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Scale-up of the process to obtain functional ingredients based in plasma protein concentrates from porcine blood

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ABSTRACT

The feasibility of a scaled-up process to obtain two protein concentrates from porcine blood plasma, i.e. serum and albumin, for use as functional food ingredients was assessed. The process consisted of fractionating plasma proteins by salting out, concentrating and purifying fractions by means of membrane technology, and subsequently dehydrating through spray-drying. The fractionation process allowed a good isolation of the desired proteins, which were then concentrated and desalted in a tangential flow filtration (TFF) process combining ultra and diafiltration. Purification, pre-concentration and dehydration were successfully achieved. The functional properties of dehydrated serum and albumin were determined. As compared to the same hemoderivatives obtained by a lab-scale production system, serum maintained the gelling properties; albumin exhibited similar foaming properties; and both serum and albumin concentrates showed slightly improved emulsifying properties.

1. Introduction

Animal blood is an abundant by-product of the meat industry derived from the activity of industrial slaughterhouses. It shows a high potential for utilisation, especially for the manufacture of foodstuff, due to its useful functional proteins. Approximately 60% of blood consists of plasma, a naturally occurring protein mixture.

To date, much research has studied the functionality of plasma proteins. Their ability to gel under thermal treatments and to form emulsions and foams are their best properties, hence they can be considered as good functional ingredients for the food industry (Alvarez, Bances, Rendueles, & Díaz, 2009; Chen & Lin, 2002; Parés & Ledward, 2001; Parés, Saguer, Saurina, Suñol, & Carretero, 1998; Saguer, Fort, Parés, Toldrà, & Carretero, 2007; Toldrá, Aristoy, Mora, & Reig, 2012). It is well-known that plasma has excellent gelling properties and water-binding capacity, thus food grade whole plasma can be used in meat products, for example, to improve texture and sliceability or to reduce drip and cooking losses or storage purges (Cofrades, Guerra, Carballo, Fernández-Martin, & Jiménez-Colmenero, 2000; Hurtado, Saguer, Toldrà, Parés, & Carretero, 2012; Viana, Silva, Delvivo, Bizzotto, & Silvestre, 2005). Recently, porcine plasma has also been used to produce protein-based biodegradable films and food coatings (Nuthong, Benjakul, & Prodpran, 2009).

The complex mix of plasma proteins can be gathered into three major groups according to a relevant content for functionality studies. This classification includes: fibrinogen, albumin, and globulins.

Fibrinogen, a single protein that represents 3% of the total content, is a multidomain fibrous protein of 340 kDa and isoelectric point (*pI*) near 5.5, involved in blood coagulation. A cold binding system based on fibrinogen from animal blood, Fibrimex™, has been developed in the Netherlands and is commercialized in the U.S. by a Nebraska based company. Good results for Fibrimex were reported in a recent study comparing different binding agents in re-formed beef steaks (Lennon, McDonald, Moon, Ward, & Kenny, 2010) or as a cold-set binder in both chilled and raw meat products (Ofori & Hsieh, 2011), thus demonstrating that using fibrinogen for this purpose is technologically and economically feasible.

Once fibrinogen is removed from plasma, the remaining solution, serum, mainly contains globulins and albumin. Albumin, a globular protein with a molecular weight of 66–69 kDa and *pI* around 4.8, is the most abundant plasma protein, representing up to 60% of the protein content. Globulins comprise a heterogeneous group of globular proteins that include enzymes, carrier proteins and immunoglobulins, and account for 40% of the plasma protein content. The range of molecular weights of globulins spreads from a few to hundreds of kDa and they have a *pI* mostly between 5 and 7 (Cheftel, Cuq, & Lorient, 1985; Putnam, 1975).

In previous studies conducted in our laboratory the functionality of isolated plasma protein fractions as well as their contribution to the global functionality of plasma via protein–protein interactions, were investigated (Dàvila, Parés, Cuvelier, & Relkin, 2007; Dàvila, Parés, & Howell, 2006; Dàvila, Saguer, Toldrà, Carretero, & Parés, 2007, Saguer, Alvarez, & Ismail, 2012). The results showed that most of the protein fractions had good solubility, emulsifying, foaming and gelling properties at varying pH's. Taking advantage of this knowledge would allow the formulation of plasma-based ingredients with enhanced functionality to meet specific requirements.

In the work cited above, the salting out fractionation together with a membrane dialysis technique was used to obtain the plasma protein

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fractions, which were then concentrated through lyophilisation. This was a time-consuming process that allowed production of small amounts of each ingredient for functional testing at laboratory scale, but is not suitable for production on a larger scale, due to poor performance and excessive cost.

So, the aim of the present study was to scale-up the process to obtain protein concentrates of two blood plasma protein fractions—serum and albumin—through a method closer to industrial-scale production.

For this purpose, diafiltration, a membrane-based tangential flow filtration (TFF) operation instead of dialysis was used to remove salts from the protein solutions. Dialysis procedures at industrial level can take up to several days and require large volumes of water and great manipulation of samples. The main advantage of the TFF technique as compared to dialysis, in addition to a reduced processing time, is that protein solutions can be concentrated and diafiltered to lower the concentration of salts in the system, thus minimizing the risk of sample loss or contamination and reducing the cost of the subsequent dehydration step.

Spray-drying is a common technique to produce dehydrated food materials that has usually proved not only efficient but also economic. Equipment is readily available and production costs are lower than most other methods. It has been reported that spray-drying has higher production output and costs up to several times less per kg of water removed than commercial scale freeze drying (Desmond, Stanton, Fitzgerald, Collins, & Ross, 2001, Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Moreover, it has been described as a technology that can improve emulsifying and foaming properties of globular proteins (Bernard, Regnault, Gendreau, Charbonneau, & Relkin, 2011). Therefore, considering both lower operative costs and higher production rates, spray-drying was chosen as the dehydration method instead of freeze-drying.

In order to assess the feasibility of the production system proposed to obtain blood proteins showing good functionality, the proximate composition, as well as the emulsifying, foaming, and gelling properties of spray-dried serum and albumin fractions were determined and compared to the characteristics of the same ingredients produced previously at laboratory scale.

2. Material and methods

2.1. Production of blood-ingredients: serum and albumin concentrates

Commercial hygienic porcine blood with sodium polyphosphate as anticoagulant was collected from the refrigerated storage tank of a local industrial slaughterhouse (Norfrisa S.A., Riudellots de la Selva, Girona, Spain) in sterile containers. Blood was maintained under refrigeration until the plasma was separated in the laboratory by centrifuging blood at 2530 ×*g* at 4 °C for 15 min (Sorvall RC-5C Plus, DuPont Co., Newtown, CT) and decanted.

In order to find the range of variability of the production process, three independent batches of each protein concentrate were produced from 10 L of blood per batch, collected on different days but under the same conditions.

2.1.1. Fractionation

Plasma protein fractions were separated by salting out, as described by Dàvila et al. (2006). A 100% saturated solution of food-grade ammonium sulphate (E-517) in 10 mM Tris–HCl pH 7.4 at 4 °C was added drop by drop to 4 L of fresh plasma to increase the ionic strength and progressively precipitate each protein fraction. All the precipitation steps were carried out in a jacketed tank with circulating water at 0–1 °C. A stirring device (Heildolph RZH 2021, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) was installed in the centre of the container and close to the bottom to guarantee a good mixing with the precipitating solution and to prevent sedimentation of coagulated protein until the desired saturation was reached. The saturation percentages of ammonium sulphate to precipitate the protein fractions were as follows: 20% for fibrinogen and 60% for globulins.

To obtain serum concentrate, precipitated fibrinogen was removed by centrifugation (10,000 ×*g* at 4 °C for 15 min), and the supernatant was recovered and stored refrigerated until further processing.

When producing albumin, a second precipitation step was carried out after removing fibrinogen. Ammonium sulphate was added up to 60% saturation and the albumin solution was recovered after centrifugation (10,000 ×*g* at 4 °C for 15 min) and separation of globulins. Supernatant was kept refrigerated until further concentration and desalting.

2.1.2. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE electrophoresis in 12.5% homogeneous acrylamide gels (Amersham Biosciences, Uppsala, Sweden) was used to confirm the success of the separation process. Hence, not only the serum and albumin concentrates, but also samples corresponding to whole plasma and the removed proteins, fibrinogen and globulins, were analysed.

Every sample was diluted to a protein content of 10 mg mL^{-1}. Solutions for the electrophoresis analysis were prepared by mixing 1 mL of the diluted sample, 4 mL of pH 8 TRIS/EDTA buffer, 0.025 g of sodium dodecyl sulphate (SDS) and 50 μL of β-mercapto-ethanol. Samples were heated at 70 °C for 5 min and centrifuged at 9700 ×*g*. Bromophenol blue 0.01% was added as tracking dye. Molecular weights were estimated using molecular mass standards from 20 to 220 kDa (BenchMark™ Protein Ladder, Invitrogen, Carlsbad, CA, USA). All experiments were performed in a PhastSystem apparatus (Pharmacia Biotech, Uppsala, Sweden) using the following running conditions: Pre-electrophoresis (250 V^{-10} mA^{-3} W, 1 Vh and 15 °C), application (250 V^{-1} mA^{-3} W, 1 Vh and 15 °C) and separation (250 V^{-10} mA^{-3} W, 60 Vh and 15 °C). Gels were stained with Coomassie blue (PhastSystem, 1990): 8 min in the staining solution (methanol, 30%; acetic acid, 10%; distilled water, 60%; Coomassie blue, 0.1%) at 50 °C; three consecutive washes with the destaining solution (methanol, 30%; acetic acid, 10%; distilled water, 60%) for 5, 10 and 8 min at 50 °C; 5 min in the preservation solution (glycerol, 10%; acetic acid, 10%; distilled water, 80%) at 50 °C.

2.1.3. Concentration and desalting

In every production batch fractions (4 L of serum or 7 L of albumin) were first concentrated and thereafter desalted in a tangential flow filtration (TFF) process by a MMS-SW18 bench filtration system (MMS AG, Urdorf, Germany) equipped with a modified-polyacrilonitrile (PAN-M) spiral wound module membrane (GE, MW1812C-34D), with a molecular weight cut-off (MWCO) of 30 kDa and 0.32 m^2 active area. The operating conditions for the concentration and the subsequent diafiltration operations were as follows: crossflow rate 400 $L \cdot h^{-1}$; transmembrane pressure (TMP) 2.0 bar; temperature ≤ 25 °C. In order to obtain 2 L of each protein concentrate, the volumetric concentration ratios (VCR) were 2 and 3.5, for serum and albumin, respectively. Changes in the flux rates were surveyed by recording the weight of permeate every 2.5 min throughout the TFF process.

The desalting operation consisted of discontinuous diafiltration. The solution was first diluted by adding 500 mL of distilled water and then concentrated back to the starting volume. This process was repeated until the required ionic strength in the retentate tank was reached. The amount of salt removed is related to the filtrate volume generated, which is reported in terms of diafiltration volumes (DFV), being 1 DFV the volume of retentate when diafiltration is started.

The desalting process was monitored from conductivity measurements of retentate samples during diafiltration. The processes were carried out until conductivities around 16 mS cm^{-1} were reached, which corresponded to the conductivity values of blood plasma before fractionation.

The concentrated and desalted serum and albumin solutions were analysed to determine their proximate composition.