Food Research International 56 (2014) 146–158

Contents lists available at ScienceDirect

Food Research International

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

Assessment of agave fructans as lyoprotectants of bovine plasma proteins concentrated by ultrafiltration

Laura T. Rodriguez Furlán [a], Pamela Aldrete Herrera [b], Antonio Pérez Padilla [a], Rosa I. Ortiz Basurto [b], Mercedes E. Campderrós [a,*]

[a] Instituto de Investigaciones en Tecnología Química (INTEQUI-CONICET), Facultad de Química, Bioquímica y Farmacia (UNSL), Chacabuco y Pedernera - 5700 San Luis - Argentina
[b] Instituto Tecnológico de Tepic (ITT), Laboratorio Integral de Investigación en Alimentos (LIIA), Nayarit - México

ARTICLE INFO

Article history:
Received 24 October 2013
Accepted 11 December 2013

Keywords:
Agave fructans
Freeze-drying
Shelf life
Functional properties
Ultrafiltration

ABSTRACT

The effectiveness of fructans as protectant agent to prevent protein denaturation was evaluated during freeze-drying and storage. Native agave fructans and two fractions obtained by ultrafiltration were assessed as protective agents. Since the protein denaturation during freeze-drying can alter the functional properties of proteins, the evaluation of bovine plasma protein properties was performed with different concentration of fructans, pH and freezing temperatures. The results showed that the incorporation of fructans as lyoprotectants improved functional properties, due to the reduction of protein denaturation with maximum stabilization of plasma bovine protein at a fructan concentration between 5% and 10% (w/v) for native fructans and 10% (w/v) for the fractions of fructans at −40 °C. Moreover, when freezing at −4 °C, fructans fraction at 15% (w/v) prevented protein denaturation while native fructans exerted no acceptable protection ($P < 0.001$). This behavior could be explained by a microstructure study of fructans, where the higher heterogeneity in size of native fructans may be the reason for the lower protective effect. Furthermore, the higher heterogeneity of the samples affected the extent of non-enzymatic browning in the range of the temperatures assessed. The shelf life of freeze-dried proteins was improved from 1.7 months for the control sample to 7–11, depending on the saccharide considered. Taking into account the health benefits of fructans, since they are categorized as prebiotic, the protein–saccharide mixture may be valuable functional ingredients for food formulations.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Fructans as non-digestible fermentable saccharide are among the most studied and well established prebiotics (Gibson, Probert, Van Loo, Rastall, & Robertfroid, 2004; Henelly, Dunne, O'Sullivan, & O'Riordan, 2006; Niness, 1999). A natural source rich in these compounds are the *Agave plants*, abundant in arid regions of Latin America, being Mexico considered as the center of origin and biodiversity of *Agave* species. The taxonomic diversity of that country has led to the development of industries for the production of alcoholic beverages, and recently, the production of syrup and agave fructans. The production of *agave* oligofructans and/or syrup is carried out by the hydrolysis of agave fructans (Ávila Fernández, Galicia-Lagunas, Rodríguez Alegría, Olvera, & López Munguía, 2011). *Agave tequilana* Weber var. blue is the most used for syrup and fructan production. This plant accumulates between 13% and 17% (w/w) fresh weight fructan in mature plants, being similar to the amount found in chicory (15.2–20.5% (w/w) fresh weight), the current source of inulin (Ávila Fernández et al., 2011; Mellado Mojica & López, 2012; Van Loo, Coussement, de Leenheer, Hoebregs, & Smits, 1995). While chicory inulin is a rather linear fructan in which fructose molecules are joined through β(2–1) linkages, fructans present in agave, particularly in *A. tequilana*, have a degree of polymerization (DP) ranging from 3 to 29 (López, Mancilla Margalli, & Mendoza Diaz, 2003; Mellado Mojica & López, 2012).

Fructans have many technological advances as food additive. Thus, higher DP fructans are more suitable as fat replacers (Verraest, Peters, Van Bekkum, & Van Rosmalen, 1996). Van den Ende et al. (2006) reported that different applications required fructans with a different DP and Gonzalez Tomas, Coll Marqués, and Costell (2008) reported how the different viscoelasticity of inulin-starch based dairy system was influenced by the inulin average chain length.

It is known that the presence of saccharides during the freeze-drying process preserves the native structure of proteins in the dried state. The mechanisms by which saccharides stabilize proteins during this process are due to their ability to form a glassy state that inhibits crystallization and influences the kinetics of deteriorative reactions upon storage (Buera, Schebor, and Elizalde (2005); Carpenter, Prestrelski, and Arakawa (1993); Minson, Fennema, & Amundson, 2006). The saccharides should have a high glass transition temperature (T_g) for a better protection (Rodriguez Furlán, Lecot, Pérez Padilla, Campderrós, & Zaritzky, 2011, 2012). They also stabilize proteins by direct interaction,

* Corresponding author. Tel./fax: +54 266 4426711.
E-mail address: mcamp@unsl.edu.ar (M.E. Campderrós).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.12.014

through hydrogen bonding (Wolkers, van Kilsdonk, & Hoekstra, 1998). Previous studies demonstrated that oligosaccharides like fructans stabilize cellular membranes during dehydration (Hincha et al., 2007).

In many regions of the world, especially in countries such as Argentina and Mexico, the majority of livestock production corresponds to cattle, being the animal blood the main by-product. Taking into account that, on average, 17% (w/w) of blood are proteins, the need to recover and use this resource is understood (Cheftel, Cuq, & Lorient, 1989). Bovine plasma production requires the separation of components by centrifugation of two fractions: the plasma and red blood cells. The proper treatment of these proteins increases profitability in the meat industry. Bovine plasma proteins are valuable from the nutritional point of view due to the presence of amino acids that are essential in small amounts in other foods. Moreover, due to the good functional properties of the plasma fraction, it is used as ingredient in a variety of foodstuffs (Del Hoyo, Rendueles, & Díaz, 2008; Silva & Silvestre, 2003).

However, these proteins, as many others, are structurally unstable in solution, and are susceptible to conformational changes due to several stresses encountered during purification, processing and storage. Freeze-drying is commonly used in the manufacture of protein products, since the proteins in dried state are less prone to denaturation and precipitation during transportation and storage (Ohtake, Kita, & Arakawa, 2011).

As was previously demonstrated, inulin protects the plasma bovine protein structure during freeze-drying, preserving, thus, the functional protein properties (Rodriguez Furlán et al., 2012). Hence, the aim of this study was to assess the protective capacity of Agave native fructans (NF) and two different fractions: high performance fructans (HPF) and high degree of polymerization fructans (HDPF) during freeze-drying process. The process of freeze-drying and self-life of the matrix (or mixture) formed by the plasma protein and fructans of Agave, and the characterization of the mixture were studied in order to optimize and predict its behavior in potential food applications.

2. Materials and methods

2.1. Raw materials

Commercial products of *A. tequilana* native fructans (NF) in powder and 72°Brix was donated by the company Agaviótica SA, Monterrey, Mexico. Two fractions of fructans concentrated were obtained by tangential ultrafiltration process at the Instituto Tecnológico de Tepic, Nayarit, Mexico. To obtain the high performance fructans (HPF) and high degree of polymerization fructans (HDPF) a solution to 20°Bx was ultrafiltered in commercially membrane modules Pellicon-2 (Millipore, MA, USA), with a nominal molecular cut off (MWCO) of 1 and 10 kDa respectively (20 °C, 3 bar y 4 $L.min^{-1}$). After separation, the fructan fractions retained on each membrane was spray-dried in LPG5 Model, CIMA Industries Inc., Chinese (inlet/outlet temperature: 100/80 °C, atomizer speed: 30,000 rpm and feed flow: 17.5 mL/min), the characteristic of powders and NF are reported in Table 1 (Aldrete Herrera, 2013; Espinosa Castrejón, 2012).

Table 1
Characteristic of *Agave tequilana* fructans additives ($g \cdot 100\ g^{-1}$).

Characteristic ($g \cdot 100\ g^{-1}$)	NF	HPF	HDPF
Humidity	4.62 ± 0.2	3.01 ± 0.03	2.52 ± 0.23
Ash	0.1 ± 0.02	0.15 ± 0.05	0.1 ± 0.05
Total carbohydrate	97.5 ± 1.7	98.2 ± 0.7	98.6 ± 0.5
Reducing sugars	13.6 ± 0.5	0.8 ± 0.06	0.4 ± 0.04
Fructans	83.9 ± 0.3	97.4 ± 0.5	98.2 ± 0.3
Aw	0.41 ± 0.02	0.33 ± 0.001	0.3 ± 0.003
Enriched DP	–	4–80	24–80

HPF, high performance fructans; HDPF, high degree of polymerization fructans and NF, fructans natives.

Spray dried bovine plasma (Yerubá S.A., Argentine) has been used. The molecular weights of proteins are in the range of 15,000 to 80,000 Da. The proximate composition provided by the manufacturer was: 76 ± 5% proteins, 0.1% fat, 10% ash, 4% water, 1% low molecular weight compounds.

2.2. Plasma protein concentration by membrane technology

The micro and ultrafiltration processes show many advantages over traditional methods for protein concentration and purification (Noordman, Ketelaar, Donkers, & Wesselingh, 2002; Torres, Marín, Ramos, & Soriano, 2002). Indeed in a previous paper Rodriguez Furlán, Pérez Padilla, and Campderrós (2010a) demonstrated that the UF-DD step allows concentrating protein, eliminating insoluble macroscopic components, and reducing the saline content. In this work, improvements were made to the equipment using two membrane cassettes (Pellicon modules, Millipore, Bedford, MA, USA), doubling the membrane area to 1 m^2 employing a new centrifugal pump (Fluid-o-Tech, Milano Italy, PA 1001, caudal of 1200 L/h) so the experience of bovine plasma concentration was faster without refrigeration needs. The feed solution of bovine plasma which was dissolved in deionized water to 3% w/v using a mixer at low speed to avoid the formation of vortex and to minimize the appearance of foam. The feed solution (5 L) was driven first through a porous support (Viledon F 02431 D, Germany) to remove macroscopic aggregates, and then through to a frontal flow filter of microfiltration (MF) of 8 μm (Hidroquil, Argentina). This pretreatment process of the solution reduces fouling in the subsequent step of ultrafiltration (UF). The filtered solution was pumped to the UF module, which contains modified polyethersulfone membranes with a molecular weight cut-off (MWCO) of 10 kDa. The protein concentration by UF was carried out by continuously removing the permeate stream until the desired concentration of 4.2% (w/v) (VCR = 1.4), was achieved. The operating conditions were the following: transmembrane pressure (ΔP) of 1.8 bar, flow rate of (0.71 ± 0.08) L/min. A discontinuous diafiltration (DD) process was applied to remove salts and other contaminant of low molecular weight. For this operation the feed solution was the UF concentrate diluted to the initial volume (5 L) with deionized water in a single state and ultrafiltrated to the desired concentration range. The cleaning of the fouled membrane was performed by applying a "Cleaning in Place" (CIP) procedure according to the manufacturer's instructions. Measurements of normalized water permeability were performed in order to verify recovery of flow through the membrane and the optimal performance during the separation process.

2.3. Freeze-drying stage

The bovine plasma protein concentrate (concentration: 4.2% w/v), obtained by UF was fractioned into four fractions. A fraction as witness sample (control) was reserved and the fructans (NF = native fructans; HDPF = high degree of polymerization fructans; HPF = high performance fructans) used as protective agents were added to the rest, in concentrations of 5%, 10%, 15% and 20% (w/v). The protein concentrates were placed on stainless steel trays and frozen in a freezer at −4 °C, −20 °C and −40 °C and freeze-dried using a lyophilizer (Rificor S.A., Argentina) at 1 bar of pressure for 48 h. The samples temperature was controlled by a temperature sensor.

2.4. Determination of denatured protein content

The soluble protein content was determined after isoelectric precipitation of denatured/aggregated proteins (de Wit, 1981, 1990; Meza, Verdini, & Rubiolo, 2009; Rodriguez Furlán et al., 2010a; Verheul, Roefs, & de Kruif, 1998). Solutions of 1% (w/v) protein concentrate were adjusted to pH 4.8 using 0.1 N of NaOH and HCl. An aliquot of the solution was centrifuged (Rolco 2070 centrifuger, Argentina) at

Download English Version:

https://daneshyari.com/en/article/6396997

Download Persian Version:

https://daneshyari.com/article/6396997

Daneshyari.com