Food Chemistry 144 (2014) 2–8

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Molecular mobility, composition and structure analysis in glycerol plasticised chitosan films

Joana F. Fundo [a], Rui Fernandes [b], Pedro M. Almeida [c,d], Alexandra Carvalho [d], Gabriel Feio [d], Cristina L.M. Silva [a,*], Mafalda A.C. Quintas [a,e]

[a] CBQF – Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Centro Regional do Porto da Universidade Católica Portuguesa, Porto, Portugal
[b] IBMC/HEMS – Instituto de Biologia Molecular e Celular/Histology and Electron Microscopy Service, Universidade do Porto, Porto, Portugal
[c] ADF – Instituto Superior de Engenharia de Lisboa, IPL, Lisboa, Portugal
[d] CENIMAT-I3N, Departamento de Ciência dos Materiais, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[e] IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Braga, Portugal

ARTICLE INFO

Article history:
Available online 5 June 2013

Keywords:
Edible films
Microstructure
NMR
Molecular dynamics
Structure–function
Water behaviour

ABSTRACT

This study was developed with the purpose to investigate the effect of polysaccharide/plasticiser concentration on the microstructure and molecular dynamics of polymeric film systems, using transmission electron microscope imaging (TEM) and nuclear magnetic resonance (NMR) techniques. Experiments were carried out in chitosan/glycerol films prepared with solutions of different composition. The films obtained after drying and equilibration were characterised in terms of composition, thickness and water activity.

Results show that glycerol quantities used in film forming solutions were responsible for films composition; while polymer/total plasticiser ratio in the solution determined the thickness (and thus structure) of the films. These results were confirmed by TEM.

NMR allowed understanding the films molecular rearrangement. Two different behaviours for the two components analysed, water and glycerol were observed: the first is predominantly moving free in the matrix, while glycerol is mainly bounded to the chitosan chain.

1. Introduction

Food materials science has become more and more important area of food research, since there is an increasing need for understanding food stability. Water activity (a_w) or glass transition temperature (T_g) – a concept originated from the polymer science field – have been used for this purpose (Slade & Levine, 1991). However, these concepts, do not take in account solute–solute and solute–water interactions, which influence food systems behaviour.

Despite of the in the last years, the approach of polymer science to food systems, i.e. T_g, has been extensively applied in the food science and technology studies, nowadays, in order to better understand the molecular mobility concept nuclear magnetic resonance (NMR) has been presented as a powerful technique to understand and evaluate molecular mobility of semi crystalline systems, since it is able to provide information on molecular dynamics of different components in dense complex systems. As such, NMR spectroscopy has been extensively used to understand the structure and dynamics of complex macromolecular systems (Domjan, Bajdik, & Pintye-Hódi, 2009), specially biological systems (like foods) in solutions and in solid state (Claridge, 2009; Keeler, 2002; Yan, Mccarthy, Klemann, Otterburn, & Finley, 1996). The application of this technique may be very useful in prediction of physicochemical changes namely texture, viscosity, water migration. Specifically, ^{1}H NMR has been used to investigate water dynamics and physical structures of foods through analysis of nuclear magnetisation relaxation times (Li, Kerr, Toledo, & Carpenter, 2000). In these measurements the samples are submitted to a static magnetic field and the protons are excited by means of a radiofrequency pulse. The analysis of the signal emitted while the sample returns to equilibrium (FID) allows determining the spin–lattice (T_1) and spin–spin (T_2) relaxation. This later variable is related with the mobility of the protons in the samples matrix. This methodology has been applied in complex food systems such as crackers (Yan et al., 1996), wheat starch (Choi & Kerr, 2003), chicken meat (Li et al., 2000), carrots (Rutledge, 2001) or even a model bread crust (Nieuwenhuijzen et al., 2010).

The stability of food "matrix" (system) depends strongly on its molecular mobility (as was discussed above) but also on its microstructure. Foods are highly structured and heterogeneous materials composed of architectural elements. The types of such structural

* Corresponding author. Tel.: +351 225580058.
E-mail addresses: rfernand@ibmc.up.pt (R. Fernandes), palmeida@adf.isel.pt (P.M. Almeida), gf@fct.unl.pt (G. Feio), clsilva@porto.ucp.pt (C.L.M. Silva), mquintas@porto.ucp.pt (M.A.C. Quintas).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.05.127

units and their interactions are decisive in the food stability, since they influence water/solute interactions and hence the water availability to participate in degradation reactions. Microscopy techniques have been widely used in foods to study their architecture and microstructure (Aguilera, Stanley, & Bakerc, 2000). Transmission electron microscopy (TEM) specifically, visualises the internal structure of food samples (Kaláb, Allan-Wojm, & Miller, 1995), helping to clarify biological systems dynamics.

Edible films have been studied for a long time for their potential to improve shelf-life and safety of food products (Aider, 2010; Epure, Griffon, Pollet, & Avérous, 2011). These systems are partially crystalline/partially amorphous, easily reproducible materials and are thus very interesting food model systems to molecular mobility studies and microstructure studies. Edible films are generally prepared by evaporation of a film forming solution composed of a natural polymer, a plasticiser and water. Depending on the evaporation method and on the viscosity and molecular entanglement of the film forming solutions the obtained film may have a different composition and/or microstructure.

The addition of low molecular weight plasticisers to amorphous biopolymers increases the matrix free volume and the molecular mobility, in an effect similar to increasing temperature (Lazaridou & Biliaderis, 2002; Lefebvre & Escaig, 1993). These additives reduce the tension of deformation, hardness, density, viscosity and electrostatic charge of a polymer, at the same time increase chain flexibility, resistance and dielectric constant (Ferry, 1980). Plasticisers modify the matrix second-order interactions of materials (which are responsible for crystalline structures of polymeric materials), without altering their fundamental chemical character. This modification is achieved by forming weak second-order or covalent bonds with the polymer. Plasticisers can also migrate in the polymer leading to material recrystallisation and a loss of elasticity (Domjan et al., 2009).

Plasticisers can also affect water retention capacity (Lefebvre & Escaig, 1993). Water, considered a plasticiser, is also one of the most important solvent medium in biological systems. (Matveeva, Grinberga, & Tolstoguzov, 2000). It greatly affects the mobility of biopolymers components and is considered as an abundant and very effective solvent/plasticiser for hydrophilic materials (Lazaridou & Biliaderis, 2002). On a molecular level, water plasticisation of a polymer leads to increased free volume, decreased local viscosity an increased back-bone chain mobility (Slade & Levine, 1991).

Chitosan, a polysaccharide composed mainly of (1 → 4) linked residues of *N*-acetyl β-D-glucosamine and (1 → 4) β-D-glucosamine (Arzate-Vázqueza et al., 2012; Ostrowska-Czubenko & Gierszewska-Drużyńska, 2009; Prashanth & Tharanathan, 2007; Rinaudo, 2006; Yang, Liu, Wu, & Li, 2010), is a pseudonatural cationic polymer widely used in edible films studies. Structurally, chitosan is a semicrystalline biopolymer having a huge potential for chemical and mechanical modifications to create novel properties, functions and applications in different areas (Bangyekan, Aht-Ong, & Srikulkit, 2006; Pillai, Paul, & Sharma, 2009; Rinaudo, 2006).

Chitosan crystal structure is stabilised by intramolecular and intermolecular H-bonds, with the acetamide groups playing the major role in the formation of second-order bonds between adjacent chains (Okuyama, Noguchi, & Miyazawa, 1997), making the chitosan structure on a film very dependent on the type and quantity of plasticisers used.

The objective of this study was to understand the relationship between the composition of film forming solutions and the properties (composition and microstructure) of the obtained glycerol plasticised chitosan films, evaluating the role of water and glycerol as plasticisant agents, and the effect of its concentrations in the systems performance. Also, we aimed at analysing the molecular mobility of such studies, in order to recognise its properties and improve its suitability as models for more complex food systems. For that purpose, film forming solutions of different polymer/plasticisant concentrations were prepared and the obtained films characterised in terms of composition, molecular mobility and microstructure.

2. Materials and methods

2.1. Chitosan films preparation

Film forming solutions were prepared by dissolving different chitosan (90% deacetylation, Aqua Premier Co., Thailand) concentrations (1%, 2% and 3% w/v) in a 1% lactic acid (Acros Organics, Belgium) with three different levels of plasticising agent (10%, 50% and 90% V/W), glycerol (Panreac, Spain). A constant amount (300 ml) of the chitosan solutions was casted in 32 × 40 cm plates and dried in an incubator at 40 °C, during 3 days. Films were stored at 22 °C and 53%RH, until equilibrium was reached prior to any characterisation.

2.2. Characterisation of the obtained films

2.2.1. Chemical composition

The final composition in chitosan and glycerol of the obtained films was determined. Chitosan concentration was estimated using a spectrophotometric method (Muzzarelli, 1998). Briefly, chitosan films were dissolved in 100 ml of lactic acid solution 4%. Cibacron brilliant red 3B-A from Sigma (Milano, Italy) was used as dye. A solution of dye was prepared by dissolving 150 mg of the powder in ultra-pure water, using a 100 ml volumetric flask. Aliquots of the dye solution, 5 ml, were made up to 100 ml with 0.1 M glycine hydrochloride buffer. Spectrophotometric measurements were done at room temperature and 575 nm, with a UV – 1601; Shimadzu Co., Kyoto; Japan.

Glycerol concentration in films was determined using a quantitative enzymatic determination (Free Glycerol Determination Kit, from Sigma, Milano, Italy). Spectrophotometric measurements were done at room temperature and 540 nm using the same spectrophotometer.

2.2.2. Thickness

The thickness of the produced films was measured using a digital micrometre (Mitutoyo, Japan). From each sample (true replicates), a minimum of eight stripes (15 × 170 mm) were cut and, at least two readings were randomly taken at different positions.

2.2.3. Water activity

Measurements were performed with a dew point hygrometer (Aqualab – Series 3, Decagon Devices Inc., USA.), at 23 ± 1 °C. The sensitivity of the equipment was 0.001. Calibration was carried out before experiments with distilled water and saturated saline solutions. Water activity value of each sample (true replicates) was the average of nine readings.

2.2.4. Nuclear magnetic relaxation

A Bruker AVANCE III solid state spectrometer (300 MHz) was used to determine the samples nuclear transverse relaxation time, or spin–spin, T_2, of the protons.

These values were obtained from the exponential or bi-exponential echoes envelope of a series of Carr–Purcell–Meiboom–Gill (CPMG) multi-echo pulse sequence, which circumvents the field and sample heterogeneities and give access to the intrinsic T_2 of the protons, while the Free Induction Decay (FID) obtained from a single pulse just gives a T_2^* determined mainly by the field non-uniformity in the heterogeneous film sample contained in the NMR 5 mm tubes.