Valorization of wastes and by-products from Red-Crayfish Industry based on their protein content

Manuel Felix, Alberto Romero, Antonio Guerrero

Departamento de Ingenieria Química, Escuela Politecnica Superior, C/ Virgen de África 7, 41011, Sevilla, Spain.

Correspondence to: Manuel Felix (E-mail: mfelix@us.es)

ABSTRACT

A big amount of protein by-products and wastes are produced in food industry every year. The proper use of these protein systems may increase the economic value of these low-priced by-products. This thesis is devoted to the valorization of wastes and by-products from the red-crayfish, exploring the potential functional properties of its proteinases renewable resources in the manufacture of emulsions, gels and bioplastics. The emulsification properties were initially studied by means of interfacial properties at three different pH values (3.0, 5.0 and 8.0), followed by the development of emulsions. To provide long-term stability, a polysaccharide (Xanthan Gum, XG) was added and the interactions among proteins and XG were assessed. Subsequently, thermal gels were obtained, and the microstructure and antioxidant properties were carried out. The influence of the pH and the degree of hydrolysis in both properties were evaluated. Finally, bioplastics materials were obtained. To enhance their initial properties, two different strategies were followed. Firstly, three additives were added (sodium sulfite, bisulfite and Urea) to the blends, promoting different protein interactions. Secondly, composites materials were obtained from the mixture between PCL and Crayfish protein concentrate. Rheology was the reference technique used for the microstructural characterization of all products obtained.

KEYWORDS: Antioxidant activity; Bioplastics; Crayfish; Emulsions; Gels; Rheology

INTRODUCTION

Every year food and agricultural industry produce a big amount of surpluses and wastes which are discarded or used as a low added value by-product. A particularly relevant example of this fact is located in Andalusia (southern Spain) and it is associated to the red-swamp crayfish. This crustacean was introduced in the middle of the twentieth century and due to favorable weather conditions, abundant food and the lack of predators, it has undergone a fast widespread growth

(Kirjavainen & Westman, 1999). This rapid growth has contributed to the development of a strong local crayfish industry at the marshes of the Guadalquivir River (Geiger, Alcorlo, Baltanas, & Montes, 2005). A currently attractive way to valorize these products is taking benefits from its relatively high protein content.

Crustaceans constitute an excellent source of high-quality protein, rich not only in essential amino acids and lipids, including long-chain polyunsaturated fatty acids from ω-3, but also containing other components of functional value such as astaxanthin that possess a high antioxidant capacity, even higher than others important antioxidants such as β-carotene or vitamin E (Miki, 1991). The quality of this protein concentrate will contribute to increase the functional properties in their products and derivatives.

To obtain an useful crayfish protein concentrate for these three applications, a previous stage of characterization and optimization of different flours from crayfish pulp has been carried out. This stage was developed in collaboration with a local factory (PEVESA), who selected the temperature, pH, extraction procedure and protein-dispersion fraction in order to obtain the phase with the highest content of soluble protein.

This thesis is focused on different applications of crayfish protein systems derived from their functional properties, aiming at the achievement of high value-added crayfish-based products. The applications considered in this study will be: emulsification ability, gel formation and bioplastics processing.

First of all, one application come from food industry, which is really interested in produce stable oil-in-water (o/w) emulsions containing a protein different to egg-yolk protein as the only emulsifier in order to produce food products such as mayonnaise and salad dressings. These alternative proteins would avoid the presence of cholesterol from yolk, the development of salmonella in yolk-containing food products or allergic reactions, which nowadays are more and more frequent. Other authors have used previously myofibrillar proteins such as actomyosin to produce emulsions and have demonstrated that actomyosin from hake had higher emulsifying activity and stability than the actomyosin from chicken and pork (Cofrades, Carballo, Careche, &

Colmenero, 1996). Protein from crayfish may constitute an excellent source of protein which may contribute to stabilize o/w emulsions.

In addition, other functional property is the ability of proteins to denature form aggregates and adopt gel-structure after a controlled heating. This application contributes to design a wide variety of food systems with the desire rheological consistency, microstructure and texture. Crustacean proteins are mainly constituted by sarcoplasmic and myofibrillar proteins. Sarcoplasmic fraction with a globular and relatively simple structure, show a weak gelation capacity and, therefore, a little contribution to the texture of processed foods. On the other hand, myofibrillar proteins, specially myosin and actomyosin, constitute multiples dominions that tend to form viscoelastic networks and gels with high consistency (Damodaran, 1997). Finally, an interesting area of research is the antioxidant characterization of these gels. Due to the presence of the above mentioned functional ingredients, these gels may constitute an excellent source of protein for the human nutrition.

Finally, a currently attractive way to valorize these by-products is through the use of this protein concentrate as renewable resources in the manufacture of “green materials”, replacing hardly degradable plastic materials from oil-based synthetic polymers. Recently, some important applications for bioplastics are beginning to emerge in the areas of food-packaging, pharmaceutics, electronics, automotive industry and biomedicine. Thus, among other applications, bioplastics from proteins can be used in food packaging, fruit coating, encapsulation, textiles, absorbent materials or tissue engineering (Sharma & Luzinov, 2012; Soroudi & Jakubowicz, 2013). This wide variety of potential applications allows us to envisage an increasingly use of biobased-plastic materials in a near future.

THESIS OBJECTIVES

One of the main objectives of this study has been to evaluate the potentials of crayfish concentrate as emulsifier to obtain highly concentrated oil/water emulsions such as mayonnaise-like. First of all, it was necessary to optimize the processing and composition parameters that would lead to long-term stable emulsions. In this context, interfacial measurements were performed in order to facilitate prediction of emulsion stability. Relevant ultimate information on emulsion stability was obtained by characterizing the rheological properties and microstructure of crayfish-based emulsions. The rheological characterization has been focused on the linear viscoelastic properties of the emulsions, determined by means of small-amplitude oscillatory shear (SAOS). Microstructural parameters have been evaluated through droplet size distributions analysis. Microstructure has also been characterized by Confocal Laser Scanning Microscopy (CLSM).

Another additional objective has been to evaluate the gel ability and the bioactive potential of gels made from non-denatured crayfish protein concentrate at different pH values. To explore improvements in both, the gelation and bioactive ability, different hydrolysates were obtained with different degree of hydrolysis. Pancreatic trypsin was the protease-enzyme chosen to hydrolyse the crayfish concentrate protein. On the other hand, transglutaminase was eventually added to a selected system (based on his bioactive properties) in order to improve the mechanical properties of its gels. To characterize mechanical properties, rheological measurements of aqueous CF protein dispersions were performed in order to follow the gelation process by means of temperature ramps, as well as frequency sweep tests to obtain the mechanical spectra of CF-based gels. Finally, bioactive ability of the different gels was evaluated behind three different reference compounds.

Finally, the last objective of this study has been to evaluate the potentials of crayfish concentrate protein to produce plasticized crayfish bio-based plastic materials by means of a conventional, highly versatile and widely used polymer processing technique such as injection molding, as an alternative to plastic materials based on polymers derived from fossil fuel. Glycerol (GL) has to be used in this process as a plasticizer. Results from crayfish protein concentrate were compared with those obtained using a low valued crayfish protein flour in order to reduce bioplastics costs, since, as is widely known, these kind of products are involved in a well-developed industry and very competitive market. Furthermore, the effect of using different chemical additives on the properties of CF-based products was also evaluated. The additives assessed in this study were sodium sulphite (SS) or bisulphite (BS) as reducing agents, urea (U) as a denaturing agent and L-cysteine (LC) as a crosslinking agent. An interesting alternative to achieve this objective involves using CF protein in combination with a synthetic polymer, preferably one showing some biodegradable properties such as polycaprolactone (PCL). This polymer is classified as a biodegradable polyester from fossil source and it has been widely used as the polymer matrix in the development of a variety of new materials. As for bioplastic processing, regardless of its formulation, injection molding has been selected since it is one of the most versatile and extended polymer processing technique, being particularly useful for polymeric materials (e.g. protein) that deviate form a typical thermoplastic behavior. This process requires a previous mixing stage of polymeric materials and plasticizer (and additives, if any) to obtain homogenized blends with suitable properties for their subsequent injection. This stage was carried out by means of a mixing-rheometer that allows an exhaustive control of the blend by recording torque and temperature over mixing. Rheological and Differential Scanning Calorimetry measurements of the blends obtained were carried out in order to obtain information that may be useful in the selection of suitable processing parameters for injection molding operations (temperature and residence time in the pre-injection cylinder as well as molding temperature). Eventually, all the probes were characterized by means of dynamic mechanical analysis (DMA) and tensile strength measurements in order to obtain the mechanical characterization.

CONCLUDING REMARKS

From protein characterization

A protein concentrate (CF2L) was obtained from the crayfish (CF) meat contains a significant amount of the essential amino acids that would provide excellent nutritional quality to CF2L-containing products. Its nutritional value together with its high solubility makes this product fairly attractive as a food ingredient in different applications such as emulsions and gels. The CF2L protein system as well as their hydrolysates show a low protein denaturation, and a desirable sulfhydryl content for different applications (emulsions, gels and bioplastics). Surface hydrophobicity increases in the same way as degree of hydrolysis (Felix, Romero, Rustad, & Guerrero, 2017a).

Results from CF2L protein adsorption at liquid-liquid interfaces evidence a fast increase in surface pressure that tends to reach a pseudo-equilibrium value regardless of the pH. However, after fitting the curve to the Ward and Tordai model, the diffusion constant parameter indicates that protein diffusion takes place faster at pH 5.0 than at pH 3.0 or 8.0. This behavior may be related to the absence of net charge close to the IEP. In contrast, fitting surface pressure data to a first order equation for the penetration stage reveals that this stage is favored at pH 3.0 (Felix, Romero, & Guerrero, 2017).

From CF2L-stabilized emulsions

CF2L-based high-oleic/water emulsions exhibit lower droplet sizes at pH 5.0, as a result of the higher surface pressure. However, the highest stability corresponds to emulsions prepared at pH 3.0, which show unflocculated unimodal distributions remaining unaltered in size over 2 months. On the other hand, droplet sizes show a strong influence on ageing time for emulsions prepared at pH 5.0 and 8.0, caused by coalescence. The increase in XG attenuates this effect to a great extent, although does not prevent an eventual emulsion destabilization. Linear viscoelastic properties and steady flow properties of CF2L-based emulsions seem to be governed by protein-polysaccharide interactions that in turn depend on pH. Those interactions also seem to control the destabilization of the emulsions studied. These results support the key role of the polysaccharide in the formation of the gel-like network, either through formation of extensive flocculation (at pH 5.0 and 8.0) or through complexation with protein, favored at pH 3.0 (Felix, Romero, & Guerrero, 2017).

From CF2L-based gels

SAOS measurements from CF2L-based gels reveal a strong dependence of gelation ability and gel strength on pH. Thus, near the IEP, the absence of net charges facilitates a proper development of gel network structures leading to fairly strong gel-like viscoelastic behaviour, with higher amount of disulphide bonds and enhanced WHC. On the other hand, at pH 8.0 and 2.0, the presence of repulsive interactions among charged protein surfaces tend to inhibit S-S bonds such that gel development is dominated by hydrophobic interactions, leading to weak gels with lower viscoelastic properties and WHC, particularly at pH 2.0 at which a large increase in the relative amount of the peptide fraction was found as a consequence of acidic hydrolysis (Felix, Romero, Rustad, et al., 2017a). The influence of protein hydrolysis is highly dependent on pH:

- At low pH, hydrolysis yields a reduction in interactions that involves a decrease in viscoelastic properties.
- At neutral pH, a moderate degree of hydrolysis brings about two opposite effects, inhibition of S-S bonds and enhancement of hydrophobic interactions and hydrogen bonds, resulting in a fairly invariable gel-like behaviour. However, WHC always decrease in consonance with the reduction of S-S bonds.
- At alkaline pH, an apparent enhancement of the gel network takes place at intermediate degree of hydrolysis, as a consequence of an increase in S-S bonds.

A high degree of hydrolysis generally leads to an apparent decrease of interactions that result in a dramatic decrease in viscoelastic properties and WHC (Felix, Romero, Rustad, & Guerrero, 2017b).

The highest antioxidant activity was obtained against ABTS and the lowest when FC was used, since this reagent is specific for phenol compounds. No particular influence of pH was found for DPPH or FC activity. However, pH exerts a dramatic increase on the activity against ABTS (around one decade). In general hydrolysates systems exhibit a higher antioxidant activity except against DPPH. This behavior is remarkable for ABTS at pH 8.0 where antioxidant activity reaches twice the value for CF2L. Once again, a high degree of hydrolysis is not desirable, because antioxidant activity decreases (Felix, Romero, Rustad, et al., 2017a, 2017b).

From CF/CF2L-based bioplastics

Initially, a commercial crayfish protein powder (CF) was used for the development of bioplastics since a big amount of protein powder is required. From the experimental results of bioplastics, it may be concluded that monitoring the torque over mixing of protein-based flour, additives and plasticizer it is useful to select the more suitable conditions (e.g. mixing time and formulation) in terms of energy efficiency. The addition of reducing agent (BS or SS), denaturing agent (U) or crosslinking promoter (LC), always yields an increase in energy efficiency at the mixing stage, leading to a remarkable reduction in the linear viscoelastic properties of blends, which is also important to select suitable operation conditions for injection molding processing. As for bioplastics, the additive developing a greater effect on mechanical properties is LC, which provides specimens showing a higher value for the Young's modulus, as well as a remnant thermosetting potential for further processing at high temperature. However, it is the maximum elongation the property that is remarkably enhanced by increasing the thermosetting temperature, which takes place at the expense of the Young's modulus. The maximum stress increase only when the temperature of the mound increases (Felix, Romero, Cordobes, & Guerrero, 2015)

The combination of techniques such as mixing rheology of CF/GL/PCL, controlling torque and temperature profiles, and DSC is very useful for selecting suitable injection molding parameters for processing blends. CF/GL/PCL biocomposites show a remarkable enhancement in mechanical properties as compared to CF/GL bioplastics, even when crystalline structure remains unaltered. Furthermore, the protein/plasticizer ratio also plays a relevant role. Thus, both polymers play a significant role on the elastic properties. However, the PCL yields a dominant contribution to the elastic response, particularly under uniaxial tensile tests, and confer a higher ability to absorb energy before rupture. The feasibility of designing renewable and biodegradable composites have been demonstrated, which may be regarded as an alternative to conventional plastic materials, containing an important amount of CF, thereby finding value-added applications of these low valued by-products of the crayfish industry (Félix, Romero, Martín-Alfonso, & Guerrero, 2015). The comparison between CF and CF2L-based bioplastics indicates there are not significant differences between both protein systems for the development of bioplastics.

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REFERENCES

Cofrades, S., Carballo, J., Careche, M., & Colmenero, F. J. (1996). Emulsifying properties of actomyosin from several species. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie*, *29*(4), 379–383.

Damodaran, S. (1997). *Food Proteins and Their Applications*. Taylor & Francis.

Felix, M., Romero, A., Cordobes, F., & Guerrero, A. (2015). Development of crayfish bio-based plastic materials processed by small-scale injection moulding. *Journal of the Science of Food and Agriculture*, *95*(4), 679–687. http://doi.org/10.1002/jsfa.6747

Felix, M., Romero, A., & Guerrero, A. (2017). Viscoelastic properties, microstructure and stability of high-oleic O/W emulsions stabilised by crayfish protein concentrate and xanthan gum. *Food Hydrocolloids*, *64*, 9–17. http://doi.org/http://dx.doi.org/10.1016/j.foodhyd.2016.10.028

Félix, M., Romero, A., Martín-Alfonso, J. E., & Guerrero, A. (2015). Development of crayfish protein-PCL biocomposite material processed by injection moulding. *Composites Part B: Engineering*, *78*, 291–297. http://doi.org/j.compositesb.2015.03.057

Felix, M., Romero, A., Rustad, T., & Guerrero, A. (2017a). Physicochemical, microstructure and bioactive characterization of gels made from crayfish protein. *Food Hydrocolloids*, *63*, 429–436. http://doi.org/10.1016/j.foodhyd.2016.09.025

Felix, M., Romero, A., Rustad, T., & Guerrero, A. (2017b). Rheological properties and antioxidant activity of protein gels-like systems made from crayfish concentrate and hydrolysates. *Food and Bioproducts Processing*, *102*, 167–176. http://doi.org/10.1016/j.fbp.2016.12.014

Geiger, W., Alcorlo, P., Baltanas, A., & Montes, C. (2005). Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions*, *7*(1), 49–73. http://doi.org/10.1007/s10530-004-9635-8

Kirjavainen, J., & Westman, K. (1999). Natural history and development of the introduced signal crayfish, Pacifastacus leniusculus, in a small, isolated Finnish lake, from 1968 to 1993. *Aquatic Living Resources*, *12*(6), 387–401. http://doi.org/10.1016/s0990-7440(99)00110-2

Miki, W. (1991). Biological functions and activities of animal carotenoids. *Pure and Applied Chemistry*, *63*(1), 141–146. http://doi.org/10.1351/pac199163010141

Sharma, S., & Luzinov, I. (2012). Water Aided Fabrication of Whey and Albumin Plastics. *Journal of Polymers and the Environment*, *20*(3), 681–689. http://doi.org/10.1007/s10924-012-0504-8

Soroudi, A., & Jakubowicz, I. (2013). Recycling of bioplastics, their blends and biocomposites: A review. *European Polymer Journal*, *49*(10), 2839–2858. http://doi.org/10.1016/j.eurpolymj.2013.07.025