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Amaranth proteins foaming properties: Film rheology and foam stability – Part 2

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ABSTRACT

In this work the influence of pH and ionic strength on the stability of foams prepared with amaranth protein isolate was analyzed. The behaviour observed was related to the physico-chemical and structural changes undergone by amaranth protein as a result of those treatments. The results obtained show that foams prepared at acidic pH were more stable than the corresponding to alkaline pH. At pH 2.0 the foams presented higher times and more volumes of drainage. This behaviour is consistent with the characteristics of the interfacial film, which showed a higher viscoelasticity and a greater flexibility at acidic pH than alkaline pH value, which in turn increased by increasing the concentration of proteins in the foaming solution. It is also important to note that the presence of insoluble protein is not necessarily detrimental to the properties of the foam. Detected changes in the characteristics of the interfacial film as in the foam stability have been attributed to the increased unfolding, greater flexibility and net charge of amaranth proteins at acidic conditions.

1. Introduction

The use of proteins obtained from vegetables is presented as a prospect response to worldwide food needs [1]. For this reason, further information on the functional behaviour of proteins of vegetable origin is needed so that they can be incorporated to a greater variety of products. Amaranth, a pseudocereal growing in the American continent, is an unconventional and interesting source of proteins. It is a C4 plant, whose seeds contain a large amount (14–17%, w/w) of high nutritional quality proteins [2,3] and it can grow under soil conditions that are unfavourable to other conventional species of crops.

Many foods are dispersed systems where proteins are used for their surfactant properties, which allow the formation and/or increase their stability. Many authors have studied interfacial properties of proteins over time, understanding that a surfactant–covered interface can be seen as a two-dimension body with its own rheological properties [4–7].

A great challenge is to incorporate amaranth into existing food formulations in order to modify their functional and nutritional qualities, as well as to create entirely new products such as foam-type products. We have already studied the interfacial and emulsifying properties of amaranth proteins at acidic and alkaline pHs. The results obtained clearly showed that amaranth proteins at acidic pH have a better activity at the oil:water interface and are capable of forming stable emulsions [8]. Recently, we have studied interfacial properties of amaranth proteins. In particular [9], we studied the diffusion, adsorption and rearrangement phenomena occurring at the air–water interface, the relation between these phenomena and the protein structure and the influence of these factors in the formation of foams formulated with them. We have found that the acidic pH (treatment at pH 2, together with the increase of ionic strength) favours adsorption of proteins, reduces the time for a rearrangement and improves the foaming properties increasing foaming capacity and forming more dense and homogeneous foams [9]. The aim of this work was study the capability of amaranth proteins to stabilize foams under different pH and ionic strength conditions analysing the rheological behaviour of air–water interface; with the final objective of using the amaranth proteins in foam type-products.

2. Materials and methods

2.1. Plant materials and flour preparation

Seeds of *Amaranthus hypochondriacus*, (cultivar 9122) were obtained from Estación Experimental of Instituto Nacional de

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Tecnología Agropecuaria (INTA), Anguil, La Pampa, Argentina. Seeds were ground and sieved through a 0.092 mm mesh in Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. The resulting flour was defatted with hexane at 4 °C for 24 h (100 g/l suspension) under continuous stirring, dried at room temperature and stored at 4 °C until used. The protein content (19.8 ± 0.2% wet basis) was determined by the Kjeldahl method [10], $N \times 5.85$ [11].

2.2. Preparation of amaranth isolates

The amaranth protein isolate was prepared according to Martínez and Añón [12]. The previously defatted flour was suspended in water in a relation 1:10 and the pH of the suspension was adjusted to 9.0 by the addition of 2 N NaOH solution. The suspension was stirred during 1 h and then centrifuged at $9000 \times g$ for 20 min at 10 °C. The pH of the supernatant was adjusted to 5.0 with 2 M HCl and centrifuged at 4 °C for 20 min at $9000 \times g$. The precipitate was suspended in water, neutralized with 0.1 M NaOH and freeze-dried. The protein content of isolate was 85 ± 1% (dry basis) as determined by Kjeldahl method [10], $N \times 5.85$ [11]. The isolates used in this work were prepared at least three times, showing similar properties.

Protein isolates were dispersed at pH 2 in 0.035 M phosphoric acid–diacid phosphate buffer, and at pH 8 in 0.035 M Tris buffer. The ionic strength (IS) was adjusted to 0.5 and 0.06 with 0.5 M NaCl, obtaining four amaranth isolates named AI pH2-highIS, AI pH8-highIS, AI pH2-lowIS and AI pH8-lowIS [9].

2.3. Protein film rheology

The interfacial rheology of films obtained with solutions in the protein concentration range between 0.001–1.0 g/ml was measured by using the automated drop tensiometer (Tracker, IT-Concept. Saint-Clémenttes Places, France). The protein concentrations used were selected according to previous works [8,9]. The bubble was formed in the protein solution and the surface tension over time was calculated based on changes in drop form.

Dilatational rheology studies the interface response to sinusoidal deformations of compression/expansion (change of area but not of form). The interface response to deformation is described by the complex modulus E^*, which refers to the relation between the response in γ for a given relative area deformation.

$$E^* = \frac{d\gamma}{dA/A} = \frac{d\gamma}{d\ln A} \quad (1)$$

E^* modulus is a complex number, which can be decomposed in a real part (E', Eq. (2)), called storage modulus – that represents the elastic behaviour of the film – and an imaginary part (E'', Eq. (3)), called loss modulus – that represents the viscous component – [13]. For an entirely elastic interface, deformation is in phase with the response. However, it is generally observed that there is a discrepancy defined by an angle θ, which increases with a greater contribution of the viscous component.

$$E' = |E*| \cos\theta \quad (2)$$

$$E'' = |E*| \sin\theta \quad (3)$$

$$\tan\theta = \frac{E''}{E'} \quad (4)$$

where θ is the viscous phase angle.

The relative contribution of both components was analyzed by using the parameter $\tan\theta$.

Experimentally, rheological parameters E^*, E', E'' and $\tan\theta$ were assessed during the formation of the interfacial film for a period of 10,800 s. Sinusoidal deformations were made at a frequency of 100 mHz during 50 s, every 500 s, with an amplitude of $0.1\Delta A/A$ within the lineal viscoelasticity range.

The interfacial rheology of films in a pseudo-equilibrium state was studied using the same automated drop tensiometer. Rheological behaviour of the interface under different deformation frequencies allows analysing relaxation times of the interfacial structure. Tests were carried out 3 h after the formation of the film. Sinusoidal deformations of amplitude $0.1\Delta A/A$ were performed, covering a frequency range between 5 mHz and 300 mHz.

2.3.1. Foaming properties – foam stability

The foaming properties of amaranth proteins were determined by conductimetry using the method and device developed by Loisel et al. [14].

Protein dispersions (soluble and insoluble proteins) or protein solutions at 1.0 g/l of soluble protein were prepared in the corresponding buffers. Protein solutions were obtained after centrifugation at $10{,}000 \times g$ during 15 min at 20 °C. Protein dispersions or protein solutions were placed in the sparging chamber at the base of an acrylic column (length: 27.5 cm, internal and external diameters: 2.4 and 3.0 cm, respectively). Foam was generated by sparging nitrogen gas through porous G4 type glass disc with a pore size of 5–15 μm, at a rate of 80 ml/min into 6 ml of the sample during 30 s. The volume of initial or residual liquid under the foam was measured by conductivity through two electrodes located in the sparging chamber. Conductivity values, as a function of time (C_t) and with reference to the conductivity of the buffered test solution (C_{init}), were used to calculate half time ($t_{1/2}$). This parameter is defined as the time elapsed from the bubbling stopped until half of the liquid incorporated to the foam was drained. VLF_{10} corresponds to the volume of liquid remaining in the foam after 10 min from the bubbling stopped. This is a parameter, defined in our laboratory, that allows to estimate the foam stability via processes of gas diffusion (disproportionation) and collapse.

The foam destabilization phenomenon was interpreted, as other authors have done [15–17], as two simultaneous processes. On the one hand, the gravitational drainage of the liquid and on the other hand, the diffusion and collapse drainage.

A first order biphasic exponential model (Eq. (5)) was used to fit the experimental data [15] as follows:

$$V_{LF}(t) = V_g e^{(-t/\tau_g)} + V_{dc} e^{(-t/\tau_{dc})} \quad (5)$$

where the first term refers to the gravitational drainage process (g) and the second to the gas diffusion or disproportionation and collapse drainage processes (dc). With the application of the model, four parameters are adjusted, two correspond to the amplitude of each process (V_g, V_{dc}), and two kinetic parameters which correspond to the relaxation time of each process (τ_g, τ_{dc}). Parameter V_{LF} for different t was calculated knowing the volume of remaining liquid at time t (V_{LS}) by subtracting the volume of liquid at time zero. V_{LS} and V_{LS0} values were experimentally obtained from the conductivity data.

The model was adjusted to the experimental data with the OriginPro 8 SR0 programme (Origin Lab Corporation, Northampton, MA 01060 USA). Each experiment was performed three times.

2.3.2. Statistical analysis

The least significant difference (LSD) test (after analysis of variance, ANOVA) was used to identify pairwise differences between means. Significance was determined at $p < 0.05$.

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