such as fruit beverages, coke etc.

Hence, we wished to evaluate the applicability of potato protein as a delivery system for hydrophobic nutraceuticals, using vitamin D_3 (VD) as a model.

VD is a fat soluble vitamin of great importance for calcium and phosphorus homeostasis (Eitenmiller, Landen, & J, 1999; Holick, 2006; Vieth, 1999;). It was found that maintaining proper levels of VD helps preventing a host of illnesses, ranging from muscle weakness, cardiovascular diseases (Holick, 2004a; Wang et al., 2008), cancer (Touvier et al., 2011) and diabetes (Holick, 2004a; Wang et al., 2008). VD is also associated with regulation of immune function and decreased risk of autoimmune diseases (Holick, 2004a).

According to the Institute of Medicine of the United States National Academies, the adequate VD intake for infants (below 12 months) is 10 μg per day, and the recommended daily allowance between 1 and 70 years of age is 15 μg per day, and for the elderly above the age of 70 years, 20 μg per day (Institute of Medicine, 2011).

Humans can synthesize VD in the skin upon exposure to ultraviolet type-B radiation (Holick, 2006, 2007b). Nevertheless, about 1 billion people worldwide are VD deficient or insufficient (Holick, 2007a), mainly due to avoidance of sun exposure to prevent melanoma, the use of sunscreen which blocks VD synthesis and low dietary intake (Holick, 2004b). There are scarce natural dietary sources, including certain oily fish (e.g. salmon and sardines), egg yolk and cod liver oil. Over the counter supplements in pill or liquid form are available, but most people are unaware of the problem, or are aware, but do not take supplements. The intake levels of females over 12 and of males over 50 in the USA are inadequate, and existing fortification (mainly of milk and cereals) are insufficient (Calvo, Whiting, & Barton, 2004). For these reasons, there is an urgent and high need for VD fortification of staple food and popular beverages.

VD insolubility in aqueous systems, poses a challenge for providing the vitamin in low- and nonfat products. Moreover, VD is sensitive to light, oxygen and high temperatures, which induce its isomerization and degradation into its inactive forms (Bell & Lawson, 1978; Eitenmiller et al., 1999). It is also adversely affected by acids (Ottaway, 2010) and rapidly degrades under stomach conditions if unprotected (Markman & Livney, 2012).

The objective of the current work was to explore the potential of potato protein as a nanovehicle for VD, and to evaluate its performance in terms of dispersion and protection capabilities, for food and beverage applications.

2. Materials and methods

2.1. Materials

VD3 in powder form (USP grade) was purchased from Sigma–Aldrich (Rehovot, Israel). Potato protein isolate powder (97% protein) was purchased from Proteinfactory® and manufactured by Theta Brothers Sports Nutrition (Brick, NJ; lot # 90607-1).

2.2. Methods

2.2.1. Solution preparation

A 25 mM phosphate buffer solution (target pH 2.5) was prepared as follows: 1.56 g $NaH_2PO_4 \cdot 2H_2O$ and 880 μl H_3PO_4 were dissolved in 1000 ml HPLC grade water. The buffer was used without further pH correction. The exact final pH of the solution was 2.5.

Potato protein isolate was dissolved in pH 2.5 buffer and shaken mechanically at room temperature (21–25 °C) overnight. The next day, the solution was filtered through a 0.45 μm filter, to remove any undissolved matter. The remaining protein concentration was quantified by absorbance at 277 nm based on a predetermined calibration curve. All spectroscopic measurements in this study were performed using an Ultrospec 3000 spectrophotometer (GE Healthcare, Waukesha, Wisconsin, USA), using a 1 cm path length cuvette. The final concentration of the potato protein stock solution was 1 mg/ml. The potato protein molar concentration was estimated assuming that the averaged molecular mass of the potato protein isolate used was 12.7 KDa (based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (Tricine-SDS–PAGE)- see supplementary data) indicating a certain degree of hydrolysis during its production.

To incorporate the VD in the protein nanoparticles we employed a method we used in previous studies in our group (David, Zagury, & Livney, 2015; Haham et al., 2012; Semo et al., 2007): VD stock solution was prepared at the desired concentration in pure ethanol. The stock solution was added dropwise to phosphate buffer or to potato protein solution while vortexing, bringing the sample to a final ethanol concentration of 2%. The samples were kept at 4 °C for two hours for equilibration.

2.2.2. Solubilization and transparency characterization

Solutions of increasing concentrations of VD in phosphate buffer with and without 1 mg/ml (79 μM) potato protein were prepared. Final VD concentrations were 10, 25, 50, 75 and 100 μg/ml. The molar ratios examined were 0:1, 0.3:1, 0.8:1, 1.7:1, 2.5:1 and 3.3:1 VD:potato protein. The absorbance of the solutions at 600 nm was measured. The blank solution was 25 mM phosphate buffer pH 2.5 with 2% ethanol.

2.2.3. UV absorbance spectra

0.5 mg/ml potato protein was prepared with 10 μg/ml VD (26 μM) (0.7:1 VD:potato protein molar ratio) in pH 2.5 phosphate buffer with 2% ethanol, as described above, and its absorbance