Journal of Thermal Analysis and Calorimetry, Vol. 93 (2008) 2, 595–598

PHYSICAL AGEING OF CRAYFISH FLOUR AT LOW MOISTURE CONTENTS

A. Farahnaky[1*], *A. Guerrero*[2], *S. E. Hill*[3] *and J. R. Mitchell*[3]

[1]Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Iran
[2]Departamento de Ingeniería Química, Universidad de Sevilla, Spain
[3]Division of Food Sciences, School of Biosciences, University of Nottingham, UK

Glass transition temperature of red crayfish flour (moisture 3.56%) was determined using a phase transition analyser (Wenger Technical Centre, USA). Due to the importance of physical ageing in functional properties of red crayfish flour (with 65% protein) the possible occurrence of physical ageing in dry powder of crayfish flour was studied at different temperatures below and close to the glass transition.

Endothermic peaks that corresponded to relaxation enthalpy were observed for a commercial crayfish flour with 4.5% moisture. Enthalpy and peak temperature increased on storage of crayfish flour when it was held in the glassy-state at different temperatures (5, 15, 25°C).

Keywords: *DSC, physical ageing, red crayfish flour*

Introduction

Physical ageing is a well-known phenomenon in polymer glasses and occurs when a polymer is rapidly cooled from the equilibrium rubber or liquid-state into the non-equilibrium glassy-state. This glass will gradually relax towards equilibrium. The relaxation of the polymer manifests itself as changes in its mechanical properties, such as density, brittleness and also dielectric loss. A change in the thermodynamic properties, such as enthalpy, specific volume and refractive index, also occurs. A convenient measure of the amount of physical ageing is to measure the enthalpy relaxation by differential scanning calorimetry (DSC). A decrease in enthalpy as a result of the relaxation process can be analysed as an enthalpy recovery peak (or 'overshoot') during a heating DSC scan. The intensity and the position of the enthalpy recovery peak depend on both the nature of the sample and how the sample was prepared, i.e. its thermal history [1–3].

Physical ageing has implications for the stability and conservation of performance for many materials stored at low moisture contents. The possible occurrence of physical ageing in fish proteins may increase the loss of its solubility. Crayfish flour from red crayfish (*Procambarus Clarkii*) may be obtained as a by-product in the red crayfish industry in large quantities. Crayfish flour is a good source of high quality protein (about 65%), rich in essential amino acids, and lipids (ca. 19%), containing other components of functional value. The potentials of crayfish proteins as a functional ingredient in food products has been recently explored by Cremades *et al*. [4, 5]. Romero *et al*. have recently studied the ability of crayfish flour in the stabilization of high internal phase ratio oil/water emulsions [6, 7]. However the reduction of the solubility of crayfish protein on storage even at low moisture levels (5%) would be expected to decrease functional properties such as gelation, emulsifying and foaming ability.

Phase transition analyser (PTA) is a technique that has been recently developed to provide information both about the glass transition temperature and the melt rheology of biopolymer systems, particularly within the context of extrusion processing [8]. In the PTA a piston applies pressure to material initially in a closed chamber and the temperature is increased. The glass transition temperature is associated with the point where the material softens, giving rise to increased movement of the piston. A flow temperature, T_f, can also be determined by replacing the closed die at the base of the chamber by one containing a small hole. T_f is taken as the temperature where the material flows as evidenced by a continuous movement of the piston at a constant temperature. For maize these two temperatures have been shown to differ for crops grown in different locations and have been related to extrusion behaviour [8].

The aim of this current study was to explore the occurrence of physical ageing in the red crayfish flour and determine the extent of structural relaxation.

* Author for correspondence: farahnak@shirazu.ac.ir

Experimental

Materials and methods

Red crayfish flour supplied by Alfocan (Sevilla, Spain) with the moisture content of 4.5% (dry basis) was sieved and the particles larger than 600 μm were removed. After the sieving the red crayfish flour was used for physical ageing study without any further treatments.

All chemicals used in this research were of analytical grade unless otherwise mentioned.

Chemical composition of red crayfish flour

Moisture and the chemical composition of the red crayfish flour including protein, fat, ash and total carbohydrate were measured according to the AOAC approved methods.

PTA for determination of glass transition

The PTA is a closed-chamber capillary 'rheometer'. One of the main differences between PTA and a capillary rheometer is that the latter extrudes at constant piston speed while the PTA performs at constant pressure.

It consists of two sealed chambers, top and bottom, separated by an interchangeable capillary die. The two chambers house electric heaters and contain a hollow cavity that allows a cooling fluid to be used. The pistons, mounted together as sidebars, are held in a fixed position during testing. Air cylinders, mounted to the bottom of the PTA, maintain constant pressure on the sample. A linear-displacement transducer measures the samples' deformation, compaction, and flow relative to initial sample height [8]. In this study the measurements of T_g was carried out at a heating rate of 5°C min^{-1} and an applied pressure of 150 bar.

Structural relaxation using DSC

A DSC-7 (Perkin-Elmer, Beaconsfield, UK), calibrated with indium and cyclohexane, was used to analyse crayfish flour samples (10–15 mg). High pressure stainless steel pans containing crayfish flour samples were scanned at a heating rate of 10 K min^{-1}, from –30 to 100°C. After cooling (50°C min^{-1}) the samples were heated once again using the same conditions to measure their glass transition temperatures.

For physical ageing study after eliminating the thermal history of crayfish flour by heating in the DSC, the sealed pans containing crayfish flour samples were held at different temperatures (5, 15 and 25°C) for up to 168 h and rerun in the DSC to explore possible physical ageing phenomenon. An empty stainless steel pan was used as reference. Pyris software (Perkin-Elmer) was used to analyse the DSC traces.

Results and discussion

Chemical composition of the red crayfish flour presented in Table 1 shows that the amount of protein, fat and ash as the main components of the powder were 65.44, 18.86 and 13.43%, respectively. The high level of protein in the composition of the red crayfish flour reveals the importance and the need to study this component for optimising its functional properties. The ash content of the powder was high compared to plant protein concentrates e.g. soy protein and this can affect its applications. Lipid oxidation in red crayfish protein because of its high fat content is another limitation of using the red crayfish flour 'as is' in human foods and further treatment might be a must in this regard.

Table 1 Chemical composition of red crayfish powder (particle size <600 μm)

Chemical composition of red crayfish flour	
Nitrogen/%	10.47
Protein (N·6.25)/%	65.44
Ash/%	13.43
Moisture/%	3.56
Fat/%	18.86
Carbohydrate/%	<0.01
Energy/kcal $(100\ g)^{-1}$	426

The DSC technique has been capable of providing traces with clear glass/rubber transition for many proteins at low moisture contents in particular globular proteins (preliminary study on T_g of red crayfish flour using DSC confirmed this). In this study it was tried to measure the glass transition of the red crayfish flour using two methods: DSC and PTA. As the DSC traces obtained were not very clear in determining the T_g of the powder even at high heating rates, the PTA data were used. The PTA showed a clear peak on the graph of derivative (mm $°C^{-1}$) *vs*. temperature (Fig. 1b). The glass transition of red crayfish flour was 38.5°C and therefore for physical ageing study three temperatures (5, 15 and 25°C) below and close to the T_g of the flour were chosen. This value is lower than those found by Hashimoto *et al*. [9] that reported T_g values about 60 and 70°C for 5 mass% moisture dried bonito and cod fish muscle, using DSC measurements. These authors also reported much higher values of T_g for sarcoplasmic (95–100°C) and myofibrillar protein fractions (110–120°C). They attributed the difference to the plasticizing effect of low molecular mass materials contained in the muscle (e.g. salts, sugars, minerals). The glass rubber behaviour seen for the red crayfish flour is related to its protein as the main fraction of the powder. Crayfish flour protein fraction, as in other crustaceans, mainly con-

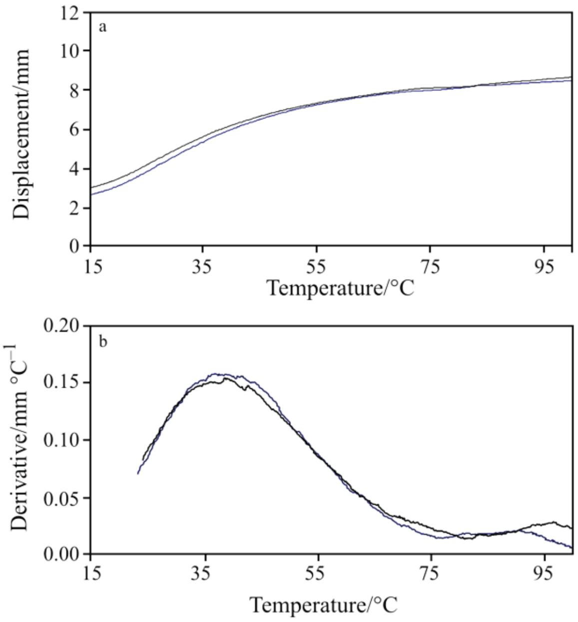

Fig. 1 a – Displacement–temperature plots from PTA indicating position of T_g. b – Derivative of displacement–temperature, the peak shows the T_g. Applied pressure: 150 bar. Heating rate: 5°C min^{-1}

sists of a mixture of water-soluble sarcoplasmic proteins (about 30 mass%) and salt-soluble myofibillar proteins (60–70 mass%), with a minor amount of insoluble stromal proteins [10]. However, the high content in lipids and other low molecular materials of crayfish flour, which would contribute to the above mentioned plasticizing effect, must be responsible for the remarkable reduction in the glass transition.

For physical ageing study as thermal history and cooling rate of a glassy polymer affects the rate at which a system reaches equilibrium, it is essential to prepare the samples with similar thermal histories. The red crayfish flour samples with moisture content of 4.5% were sealed in the DSC pans and heated in the DSC up to 100°C at 10°C min^{-1} and cooled down at 50°C min^{-1}. The sealed red crayfish flour pans were then stored for different times ranging from 2 to 168 h at three temperatures 5, 15 and 25°C. These temperatures were used to represent a range of temperatures below the glass transition temperature of the red crayfish flour samples. After the storage, the pans were rerun in the DSC and the curves were obtained. After subtracting the DSC trace of each sample before and after storage, as seen (Fig. 2) there is a low temperature peak that increases with the ageing time and ageing temperature. When amorphous polymers are stored below their glass transition temperatures, a spontaneous decrease in volume or enthalpy known as physical ageing is observed. On storage there is a relaxation towards the equilibrium state. This enthalpy loss is regained on heating and can be observed as an endotherm appearing on the DSC curve [11, 12]. It is therefore possible that the peak being observed corresponds to physical ageing.

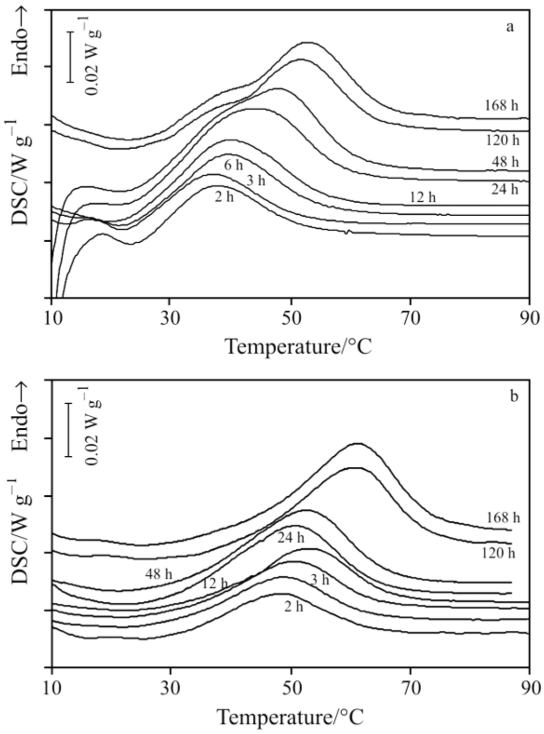

Fig. 2 DSC curves of crayfish flour samples aged at a – 15 and b – 25°C for up to 168 h. The growth of an endothermic peak with storage time can be seen in the temperature range of 25–65 and 30–80°C for storage temperatures of 15 and 25°C, respectively

Plots of peak temperature *vs.* logarithm of ageing time (min) are shown in Fig. 3 for the red crayfish samples aged at 5, 15 and 25°C. There is a linear dependence between the peak temperature and the logarithm of ageing time, this is a well-reported characteristic of physical ageing of synthetic polymers and is in agreement with the findings on starch and starch sugar systems [13]. The storage temperature does not seem to

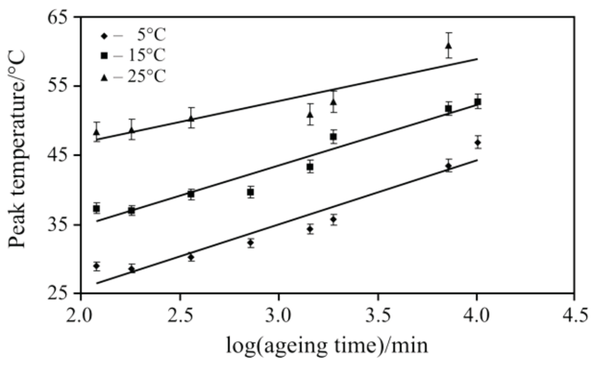

Fig. 3 Dependence of the peak temperature on the logarithm of the ageing time (min) for crayfish flour samples aged at 5, 15 and 25°C. Each bar is ±SD

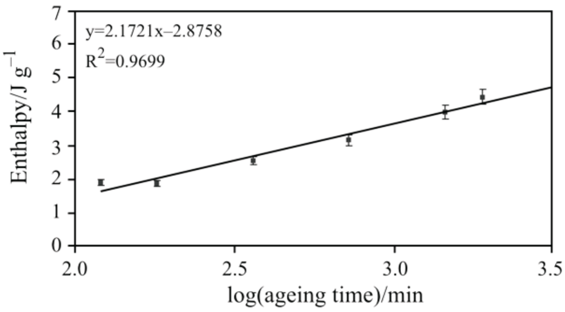

Fig. 4 Ageing endothermic energy of red crayfish flour samples containing 3.56% moisture on storage at ageing temperature of 15°C. Each bar is ±SD

influence the slope. The temperature at which the endothermic peaks appear depends on the storage temperature as is shown in Fig. 3, the higher the storage temperature the higher the peak temperature of the endotherm. For all three ageing temperatures, after 3 h of ageing, the peak temperatures appeared approximately 25°C above the temperature of storage. The plot of enthalpy *vs*. log of ageing time in Fig. 4 reveals that enthalpy increases linearly with log of ageing time, and this is a well reported typical characteristic of polymers during physical ageing process.

Although some chemical change or relocation of water or other physical change may explain the results, the position and the growth dependency of the low temperature peak does resemble those known as enthalpy relaxation for other polymers.

Conclusions

An endothermic peak was seen for red crayfish flour occurring in the region of the observed T_g and the properties of these peaks were similar to those reported for endothermic peaks derived from physically aged synthetic polymers such as poly(vinyl pyrrolidone) and poly(methyl methacrylate) [14, 15].

The results from this study would suggest that red crayfish flour undergoes substantial physical ageing, and it is worth considering why this could be so. This phenomenon could be related to the protein fraction as the main component of the flour. The flexibility of the protein molecules would be low at a molecular level, as so much of the backbone is organised into higher orders of structure. Mobility and change of side chains could be possible even when the backbone of the molecule is in the glass. This local movement could explain the changes in the glass.

Changes in solubility were seen on storing for dry bovine serum albumin samples (10% moisture) used in the previous study on physical ageing of BSA [3], although in this current study no measurement of molecular mass and protein solubility was carried out, increase in molecular mass and loss of solubility of red crayfish may be expected during storage. Physical ageing could be considered as the cause of the loss of functionality and solubility as reported as a common problem with red crayfish flour. This could have practical implication for better use of maximum functionality of red crayfish proteins as a large and expanding market, therefore requires further studies.

References

1 Y. I. Matveev, V. Y. Grinberg, I. V. Sochava and V. B. Tolstoguzov, Food Hydrocol., 11 (1997) 125.
2 R. Wungtanagorn and S. J. Schmidt, J. Therm. Anal. Cal., 65 (2001) 9.
3 A. Farahnaky, F. Badii, I. A. Faraht, J. R. Mitchell and S. E. Hill, Biopolym., 78 (2005) 69.
4 O. Cremades, J. Parrado, M. C. Alvarez-Ossorio, M. Jover, L. Collantes, J. Gutierrez and J. Bautista, Food Chem., 82 (2003) 559.
5 O. Cremades, E. Ponce, R. Corpas, J. Gutierrez, M. Jover, M. C. Alvarez-Ossorio, J. Parrado and J. Bautista, J. Agric. Food Chem., 49 (2001) 5468.
6 A. Romero, F. Cordobés, M. C. Puppo and A. Guerrero, Food Hydrocol., accepted.
7 A. Romero, F. Cordobés, M. C. Puppo, C. Bengoechea and A. Guerrero, Food Hydrocol., accepted.
8 B. Plattner, B. Strahm and K. Rausch, ASAE Annual International Meeting, Sacramento, California, USA; ASAE, St. Joseph, MI; ASAE Meeting 2001, Paper Number 01-6067.
9 T. Hashimoto, T. Suzuki, T. Hagiwara and R. Takai, Fish Sci., 70 (2004) 1144.
10 T. Suzuki, Fish and Krill Processing Technology, Applied Science Publishers, London 1981.
11 S. Montserrat, J. Polym. Sci. Part B: Polym. Phys., 32 (1994) 509.
12 H. J. Thiewes and P. A. M. Steeneken, Carbohydr. Polym., 32 (1997) 123.
13 C. Martinet, Ageing of Starch and Starch-sugar Systems in the Glassy State, Ph.D. Thesis, Nottingham University, UK 2001.
14 M. T. Kalichevsky, E. M. Jaroszkiewicz and J. M. V. Blanshard, Int. J. Biol. Macromol., 14 (1992) 257.
15 B. C. Hancock, S. L. Shamblin and G. Zografi, Pharmacol. Res., 12 (1995) 799.

Received: July 30, 2007
Accepted: February 12, 2008

DOI: 10.1007/s10973-007-8655-x