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Effect of whey protein agglomeration on spray dried microcapsules containing *Saccharomyces boulardii*

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ABSTRACT

This work investigates the effect of whey protein agglomeration on the survivability of *Saccharomyces boulardii* within spray dried microcapsules. It attempts to go beyond phenomenological observations by establishing a relationship between physicochemical characteristics of the polymeric matrix and its effect on probiotic endurance upon spray drying. It is well known that this type of thermal shock has lethal consequences on the yeast cells. To avoid such undesirable outcome, we take advantage of the early agglomeration phenomenon observed for whey protein by adjusting the pH value of preparations close to isoelectric point (pH 4–5). During the subsequent process of spray drying, development of whey protein agglomerates induces formation of an early crust, and the protein in this molten globular state creates a cohesive network encapsulating the yeast cells. It appears that the early crust formation at a given sample pH and temperature regime during spray drying benefits the survivability of *S. boulardii* within microcapsules.

1. Introduction

Whey protein is a valuable by-product of the cheese industry and finds application in a wide range of dairy/non-dairy beverages, desserts and meat products (Anandharamakrishnan, Rielly, & Staply, 2007). It is mainly a mixture of β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins with an isoelectric point at pH ~ 5.0 (Fitzsimons, Mulvihill, & Morris, 2007). Whey protein physiochemical properties make it suitable for nutraceutical applications, including controlled or targeted delivery in the release of bioactive compounds/substances (Gunasekaran, Xiao, & Ould Eleya, 2006). It possesses an excellent balance of essential and sulphur containing amino acids, and this nutritional quality is often highlighted in health and sports supplements. However, various processing conditions compromise this nutritional quality and the proper reconstitution upon hydration following thermal denaturation, thus, it seldom exhibits both techno- and biofunctionality in chorus (Daemen & van der Stege, 1982).

The native state of globular proteins occurs from the primary to the quaternary conformation through stabilised interactions between adjacent peptide chains. Changes in environmental conditions, including temperature or pressure increase and variation in pH, disrupts this native globular conformation, which renders the protein biologically inactive. The unfolded protein state allows enhanced hydrophobic forces, hydrogen bonding and disulphide–sulphydryl interactions, which result in aggregation, coagulation or even precipitation (Pelegrine & Gasparetto, 2005; Terebiznik, Buera, & Pilosof, 1997). To date, the precise effect of microencapsulation via, for example, spray drying on molecular aspects of the protein structure is a subject of considerable interest. Microencapsulation protects and then releases the core inclusion depending on the physiochemical property of the wall material (Fang & Bhandari, 2010).

Spray drying is the most common and cost effective method to produce encapsulated food and pharmaceutical products; in comparison to freeze drying, it is 30 times cheaper for product manufacture (Desobry, Netto, & Labuza, 1997). As proposed by Shahidi and Han (1993), the reason of using encapsulated products in the food industry is to isolate and protect the core material from the outside environment, to reduce the transfer rate of core material to the exterior, and to mask the sensory attributes of the core substance. Encapsulation through spray drying has been carried out on probiotic bacteria (lactobacilli, bifidobacteria, etc.) and the relatively heat stable yeast (Favaro-Trindade & Grosso, 2002; Picot & Lacroix, 2004).

Saccharomyces boulardii is a non-pathogenic yeast and is considered as a biotherapeutic agent. Clinical studies have proven its efficacy in treatment or prevention of intestinal disorders including antibiotic associated diarrhoea (Kotowska, Albrecht, & Szajewska, 2005), traveller's diarrhoea (Elmer & McFarland, 2001), and recurrent *Clostridium difficile* disease (Elmer, McFarland, Surawicz, Dankos, & Greenberg, 1999). To achieve the above benefits, sufficient amount of live yeast cells need to reach the large intestine but,

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in reality, many of the cells are inactivated by the extreme conditions within the gastrointestinal tract, i.e., gastric acidity and bile to name but a few (Klein, Elmer, McFarland, Surawicz, & Levy, 1993). Microencapsulation of yeast with an appropriate encapsulant can serve as an efficient method to deliver sufficient live and active cells to the large intestine.

The present research reports on avenues that may be used for attaining maximum survivability of yeast within whey protein microcapsules obtained by spray drying. The thermal treatment during spray drying has a lethal effect on the yeast cells as well on the globular conformation of protein molecules that undergo denaturation. We have attempted, therefore, to manipulate the pH range of the feed solution (from 4 to 6) in order to produce microcapsules that possess viable yeast cells within a moderately denatured protein matrix.

2. Materials and methods

2.1. Materials

2.1.1. Whey protein isolate

The material used was a product from Fonterra, Waikato, New Zealand. According to the supplier, the bulk density of the whey protein powder was 0.45 g/ml with a microbial count of 9900 cfu/ml. As per product specification, it contains 91.3% protein, 0.7% fat, 3.5% moisture, 3.8% ash and 0.44% lactose.

2.1.2. S. boulardii

Lyophilised yeast cells were obtained from Biocodex (Gentilly, France). Yeast cells were cultured to a concentration of $10^{7.5}$ cfu/ml (peak of the log phase) in yeast nitrogen base broth (YNB) containing 1% D-glucose (BDH, Merck, UK).

2.1.3. Yeast malt extract agar (YMA)

This was obtained from Sigma–Aldrich Private Ltd., New South Wales, Australia. It contains peptic digest of animal tissue (0.5%), yeast extract (0.3%), malt extract (0.3%), dextrose (1%) and agar (2%), and it was used presently for further growth and identification of the yeast colonies.

2.2. Sample preparation and analysis

2.2.1. Whey protein dispersions

These were prepared by mixing the protein powder (20%, w/w) in distilled water at ambient temperature (~24 °C) to produce a solution of unbuffered "natural" pH of 6.6–6.7. Dispersions were made using a magnetic stirrer for 30 min to ensure proper dissolution and stored at 4 °C overnight to achieve thorough hydration. Dropwise addition of 1 M NaOH or 1 M HCl solution was used to obtain samples at the required pH range.

2.2.2. Rheological analysis

Denaturation and subsequent structure formation of whey proteins (20%, w/w) were examined using a controlled strain rheometer AR-G2 (TA Instruments, New Castle, DE). The viscoelastic property of the whey protein gel as a function of temperature was characterised by small-amplitude oscillation in shear using a parallel-plate geometry of 40 mm in diameter. Samples were loaded on a Peltier plate at 25 °C with the outer edges being covered in silicone oil from BDH (50 cS) to minimise moisture loss. The temperature of the system was raised to 85 °C at a scan rate of 1 °C/min followed by an isothermal run of 20 min at the same temperature. A constant angular frequency and strain of 1 rad/s and 0.1%, respectively, were maintained throughout the experimental routine.

2.2.3. Differential scanning calorimetry

These experiments were performed using Setaram Micro DSC VII (Setu-rau, Caluire, France). To analyse the effect of temperature on the chemistry of protein molecules, native whey protein samples at pH 4, 5 and 6 (20%, w/w) were placed in 850 mg cell with an equal amount of distilled water in the reference pan. Samples were stabilised for 1 h before the experimental run from 25 to 95 °C and a scan rate of 1 °C/min. The mid-denaturation temperature (T_{mid}) that corresponds to the maximum of the transition peak was determined using Setaram proprietary software.

To determine the extent of protein denaturation during spray drying, the microcapsules of whey powder from the spray dryer were resuspended in distilled water to a concentration of 20% (w/w) with the aid of a magnetic stirrer, and refrigerated at 4 °C for 12 h. Samples were then subjected to thermal treatment at the same temperature regime as for the whey protein gels of the preceding paragraph.

2.2.4. Fourier transform infrared spectroscopy

Conformational aspects of the protein molecules, obtained in the form of a white powder after spray drying at different inlet temperatures, were examined using a Perkin Elmer Spectrum 100 FTIR spectrometer (MA, USA). Sample spectra were recorded in the absorbance mode. For each spectrum, an average of four scans was recorded at 8 cm^{-1} resolution in the wavenumber range of 800–4000 cm^{-1} after background subtraction to ensure elimination of extraneous artifacts. The Perkin Elmer Spectrum 100 utilises a "smart, plug-play-and-go" Universal-ATR (UATR) technology, which ensured that sample loading was consistent throughout our work.

2.2.5. Isolation and culturing of yeast cells

Commercially available freeze-dried cells of *S. boulardii* (0.1 g) were transferred into 10 ml of a sterile YNB broth (a nutritious material used for the growth of yeast cells) to incubate for 24 h at 37 °C. The outcome is a cloudy liquid medium, and 1 ml of that sample was serially diluted in 9 ml of saline solution (0.85% of NaCl) and plated onto YMA culture media to incubate for 48 h at 37 °C; this clear and solid-like medium is needed to identify the yeast colonies and assist with the enumeration of the cell mass.

Colonies of yeast that have been identified as *S. boulardii* were transferred to 10 ml of YNB broth, by tipping a glass rod in YMA media, for further growth; thorough mixing was implemented with a vortex mixer. An aliquot of 1 ml of the above broth was inoculated to the same broth line (YNB, 99 ml), and incubated at 37 °C for 12 h (peak of the log phase of *S. boulardii*). This served as the seed culture of the probiotic for subsequent microencapsulation work.

2.2.6. Microencapsulation of yeast with spray drying

Microencapsulation was performed by spray drying a whey protein suspension containing the culture of *S. boulardii*. In doing so, yeast cells were harvested from the late log growth phase, and 100 ml of broth containing yeast cells were centrifuged at a relative centrifugal force of 3944*g* at 4 °C for 10 min. Precipitated pellets obtained after centrifugation were washed using a saline solution and then added to whey protein (20%, w/w) dispersions, with an average cell count of $\sim 2 \times 10^8$ cfu/ml.

Spray drying of the samples was performed using LabPlant Spray Dryer SD-Basic (Labplant UK Ltd.) with the help of a peristaltic pump and controlled settings of air pressure at 4.0 kg/cm^2 with a flow rate of 8.0–12.0 ml/min. The inlet temperatures varied between 70 and 90 °C in accordance to our experimental design. The reading of outlet temperature on the instrument's control panel was within the range of 45–50 °C. To ensure an even distribu-