Innovative Food Science and Emerging Technologies 36 (2016) 83–91

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Development of innovative biodegradable films based on biomass of *Saccharomyces cerevisiae*

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ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 18 May 2016
Accepted 2 June 2016
Available online 3 June 2016

Keywords:
High pressure homogenisation
Yeast
Saccharomyces cerevisiae
Biodegradable film
Characterisation

ABSTRACT

Biodegradable alternatives to petroleum-based polymers are being developed for food packaging. *Saccharomyces cerevisiae* has been widely used for the production of fermented beverages and leavened foods. In this work, the application of high pressure homogenisation and a thermal treatment to the yeast biomass was studied to develop biodegradable films. The highest dispersibility indexes of protein (84.5 ± 3.3%), carbohydrates (24.3 ± 1.1%), RNA (40.6 ± 0.9%) and soluble solids released were found at high homogenisation pressure (125 MPa). Combinations of one or two homogenisations and a thermal treatment at 90 °C during 20 minutes were applied and dispersions were fully characterised, focusing on their film-forming capacity. The best combination was homogenisation, heat treatment and a further homogenisation, since it produced films that presented good attributes, great continuity and homogeneity without small cracks. However, hydration of films was increased from 0.31 to 0.48 $gH_2O/g.d.m$ with the number of homogenisation processes applied. Results revealed that yeast biomass is a viable source to be used in biodegradable films.

Industrial relevance: Saccharomyces cerevisiae yeasts have many applications in food industry. The development of biodegradable films based on yeast biomass carries many advantages such as, the possibility of using commercial pressed baker's yeast or an industrial residue from brewing industry, the use of a low-cost sources and the application of environmentally friendly procedures. The methodologies applied for the development of the film forming dispersion, high pressure homogenisation and thermal treatment, are able to be scaled-up to an industrial level.

1. Introduction

Food quality deterioration due to physicochemical changes or chemical reactions is often caused by the interaction between food and the surrounding medium or inside the product (Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002). Food packaging protects products from the environment and their developments are trending towards the use of biopolymers to manufacture food contact materials in order to increase products shelf-life. These developments also respond to consumer demand to the use of natural products with low environment contamination. Biopolymers used to develop food packaging materials are polysaccharides, proteins and lipids (Janjarasskul & Krochta, 2010; Campos, Gerschenson, & Flores, 2011 and Pan, Jiang, Chen, & Jin, 2014). Polysaccharides and proteins are generally used for their ability to establish polymer interactions and create a continuous network responsible for the functional properties of the biodegradable films (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011 and Giancone, Torrieri, Masi, & Michon, 2009). The main polysaccharides from agricultural or industrial by-products sources used are chitosan, starch, alginate, carrageenan, modified cellulose and pectin (Han & Gennadios, 2005). Casein, gluten, whey, soy proteins, gelatine, etc. are those proteins mostly studied to manufacture edible films and packaging materials (Ciannamea, Stefani, & Ruseckaite, 2015; Krochta, 2002 and Pérez-Gago, Nadaud, & Krochta, 1999). In this sense, animal and vegetable proteins are of great interest for the production of food packaging films because of their relatively low cost, high availability as by-products of food industry and agriculture and inherent biodegradability (Ciannamea, Stefani, & Ruseckaite, 2014; Pereda, Aranguren, & Marcovich, 2008 and Caprioli, O'Sullivan, & Monahan, 2009).

While waxes, like beeswax or paraffin wax are examples of lipids components for these systems (Morillon et al., 2002). These types of materials are useful to control moisture, gases and lipid migration.

Cells of *Saccharomyces cerevisiae* can be regarded as food-grade, low cost and abundant food ingredient. Traditionally, these unicellular fungi

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http://dx.doi.org/10.1016/j.ifset.2016.06.002
1466-8564/

have been used by the food industry principally for their production of ethanol and carbon dioxide which are important to the brewing, wine, distilling and baking industries (Ferreira, Pinho, Vieira, & Tavarela, 2010). Nowadays, there are other applications that are gaining more attention. Yeast biomass, as a by-product of food industry, contains about half of its dry weight as proteins and as polysaccharides that could be isolated for the upgrading of yeast production (Otero, Wagner, Vasallo, García, & Añón, 2000 and Otero et al., 2002).

Wagner, Sceni, and Otero Rambla (2009) explained that a yeast cell suspension can be adequately treated and that is possible to isolate individual components of interest. This treatment is determined depending on the location of the compound, intracellular or extracellular. When the compound is located extracellular is possible to separate by centrifugation or filtration. On the other hand, if the compound is located intracellular is necessary to break the cell, and then separate the insoluble and soluble fraction. Two types of rupture are described in bibliography: mechanical and non-mechanical (Liu, Zeng, Sun, & Han, 2013). The mechanical methodologies comprise high pressure homogenisation (liquid shear), bed mill (solid shear), etc. The non-mechanical ones are lysis cellular by osmotic shock-heat treatment, the addition of solvents (toluene) or detergents and/or the use of enzymes (Freimund, Sauter, Käppeli, & Dutler, 2003 and Bzducha-Wróbel et al., 2014). Sceni et al. (2009) described the fractionation of yeast cell by its rupture with glass beads grinder and they characterised each fraction, soluble and insoluble.

Yeasts contain approximately 40–60% of proteins and they are obtained as concentrates and isolates exhibited some functional properties such as hydration, rheology and surface agents (Sceni et al., 2009; Pacheco & Sgarbieri, 1998 and Kinsella, 1986). Also, polysaccharides are basic components of yeast cell walls. Isolated insoluble β-glucan has been proven to strengthen the immune system (Brown & Gordon, 2001 and Wu et al., 2013), they showed anti-bacterial, anti-tumor and anti-viral effects and accelerate wound healing (Chan, Chan, & Sze, 2009). In addition, they could be used as a food thickener (Paul, Morin, & Monsan, 1986 and Xiu, Zhou, Zhu, & Zhang, 2011).

Applications of yeast cell components for the preparation of biodegradable films were described by Novák et al. (2012). Yeast cell wall polysaccharide suspensions (β-(1-3)-(1-6)-*D*-glucan) were isolated from baker's yeast to prepare films with the addition of glycerol as plasticizer. Those prepared films were water-insoluble, compact, non-porous and non-crystalline. On the other hand, there are no descriptions concerning the use of the whole cell, cell wall and cytoplasm, to produce films. Therefore, we consider to study this further, to take profit of the interactions between polysaccharides and proteins to develop a film.

The aim of this work was to use the integral yeast cell biomass to produce films intended for food contact materials, using homogenisation to break the cell wall and release intracellular materials followed by subsequent treatments. Characterisation of dispersions and films was carried out to study the obtained films and their relationship with treatments performed on dispersions.

2. Materials and methods

2.1. Materials

Commercial samples of pressed bakers' yeast (*Saccharomyces cerevisiae*) were obtained from CALSA (AB Mauri, Argentina). Reagents and salts used in this work were of analytical grade.

2.2. Study of the homogenisation process on the yeast cells rupture

Commercial baker yeast (500 g, 30 g/100 g dry matter) were dispersed in 2 L of distilled water and washed by centrifugation at 4 °C and 1380 g (Beckman Centrifuge J2-MC, Beckman Coulter Inc., USA) during 20 min. Afterwards, a mother dispersion of 10% wt/v of clean yeast cells was prepared in distilled water and subjected to a continuous two-stage homogenisation under different pressure conditions from 75 to 150 MPa, during 9 min in a valve homogeniser (Panda 2 K NS1001L, GEA Niro-Soavi, Italy).

2.3. Determination of particle size

The particle size distribution (PSD) of dispersions was measured by laser diffraction by using a Malvern Mastersizer® 2000E (Malvern Instruments, Worcestershire, UK) in the range of 0.1–1000 μm. The volume used for measurements was 0.2 mL of dispersion in 600 mL of water and stirred at 2000 rpm (Hydro Pump 2000MU, Malvern Instruments, Worcestershire, United Kingdom) and room temperature. Experiments were performed three times in triplicate. Results were reported as volume and number frequency (×100). Optical parameters applied were: relative refractive index of dispersed particle and water, 1.52 and 1.33, respectively and adsorption coefficient: 0.1.

2.4. Evaluation of the material composition after homogenisation

2.4.1. Dispersible matter and water retention capacity (WRC)

Dispersible matter was determined gravimetrically after centrifuge the dispersion at 19,837 g during 15 min (rotor JA-14, Beckman Centrifuge J2-MC, Beckman Coulter Inc., USA). In addition, WRC was determined gravimetrically by calculating solid residue of the sediment obtained after centrifugation. The supernatant and the sediment were dried at 105 °C for 1 h and weighed in an analytical balance ±0.001 until constant weight. Dispersible matter was determined after performing experiments in duplicate after three repetitions.

2.4.2. Protein (P), carbohydrates (CH) and ribonucleic acid (RNA) concentration

Whole cell dispersion composition was determined. Protein concentration was determined by micro-Kjeldahl method as described by Nkonge and Murray-Ballance (1982). This methodology consists in an acid digestion of the sample followed by the determination of the released ammonium by colorimetry. For the digestion, a standard solution was prepared by mixing 47.2 mg de $(NH_4)_2SO_4$ (p.a), 0.11 g of a catalysis mix (92.8% K_2SO_4, 2.8% TiO_2, 4.4% $CuSO_4 \cdot 5H_2O$) and 3 mL of concentrated sulfuric acid. The mixture was heated until completely digested, cooled, and distilled water was added up to 100 mL. Nitrogen content in the solution was 100 μg/mL.

The blank was performed following the same procedure but without the addition of $(NH_4)_2SO_4$. The standard curve was prepared by mixing different volumes of standard solution and blank, to obtain 0.5 mL of solutions with nitrogen concentrations of 0 to 35 μg/mL.

For samples digestion the same procedure was followed, but replacing the $(NH_4)_2SO_4$ by a certain amount of sample, between 200 and 1000 mg, ensuring nitrogen content within the limits of the calibration curve in 0.5 mL of the digestate.

Carbohydrate (CH) content was determined by *Dubois method* (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). This is 1 mL of the sample was mixed with a solution of phenol al 5% p/v (0.5 mL) and homogenised with a vortex. Afterwards, 2.5 mL of H_2SO_4 concentrated was added and homogenised again. The mixture was incubated during 10 min at room temperature and 15 min at 37 °C. After cooling, the absorbance at 490 nm was measured. A calibration curve was prepared using a glucose solution as standard in a range from 0 to 80 μg/mL. CH percentage in dispersion and supernatant were calculated with Eq. (1) and Eq. (2), respectively.

$$\%CH_{dispersion} = \frac{(Abs - b)}{a} \times \frac{m_{dispersion}}{m_{dry}} \times \frac{1g}{10^6 \mu g} \times 100 \quad (1)$$

$$\%CH_{supernatant} = \frac{(Abs - b)}{a} \times \frac{m_{supernatant}}{m_{dry}} \times \frac{1g}{10^6 \mu g} \times 100 \quad (2)$$

where a and b are the slope and the intercept of the calibration curve respectively; $m_{dispersion}$ is the dispersion mass; m_{dry} is the dispersion dry mass and $m_{supernatant}$ is the supernatant mass ($m_{dispersion}$–m_{dry}).

RNA concentration was determined following *Rut method* (Rut, 1973) with some modifications (Sceni et al., 2009). One milliliter of sample (supernatant) and 5 mL of $HClO_4$ 0.5 M were added in a glass tube. After heating 20 min at 90 °C, a centrifugation step was applied at 750 *g* for 15 min. Supernatant absorbance was determined at 270 and 290 nm. RNA percentage was determined with Eq. (3).

$$\%RNA_{Supernatant} = \frac{(Abs_{270} - Abs_{290})}{163000} \times vol_{final} \times \frac{m_{Supernatant}}{m_{dry}} \times 100 \quad (3)$$

where Abs_{270} and Abs_{290} are the absorbances at 270 and 290 nm respectively; vol_{final} is the total volume of the tube (6 mL); m_{dry} is the supernatant dry mass and $m_{supernatant}$ is the supernatant mass that is obtained by the difference between the dispersion mass and the mass of the dry sediment.

Same determinations were performed to the supernatants obtained from centrifugation of dispersions treated and non-treated. From these values, the dispersible index (DI %) was calculated using Eq. (4). All described assays were realized in triplicate with two repetitions.

$$DI(\%) = (\%\text{component in supernatant}/\%\text{component in native yeast}) \times 100. \quad (4)$$

2.5. Assessment of the combination of different treatments on yeast dispersions

Dispersions at 10% wt/v were subjected to different treatments that combined homogenisation and a thermal treatment at 90 °C in a water bath for 20 min, considering that at this temperature proteins are denatured as demonstrated by Otero et al. (2002). The homogenisation conditions selected were 125 MPa, in agree with results obtained in a previous section. To minimize a temperature rising, the homogenate were cooled between passes with an ice-water bath. The different procedures were combined by doing one treatment before the other as follows:

No treatment, whole cells (**W**); only heating (**T**); only homogenised at 125 MPa (**H**); homogenised at 125 MPa followed by heating (**HT**); heating followed by homogenisation at 125 MPa (**TH**) and homogenised at 125 MPa followed by heating and further homogenisation (**HTH**).

2.6. UV–Vis spectra of yeast dispersion

UV–Vis spectra of yeast dispersions resulting from different treatments were obtained using a UV–Visible spectrophotometer (T60, PG Instruments, United Kingdom). From the spectrum of each sample at adequate dilution, values of turbidity were obtained by absorbance at 500 nm. Determinations were performed in triplicate.

2.7. Preparation of yeast films with treated dispersions

Films based on yeast biomass were performed by casting method using dispersions described in Table 1. Twenty milliliters of each aqueous dispersion were poured into plastic Petri dishes of 90 mm of diameter. Evaporation of the water was done at 55 °C in a ventilated oven (Sanyo MOV 212F, Japan) until the remaining water content of the films was between 10–15%. Samples were stored at 43% relative humidity (r.h.) and 21 °C.

Table 1
DI (%) of carbohydrates, proteins and RNA after homogenisation process. **W**: No treatment, whole cells; **H**: homogenised dispersion, subscripts indicate the reached pressure.

Sample	Dispersibility index (DI %)		
	CH	P	RNA
W	3.8 ± 0.4	2.3 ± 0.2	2.4 ± 0.1
H_{75}	21.2 ± 2.4	60.5 ± 2.5	24.8 ± 0.2
H_{100}	24.0 ± 0.7	82.6 ± 2.8	38.6 ± 0.8
H_{125}	24.3 ± 1.1	84.5 ± 3.3	40.6 ± 0.9
H_{150}	24.4 ± 0.8	82.7 ± 3.0	38.1 ± 0.2

CH: carbohydrates; **P**: protein; **RNA**: ribonucleic acid.

2.8. Yeast films characterisation

2.8.1. Quality evaluation

The quality of the films obtained was assessed using the method proposed by Gontard (1991) and modified by Moraes, Scheibe, Sereno, and Laurindo (2013). Quality aspects considered for the evaluation were handleability, homogeneity and continuity. Handleability informs if the film can be manipulated without being broken, continuity expresses the absence or presence of breaks in the film and uniformity informs about the homogeneity of the film. This evaluation was carried out with three independent replicates.

2.8.2. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was carried out on the dispersions after each treatment. Samples without previous dilution were collected on copper grids (Formvar/carb, 300 mesh) and subsequently stained using saturated uranyl acetate solution for 1 min. The electron microscope (Phillips EM-301, Netherlands) was operated at 60 kV.

2.8.3. Hydration properties

2.8.3.1. Water sorption isotherms. Water sorption isotherms allow to study the equilibrium water uptake as a function of relative humidity (r.h.) or water activity (a_w = % r.h./100) of the environment. Sorption isotherms of films were determined gravimetrically according to standard procedure (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007). We use seven sorption containers of 1.5 L, each one containing a saturated salts solution of LiCl, $MgCl_2$, K_2CO_3, NaBr, NaCl, KCl, and $BaCl_2$, to generate a_w of 0.11, 0.33, 0.43, 0.57, 0.75, 0.84 and 0.90, respectively (Spiess & Wolf, 1983). Dried atmosphere of 0% r.h. was generated using silica gel. Film samples of approximately 58 cm^2 and 0.03 cm of thickness were placed in desiccators containing saturated solutions or silica gel. Weight of samples was made using analytical balance ($\pm 10^{-4}$ g). The water content or intrinsic hydration *h* (g of water per g of dried mass) was evaluated as function of a_w taking the difference between the mass of the hydrated film and that of the fully dried film. Sorption tests were done in duplicate at each relative humidity. Experiments were performed at 24 °C.

Isotherms were fitted using Guggenheim-Anderson-De Boer (GAB) model (Guggenheim, 1966) trough Eq. (5):

$$h(a_w) = \frac{N.c.k.a_w}{[(1 + (c-1)k.a_w)(1-k.a_w)]} \quad (5)$$

where *N* is the monolayer water content (g of water per g of dried mass) related to primary binding sites of water molecules, *c* is a parameter related to the sorption heat monolayer, also can be interpreted as a parameter related to the force of the water binding to monolayer. Finally, *k* is a factor related to sorption heat multilayer (Eim, Rosselló, Femenia, & Simal, 2011) and represents the capability of water to bind to the multilayer (Salvay, Colombo, & Grigera, 2003).

2.8.3.2. Kinetics of water sorption. Kinetics of water vapour sorption was measured at 24 °C. Fully dried film samples were placed into the sorption containers at 90% r.h. The samples were removed at specific intervals of time and weighed for data collection. The water content h as function of time t was fitted with a first order kinetics model, using a biexponential function that take account two rates for water uptake (Salvay et al., 2003):

$$h(t) = h_0 + A_1\left[1-\exp\left(-\frac{t}{\tau_1}\right)\right] + A_2\left[1-\exp\left(-\frac{t}{\tau_2}\right)\right] \quad (6)$$

where h_0 is the initial water content, A_1 and A_2 are constants, and τ_1 and τ_2 are the time constants for water uptake of process 1 and 2 respectively.

3. Results and discussion

3.1. Effect of homogenisation process on the yeast cells rupture

Homogenisation process breaks yeast cell wall and leads to the release of intracellular material and both contribute to the optimum film-forming dispersion.

Composition of native yeast was RNA (%): 7.72 ± 0.04; proteins (%): 41.2 ± 0.4 and carbohydrates (%): 46.6 ± 1.2. Values of dispersibility index of proteins, carbohydrates and RNA calculated with Eq. (4) are showed in Table 1. Proteins were the most important components in the dispersible solid fraction and their concentration in dispersions processed at 125–150 MPa seemed to be higher than at lower pressures. A maximum concentration of proteins and nucleic acids were determined when processing in the range of 100–150 MPa.

More data of the evaluation of different homogenisation pressures is very well described in Supplementary material.

Results from this study demonstrated that homogenisation at 125 MPa is the most suitable condition to process yeast cell that lead to the release of a convenient amount of intracellular material, an appropriated rupture of the cell wall and a good water retention.

3.2. Yeast dispersions treated by homogenisation and heating and combined treatments

The different treatments and their combinations applied to yeast dispersions lead to suspensions with characteristics and properties that conduct to different products with filmogenic properties. High pressure homogenisation breaks yeast cell wall and intracellular material is released to the surrounding media (Spiden, Scales, Kentish, & Martin, 2013) as it was demonstrated in previous sections. Meanwhile, heat treatment produces enzymatic reactions inactivation, protein denaturing and unfolding of the triple helix of β-glucan present in the cell wall (Novák et al., 2012). Protein denaturation is guaranteed at the selected temperature (90 °C), as it was demonstrated in previous studies using the whole cells of *S. cerevisiae* and denaturation temperature peak was approximately 66 °C for these cells (Otero et al., 2000).

3.2.1. Particle size of treated yeast dispersions

Particle size distributions of treated dispersions are showed in Fig. 1. Both dispersions T and H displayed in % Volume (Fig. 1a), presented a main size distribution peak is centred at 5 μm. At this stage, the cells may keep their natural morphology but with some fissures. However, sample T presented another population centred at 120.2 μm indicating that agglomerates were formed during the heat treatment. In these dispersion related to those cells. In sample HT, intracellular material has been released to the surrounded media and then, after the thermal process, protein denaturation and aggregation occurred (Wagner et al., 2009). This is reflected through a broad peak extended up to 200 μm. When the thermal treatment was applied before homogenisation (TH sample), results showed a bimodal size distribution similar to

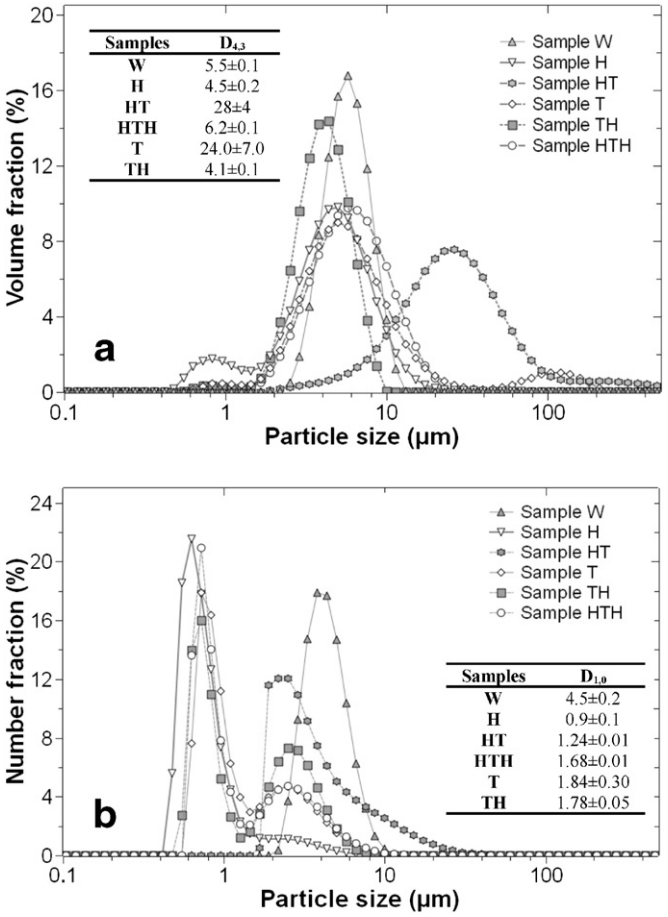

Fig. 1. Comparison of A) % Volume fraction and B) % Number fraction of samples with different treatments. **W:** No treatment, whole cells; **T:** only heating; **H:** only homogenised; **HT:** homogenised followed by heating; **TH:** heating followed by homogenisation; **HTH:** homogenised followed by heating and further homogenisation.

sample H, described in Supplementary material (H = H_{125}). In this case, proteins denaturation takes place into the cytoplasm and aggregation is limited by the cell wall. Afterwards, homogenisation breaks these aggregates and the final process determined the profile of the distribution. Thus, the order of the treatment is very important, even if the treatments applied were identical, HT or TH. Sample HTH showed a similar distribution profile to TH. In both samples, aggregates were broken due to the final homogenisation. Mean diameter over volume ($D_{4,3}$), also called de Brouckere mean, is more sensitive to aggregation processes (Palazolo, Sobral, & Wagner, 2011). As showed in Fig. 1a, $D_{4,3}$ values were different between samples, probably due to the fact that protein denaturation occurred inside the cell in TH sample and outside the cell in HTH sample. Fig. 1b shows particle size distribution in %Number with number mean ($D_{1,0}$) or arithmetic mean, this value is sensitive only with number of particles. Fig. 1b shows that dispersion H, T and HTH presented a population centred at 1 μm corresponding to small particles coming from the broken cell wall due to homogenisation and, in the case of T dispersion, because of the solubilisation of some components from cell wall and from the cytoplasm extracted from small fissures produced by heat treatment.

3.2.2. Turbidity of yeast dispersions with combined treatments

Turbidity measurements of yeast dispersions represent the amount of particles that are suspended into the media. This information gives a clue of the micronisation degree of the cell and the presence of protein aggregates formed during the heating process and afterwards reduced by homogenisation. Table 2 shows the absorption at 500 nm indicating

Table 2
UV spectra at 500 and dispersibility index DI(%) of the supernatant after treatments. **W:** No treatment, whole cells; **T:** only heating; **H:** only homogenised; **HT:** homogenised followed by heating; **TH:** heating followed by homogenisation; **HTH:** homogenised followed by heating and further homogenisation.

Sample	UV–Vis spectra	Dispersibility index (%)–DI(%)		
	A_{500}	CH	P	RNA
W	0.99 ± 0.01	3.8 ± 0.4	2.3 ± 0.2	2.4 ± 0.1
T	0.81 ± 0.01	25.5 ± 1.3	17.5 ± 0.9	31.9 ± 1.6
H	0.38 ± 0.03	24.3 ± 1.2	84.5 ± 4.2	40.6 ± 2
TH	1.02 ± 0.03	45.9 ± 2.3	18.5 ± 0.9	36.2 ± 1.8
HT	0.62 ± 0.07	51.1 ± 2.5	18.7 ± 0.9	47.8 ± 2.4
HTH	0.74 ± 0.01	58.0 ± 2.9	20.0 ± 1	48.8 ± 2.4

CH: carbohydrates; P: protein; RNA: ribonucleic acid.

the turbidity of the samples. Fig. 2 shows the TEM micrographies of treated dispersions. When yeast dispersion is only heated (T), there is certain turbidity due to the release of some cell wall components. In this case, protein aggregation happened inside the cell; therefore the formed aggregates are tight and thick to be confined in the internal cell space (Fig. 2a). When this sample is homogenised, sample TH is obtained and turbidity increased as expected due to the cleavage of the aggregates, but the structure is still closed (Fig. 2b). Regarding the sample H (Fig. 2c) there is a decrease in the turbidity value respect to W due to the solubilisation of intracellular components. When sample H is heated, HT dispersion is obtained and the turbidity value is increased due to a higher interaction between proteins and aggregates formed. The structure of these aggregates is open and highly hydrated since they are formed in an aqueous media (Fig. 2d). The last homogenisation in sample HTH raised the turbidity value due to the increase of the number of suspended particles, produced by the size reduction of aggregates created during the heating step. Indeed, in this case the structure is even more open (Fig. 2e,f).

The WRC was also determined from the insoluble fraction separated by centrifugation of treated dispersions. This parameter represents the capability of the material to form a network that retains water. This behaviour is desired when using a biopolymer as a film-forming material. Butler, Vergano, Testin, Bunn, and Wiles (1996) explained that the most important mechanism of film formation is a previous loss of the polymer chain and then a reforming of the chain into a matrix with the water retained informed network.

Fig. 3 shows results of WRC (%) and dispersible matter of the treated dispersions. It could be observed that both parameters were decreased in dispersion HT respect to H because of the protein aggregation after heating. Meanwhile, dispersion HTH presented an increase in dispersible matter but value of WRC (%) did not change respect to HT. Dispersion of cell wall components and some cytoplasmatic material happened in dispersion T evidenced by the increase in dispersible matter respect to W, however WRC (%) is decreased due to protein coagulation inside the cell. Dispersible matter is augmented after the homogenisation of the heated dispersion (TH) corresponding to smallest particles dispersed as showed in Fig. 1b. Table 2 reports the percentage of each component in the supernatant obtained after the centrifugation of treated dispersions and dispersible index was calculated as explained in Eq. (4).

Table 2 demonstrates that heating after homogenisation produced a reduction in DI (%) of P, but values for CH and RNA were increased. This indicates that the main reaction is the protein aggregation. Comparing samples W and T there is an increase in DI (%) of P with the heating treatment due to the release of mannoproteins from the cell wall (Wagner et al., 2009), the same trend for carbohydrates and RNA.

Results expressed that dispersions HT and HTH presented a good balance between dispersed components, since homogenisation before heating produce a lower protein aggregation than heating the whole yeast cell (T), due to a dilution of released cytoplasmatic proteins in comparison with aggregation inside the cell, respectively. If the dispersion HT is compared to HTH, both presented the same amount of dispersible proteins; however, the latter contains a higher amount of dispersible carbohydrates that would favour afterwards the film formation.

It is clear that yeast dispersions are multi-component systems where more than one hydrocolloid is present (mostly proteins and polysaccharides) able to form a continuous and cohesive network needed to form a film as described by Giancone et al. (2009). Therefore, it is important to have a correct amount of proteins and carbohydrates released to the media that can be available to establish polymer interactions.

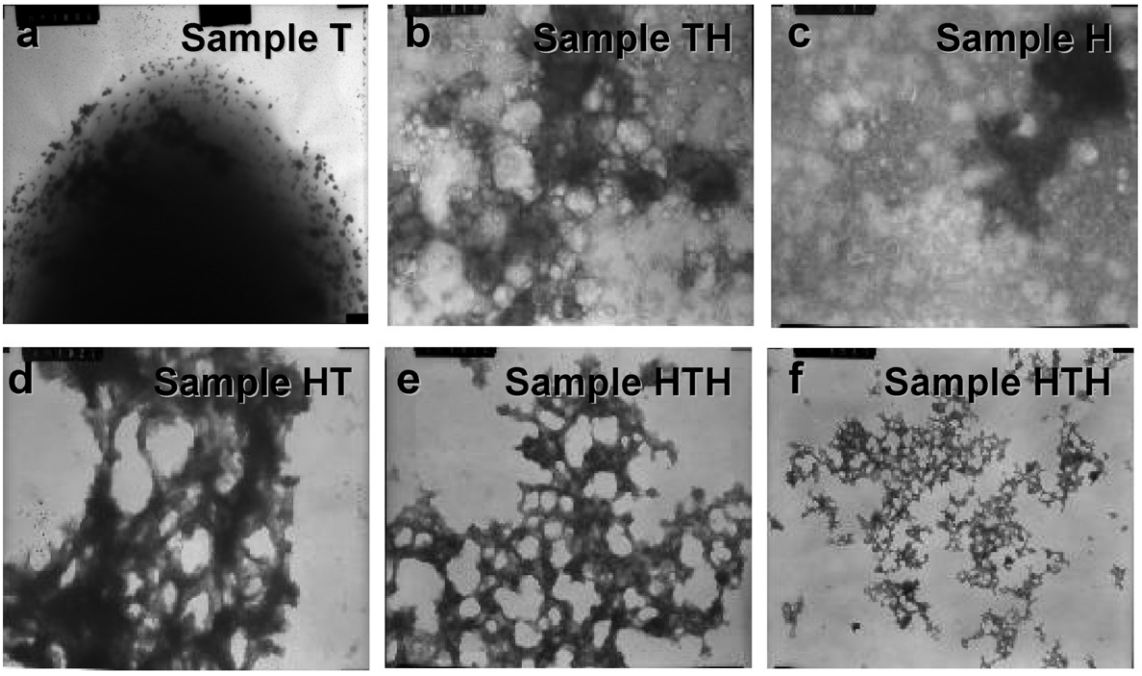

Fig. 2. TEM micrographies of treated dispersions: a) T (×35000); b) TH (×26000); c) H (×35000); HT (x35000); HTH (×35000); HTH (×10000). **T:** only heating; **TH:** heating followed by homogenisation; **HTH:** homogenised followed by heating and further homogenisation.

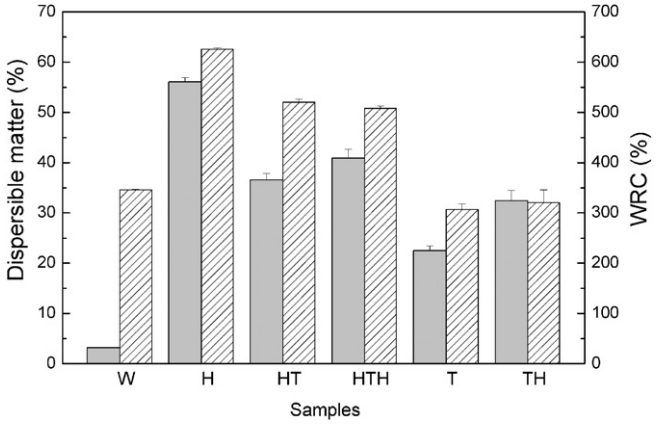

Fig. 3. Dispersible matter (%) (plain bars) and WRC (%) (scattered bars) of yeast dispersion (b) submitted to different treatments (H = H_{125}).

3.3. Characterisation yeast films obtained by treated dispersions

3.3.1. Quality evaluation

Combinations that produced a film-forming dispersion were HT, TH and HTH, this demonstrated the importance of combining homogenisation and heat treatment. Indeed, the order of the treatments had an influence on the final characteristics of the films. Best results were obtained in HTH combination with a good handleability, continuity and uniformity, TH and HT dispersions formed film but with a difficult handleability and presented some breaks in the matrix decreasing the continuity of the films. However, films obtained with HT dispersions presented higher amounts of breaks than TH film due to homogenisation process breaks the aggregates formed during heating.

When proteins coagulate in whole cells, they form aggregates inside of these ones, so the network needed to give structure to the film is not formed. On the other hand, when proteins are firstly unfolded and then aggregated, there was the possibility to interact and reorganize to form a complex network when aggregates, as demonstrated in TEM micrographies (Fig. 2). When yeast dispersions were previously homogenised, intracellular material is released by the cell wall cleavage, then the heat treatment produces β-glucan and protein unfolding, protein-water and protein-protein interactions are increased and aggregates were formed but then with the last homogenisation those aggregates are broken and new interactions are formed and so, an accurate network is obtained for film formation.

Some patents and studies postulated the use of waste brewer yeast for the production of films and coatings (Kasai et al., 2000 and Kirin Beer Kabushiki Kaisha, 2002). However, they proposed to remove the intracellular soluble material and use only the cell wall material to prepare the films. They argued that intracellular material is not capable to form films and even hinders their formation, may be proteins were very hydrolisated and they lost their capability to form a network. However, our results demonstrated the potential of dispersions of the whole yeast to prepare films intended for food packaging materials when applying treatments in a suitable order, indicating that both intracellular material and the cell wall components contribute to the film-forming material, gaining in higher yields. It is likely that in those works the intracellular material has not been taken into account because waste baker's yeast biomass did not guarantee the integrity of the proteins due to an advance hydrolysis degree of proteins.

At this point, results demonstrated that is possible to achieve film-forming dispersions using the entire yeast cell depending on the treatments applied in certain order. Then, depending on the application that formed films will cover, one or other procedure should be done. If the aim is to develop an edible coating with yeast biomass, some treatments should be done to the film-forming dispersion before the casting step, such as the removal of ribonucleic and deoxyribonucleic acids that may cause the accumulation of uric acid in the body (Lyutskanov, Koleva, Stateva, Venkov, & Hadjiolov, 1990). On the other hand, to perform food packaging materials those treatments could be avoided but some modifications in formulations such as plasticization (Vieira, da Silva, dos Santos, & Beppu, 2011), reinforcing (Pereda, Amica, Rácz, & Marcovicha, 2011) or adding an hydrophobic compound to decrease the film hydrophilic behaviour (Slavutsky & Bertuzzi, 2015) could be necessary to reach the desired functional properties.

3.3.2. Hydration properties

The reduction of the exchange of water between the food and the environment is an important role of biodegradable films, in particular to reduce drying of moist foods. Solubility of water in the film affects directly the moisture barrier properties, moreover the increasing in water content causes an augment in elongation properties and a decrease in tensile strength and elastic modulus (Cuq, Gontard, Cuq, & Guilbert, 1997). Therefore is especially important to characterise the hydration in the film matrix through kinetics of water uptake and sorption isotherms (Coupland, Shaw, Monahan, O'Riordan, & O'Sullivan, 2000). To characterise the hydration of yeasts films, samples T, TH, and HTH were studied. Film T was selected to observe the effect of a purely thermal treatment and explore the changes on hydration produced by previous and next pressure homogenisation.

3.3.2.1. Kinetics of water sorption. Fig. 4A displays representative plots of the kinetics of water uptake for the film T, TH, and HTH, at 90% r.h. Table 2 shows the water content in equilibrium at 90% r.h. ($h_{\infty,90\%r.h.}$) obtained from the fits of experimental data through Eq. 6. It was found than $h_{\infty,90\%r.h.}$ was 0.31 ± 0.01, 0.33 ± 0.01, and 0.48 ± 0.02 (g H_2O per g d.m.) for the films T, TH, and HTH respectively. These values show that the successive treatments of high pressure homogenisation of the dispersion produce an increment in the hydration of films. This is probably due to different processes. Firstly, high pressure breaks the cell wall, allowing liberation of intracellular biomolecules that expose hydrophilic groups to water. Secondly, the rupture that high pressure homogenisation produces to hydrophobic aggregates formed during heating yeast dispersions at 90 °C.

Data shows that water sorption is biexponential time-dependent for all film samples studied and demonstrate biphasic behaviour of the water uptake process. Table 2 shows, for each sample, both water sorption rates $1/\tau_1$ and $1/\tau_2$, representing a fast process and a slow process respectively. Since cytoplasmic material liberated outside the cell is more accessible to water hydration in comparison to the remaining cellular complex, it is reasonable to propose that the biphasic behaviour of the water sorption kinetics originated from intracellular material released (fast process) and from cellular complex (slow process). Furthermore we can see in Table 3 that the water sorption rates $1/\tau_1$ and $1/\tau_2$ increase with steps of homogenisation applied to the dispersion. This effect may be due to the rupture of hydrophobic aggregates, and the crack of cell wall that make more accessible water molecules to internal complex. Consequently, the film HTH hydrates more quickly and in greater quantity.

This interpretation agrees with the morphological characterisation performed by SEM showed and discussed in Supplementary material.

3.3.2.2. Water sorption isotherms. Films samples were also equilibrated at different relative humidities. Fig. 4 shows the results obtained in these experiments expressed through the water sorption isotherms. Dehydration isotherms starts from samples equilibrated at 90% r.h., and hydration isotherms starts from fully dried samples. For all films samples, a slight increase in the water content h for small values of a_w, and a significant increase for $a_w > 0.6$ was observed (Fig. 4B). This suggests a hydration mainly in forms of multilayer, with a small monolayer of hydration. In this way, the water hydration is mobile water that is not strongly bounded to the film.

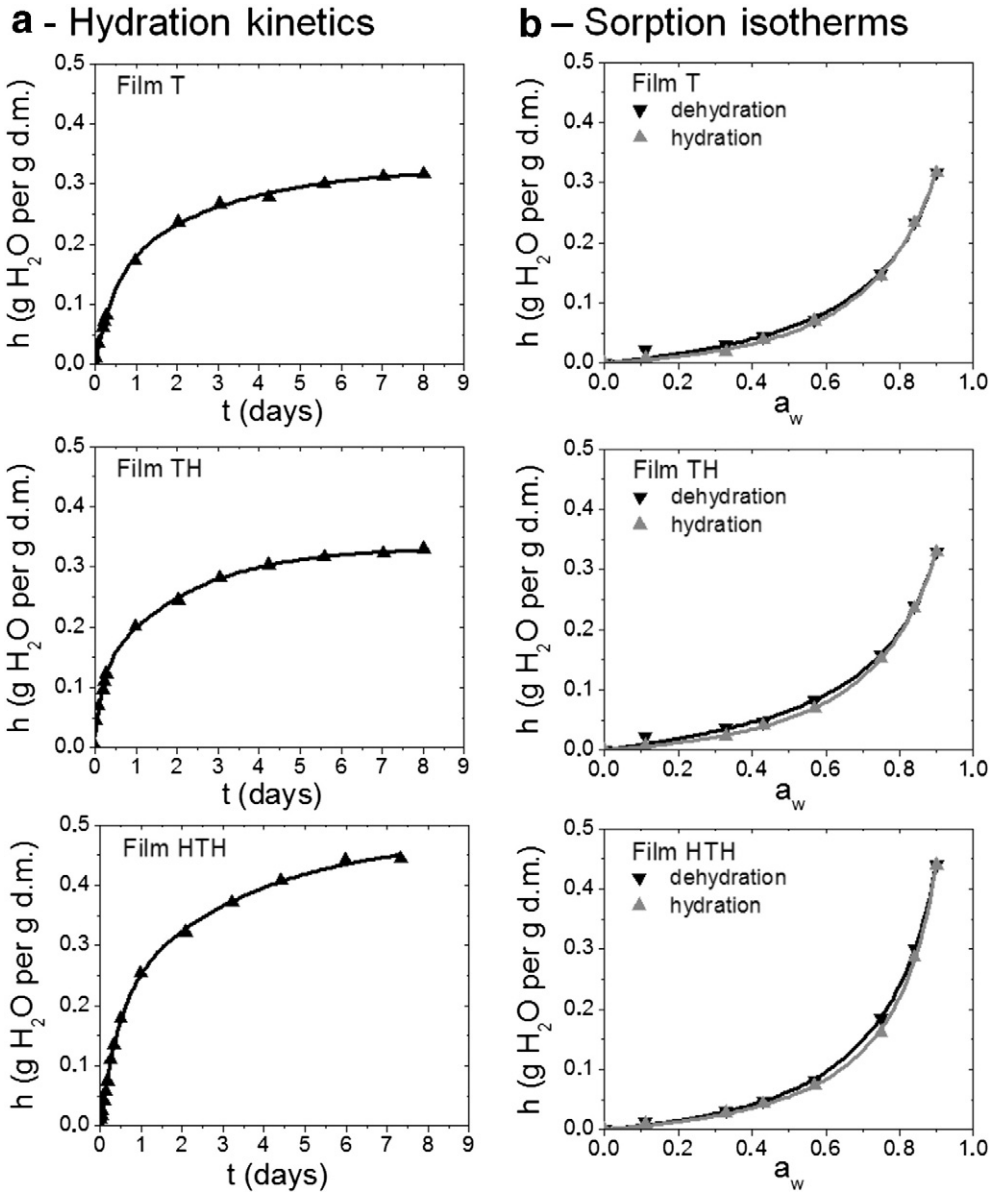

Fig. 4. Hydration curves for T, TH, and HTH yeast film. *A.* Kinetics of water uptake at 90% r.h. *B* Water sorption isotherms: dehydration isotherms (▼) and hydration isotherms (▲). **T:** only heating; **TH:** heating followed by homogenisation; **HTH:** homogenised followed by heating and further homogenisation.

Table 3
Fit parameters of water sorption kinetics at 90% r.h. and values of the parameters fitted for the sorption isotherms displayed in Fig. 4A and B, respectively. R^2 and χ^2 are statistical parameters.

Sorption kinetics (Fitted by biexponential function)					
Film	$h_{\infty,90\%r.r.}$ (g H_2O per g d.m)	$1/\tau_1$ (day^{-1})	$1/\tau_2$ (day^{-1})	R^2	χ^2 (10^{-5})
T	0.31 ± 0.01	2.2 ± 0.3	0.36 ± 0.04	0.9987	2.5
TH	0.33 ± 0.01	5.5 ± 0.9	0.45 ± 0.07	0.9946	4.2
HTH	0.48 ± 0.02	7.7 ± 0.9	0.53 ± 0.07	0.9991	2.4

Sorption isotherms (Fitted by GAB model)						
Film	Condition	N (g H_2O per g d.m)	c	k	R^2	χ^2 (10^{-5})
T	Dehydration	0.07 ± 0.01	1.0 ± 0.1	0.90 ± 0.01	0.9968	4.25
	Hydration	0.06 ± 0.01	1.0 ± 0.1	0.93 ± 0.01	0.9972	3.79
TH	Dehydration	0.07 ± 0.01	1.3 ± 0.1	0.92 ± 0.01	0.9980	2.89
	Hydration	0.06 ± 0.01	1.2 ± 0.1	0.95 ± 0.01	0.9979	3.06
HTH	Dehydration	0.09 ± 0.01	1.5 ± 0.1	0.97 ± 0.01	0.9993	1.21
	Hydration	0.08 ± 0.01	1.4 ± 0.1	0.98 ± 0.01	0.9996	1.13

Dehydration and hydration isotherms were analyzed using GAB formula described by Eq. 5 and fitted parameters are shown in Table 3. Parameter c is related to the force of the water binding to monolayer and k represents the capability to water binding to multilayer, both were increased with the number of high pressure homogenisation applied to the dispersion. These results also suggest that the film HTH is slightly more hydrophilic compared with the film samples TH and T.

The complete cycle of desorption-sorption shows a weak hysteresis effects. This reflects that the complete drying does not produce severe changes in samples. This may be due to the fact that films are constituted from biomacromolecules denatured by heat treatment at 90 °C. It has been reported that for proteins previously denatured, the complete drying does not produce remarkable effects of irreversibility and hysteresis on water sorption isotherms (Salvay et al., 2003).

The sorption isotherms obtained for the yeast films are comparable with the sorption isotherms reported in the literature for starch based films (Slavutsky & Bertuzzi, 2015 and Bertuzzi et al., 2007), hydroxypropylmethylcellulose based films (Pastor Navarro, 2010), and myofibrillar protein based films (Cuq et al., 1997).

It has been demonstrated that hydration water acts as plasticizer of film by embedding itself between the polymers chains, spacing them, lowering the glass transition temperature, and improving flexibility (Levine & Slade, 1988). In this way, hydration water affects the main structural and functional properties of the film (Cuq et al., 1997). Therefore, the greater hydration capacity showed in films obtained by HTH dispersion is could be also related to the continuity, homogeneity, and absence of cracks observed in these films.

4. Conclusions

Cells of *Saccharomyces cerevisiae* demonstrated to be capable to form films when specific treatments, in a certain order, were applied to yeast dispersion. Cytoplasmic components must be released from cells at high homogenisation pressures in the range of 100–150 MPa. This release has positive effects on the film-forming activity of yeast dispersions because proteins are available to interact between them and have noticeable consequences in water sorption and thermal resistance. Homogenisation (H) should be accompanied by a thermal treatment (T) in order to inactivate enzymes, denature proteins and unfold polysaccharides. Films obtained by HTH dispersions exhibited best attributes, a great continuity and homogeneity without small cracks in comparison with other films obtained from dispersions TH or HT. Hydration of films was increased with the number of homogenisation processes applied to yeast dispersions and could be related to the better attributes required for a film. According to the obtained results, yeast biomass must be viewed as an alternative and valuable option to traditional sources of biodegradable polymers to generate new ecological-friendly packaging films. Residual brewers' yeast biomass is an industrial valuable source of material that can be exploited after a cleaning process if cells are not extremely lysated. However, additional experiments and studies should be performed in order to obtain the films with the desired properties for the development of food contact materials. These studies are ongoing in our laboratory.

Acknowledgements

The authors of this article gratefully acknowledge the financial support from National University of Quilmes (UNQ, Argentina) through R&D program PUNQ 1008/11 and National Scientific and Technical Research Council (CONICET, Argentina) (Res.4845/2014). In addition, authors would like to express their gratitude to Gisela Maxia and Soledad Pereda from the Scanning Electron Microscopy Laboratory (National Institute of Industrial Technology, Argentina) and Edelflex Argentina S.A. for their technical support with the valve homogeniser.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ifset.2016.06.002.

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