Food Hydrocolloids 63 (2017) 429–436

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Physicochemical, microstructure and bioactive characterization of gels made from crayfish protein

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form
5 September 2016
Accepted 19 September 2016
Available online 20 September 2016

Keywords:
Bioactive properties
Crayfish proteins
Thermal gelation
Viscoelastic properties

ABSTRACT

Crayfish proteins are valuable active ingredients for food products, mainly due to its protein quality and antioxidant activity. A highly soluble crayfish protein concentrate (CF2L) obtained from crayfish surpluses was used to evaluate gelling properties at three different pH values (2.0, 6.5 and 8.0). Thermal gelation processes were monitored by Small Amplitude Oscillatory Shear (SAOS) measurements. Subsequently, gels were characterized by viscoelastic properties, water-holding capacity (WHC) and a selective solubility. All the systems exhibited a gel-like behavior, showing a strong dependence on pH.

Antioxidant measurements were performed by using three different reagents (DPPH, ABTS and Folin-Ciocalteu) and revealed an interesting potential for human food. The pH effect was evaluated, showing a remarkable dependence of ABTS on its value.

The results show that gels made from crayfish surpluses have potential for use in human nutrition, not only based on the physical properties, but also on its protein quality and antioxidant ability.

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1. Introduction

In the middle of last century, the freshwater red-swamp crayfish (Procambarus clarkii) was introduced in the Guadalquivir marshlands. Due to weather conditions, abundant food and the lack of predators, the crayfish population underwent a fast and widespread growth and was soon considered as an invasive species. The use of crayfish-meat surpluses to produce a non-denatured protein concentrate may be a useful method to preserve and utilize the crayfish for later use, from which is possible to produce different food products, e.g. in the form of emulsions or gels (Bengoechea, Puppo, Romero, Cordobes, & Guerrero, 2008; Romero, Bengoechea, Cordobés, & Guerrero, 2009a).

In the last few years, there have been an interest in the nutritional value of food industry by-products since it is possible to develop food products that are interesting in the health and snack food markets (Glew et al. 2006; Sah, Vasiljevic, McKechnie, & Donkor, 2015). These added-value may be considered on the basis of both their amino-acid composition and bioactive properties such as antioxidant or hypertensive activity (Dey & Dora, 2011).

Organic compounds in humans are made up to around 85 wt % of proteins. Thus, they are an essential part of human diet, not only in infants for growth and development, but also for adults. Protein quality can be determined by the amino acid composition and especially the content of the 9 essential amino acids (Reeds, 2000).

Additionally, in a healthy diet, a sufficient intake of antioxidant compounds is also important, since free radicals are continuously produced as part of the human metabolism. These may induce damages to biomolecules that may promote changes in DNA and, as a consequence, serious health problems (Gey, 1993). Antioxidants, present naturally in food, have also been postulated as antiaging agent (Brown, 2005). In addition, antioxidant peptides have been found in numerous food products including milk, wheat, potato and fungi (Suetsuna, Ukeda, & Ochi, 2000; Sun, He, & Xie, 2004). More recently, some studies have been focused on antioxidant peptides from fish (Elias, Kellerby, & Decker, 2008; Sakanaka, Tachibana, Ishihara, & Juneja, 2005).

Proteins may therefore be used as potential antioxidants (Saard, Sarnthima, Khammuang, & Kanchanarach, 2014). The antioxidant activity of proteins is attributed to complex interactions amongst their ability to inactivate reactive oxygen species, scavenge free radicals, chelate prooxidative transition metals, reduce hydroperoxides, enzymatically elimination specific oxidants, and alter the physical properties of food systems. As a consequence,

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http://dx.doi.org/10.1016/j.foodhyd.2016.09.025
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proteins are considered unique compared to other food antioxidants, because they can inhibit different steps in lipid oxidation (Elias et al. 2008; Irshad, Kanekanian, Peters, & Masud, 2013).

Functional properties of crayfish protein isolates such as gel and emulsifying properties have been recently evaluated (Romero et al. 2009a, 2009b, 2011). However, its nutritional value and bioactivity potential have not previously been investigated. The protein quality together with bioactive properties such as antioxidant activity are important for these ingredients to contribute to a healthy diet (FAO, 1985).

The overall objective of the present work was to evaluate the gelling properties and bioactive potentials of gels made from non-denatured crayfish protein concentrate (CF2L) at three different pH values (2.0, 6.5 and 8.0). To achieve these objectives, a physical and chemical characterization of CF2L was carried out. Subsequently, heat-induced gelation of CF2L was monitored by Small Amplitude Oscillatory Shear (SAOS) as a function of pH. Further characterization of the final gel was performed by means of WHC and selective solubility in order to evaluate the involved interactions. Finally, antioxidant activity of the different gels was evaluated by three different methods.

2. Material and methods

2.1. Materials

Fig. 1 shows the process to obtain different protein fractions from crayfish (CF) meat. Initially, CF meat was separated from the shell by grinding and sieving and, subsequently, CF pulp was kept frozen until use. This first stage was carried out by ALFOCAN (Isla Mayor, Sevilla, Spain). After thawing at 4 °C, CF pulp was homogenized and subjected to centrifugation at 15,000xg for 15 min, obtaining three different phases. A heavy phase (CF1P), which mainly consists of solid materials (some parts of the exoskeleton) and muscle tissue. This phase represents about 10 wt % of the CF pulp, while its protein content is c.a. 14 wt%, which constitutes the 8.6 wt% of the total CF-pulp protein amount. An intermediate phase (CF2P), which mainly consists of water (c.a. 70 wt % of the CF-pulp) was also obtained. The protein content of this phase is c.a. 80 wt% and represents the 89.2 wt% of the total protein amount in the CF-pulp. Eventually, a light phase (CF3P), which mainly consists of lipids (ca. 20 wt % of the CF-pulp) was obtained. The protein content of this lipophilic phase is c.a. 27 wt%, which represents the rest of the protein contributed by the CF-pulp (2.2 wt%). Moreover, some hydrophobic compounds such as astaxanthin and E vitamin are present in this phase. The CF2P was the selected phase because it is the water soluble protein fraction and shows the highest protein content. In addition, both myofibrillar proteins and sarcoplasmic proteins are present in this phase. Finally, the intermediate phase (CF2P) was freeze-dried in order to obtain a flour-fraction rich in proteins, the protein powder was named CF2L.

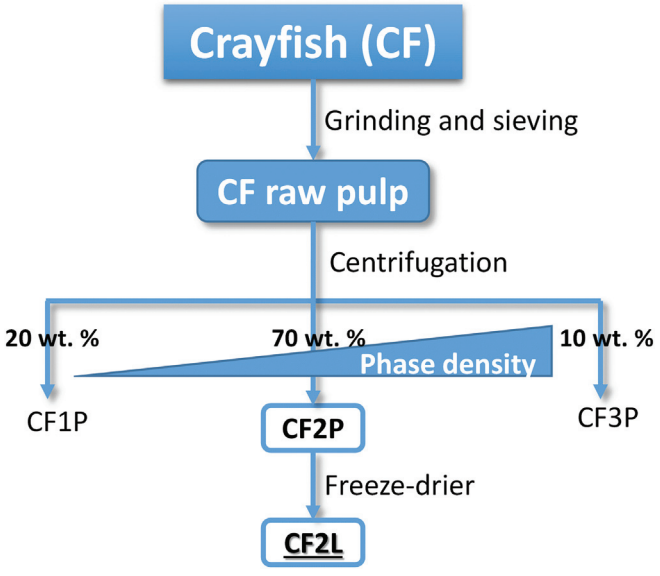

Fig. 1. Diagram of the procedure carried out in order to obtain the CF2L protein concentrate.

The protein content of the CF2L was determined in quadruplicate as % N x 6.25 using a LECO CHNS-932 nitrogen micro analyzer (Leco Corporation, St. Joseph, MI, USA). In the same way, lipid, moisture and ash contents were determined according to A.O.A.C. (1945).

DPPH, ABTS, Folin-ciocalteu, HCl, NaOH reagents were of analytical grade, purchased from Sigma–Aldrich Chemical Company (St. Louis, USA). Milli-Q ultrapure water was used for the preparation of all solutions.

2.2. Protein powder characterization

2.2.1. Amino acid characterization

CF2L samples were dissolved in 6.0 M hydrochloric acid and were incubated in an oven at 110 °C for 24 h. After hydrolysis, pH was adjusted to 7 using 6M NaOH and samples were filtered through a Whatman glass microfibre filter (GF/C). Finally, samples were diluted (1:500) by adding doubly distilled water.

Reverse phase HPLC by precolumn fluorescence derivatization with o-phtaldialdehyde (SIL-9A Auto Injector, LC-9A Liquid Chromatograph, RF-530 Fluorescence HPLC Monitor, all parts from Shimadzu Corporation, Japan) was performed using a NovaPak C18 cartridge (Waters, Milford, MA, USA). Glycine/arginine and methionine/tryptophan were determined together, as their peaks merged. By this procedure, it is only possible to detect: Alanine (Ala), Aspartic acid (Asp), Glutamic acid (Glu), Histidine (His), Serine (Ser), Glycine (Gly), Arginine (Arg), Threonine (Thr), Tyrosine (Tyr), Methionine (Met), Valine (Val), Phenylalanine (Phe), Isoleucine (Ile), Leucine (Leu) and Lysine (Lys). The other amino acids were not included in the results of this study because they are completely destroyed by acid hydrolysis or cannot be directly determined from acid hydrolyzed samples.

2.2.2. Free and total sulfhydryls

Free and total sulfhydryl groups of CF2L samples were determined using the method developed by Beveridge, Toma, and Nakai (1974). Samples were suspended (10 mg/mL) in 0.086 moL/L Tris-HCl − 0.09 moL/L glycine − 4 mmoL/L EDTA − 8 moL/L urea − pH 8.0 buffer. Dispersions were stirred at 25 °C for 10 min at 500 rpm in a thermomixer and centrifuged at 15,000xg (10 min, 10 °C). The supernatant was incubated with Ellman's reagent (DTNB) (4 mg DTNB/mL methanol) and 1 mL of 2-nitro-5-thiosulfobenzoate (NTSB) was used in the case of the total sulfhydryls. Absorbance at 412 nm was measured in a Genesis-20 spectrophotometer (Thermo Scientific, USA). The molar extinction coefficient of NTB (13,600 L $mol^{-1} \cdot cm^{-1}$) was used. Protein concentration of extracts was determined by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.2.3. Protein solubility

Protein solubility was determined as a function of pH in the range 2–10. Aqueous dispersions at 1 mg/mL were prepared and pH of different aliquots was adjusted with 6 N NaOH and 6 N HCl at

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