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Use of galactomannan edible coating application and storage temperature for prolonging shelf-life of "Regional" cheese

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ABSTRACT

The objectives of this work were to determine the influence of the application of two different coatings (galactomannan and chitosan) and of storage temperature on the gas exchange rate of "Regional" cheese; subsequently, the coating that showed the greatest influence on the cheese gas exchange and simultaneously decreased the O_2 consumption (R_{O_2}) and the CO_2 production (R_{CO_2}) rates was applied on cheese, being the shelf-life parameters monitorized through the performance of chemical and microbiological analyses. Both coatings caused a reduction of R_{O_2} and R_{CO_2} of the cheese (between 0.19- and 1.30-fold for R_{O_2} and between 0.19- and 1.50-fold for R_{CO_2}, depending on the temperatures). The cheese coated with the galactomannan coating was the one with the lower values of R_{O_2} (between 0.195 and 0.635 mL kg^{-1} h^{-1}) and R_{CO_2} (between 0.125 and 0.900 mL kg^{-1} h^{-1}). Temperature was also found to have an important effect on R_{O_2} and R_{CO_2}, its influence being well described by an Arrhenius equation with coefficients of determination, R^2, of 0.85 and above. The chemical and microbiological analyses showed that the application of the coating in cheese samples can be used to decrease the water loss and the colour changes during the storage time. The presence of the coating decreased the moisture loss of the cheese in 2.5% and 1.9%, and the weight loss in 3.8% and 3.1% at 4 °C and 20 °C, respectively. Also, the hardness of the cheese can be decreased as a result of the interaction of the presence of the coating with changes in the storing temperature. In the studied range (4–20 °C) temperature has a statistically significant effect in moisture loss, colour change, hardness and total mesophilic bacterial growth.

Overall, galactomannan coating can be used to improve "Regional" cheese shelf-life as it decreases R_{O_2} and R_{CO_2}, improves its weight and appearance and can be used to incorporate natural preservatives to reduce post contamination.

1. Introduction

Food and packaging industries have been joining efforts to reduce the amount of non-biodegradable food packaging materials, as environmental issues are a major concern of the consumer; it is therefore foreseeable that the future generation of packaging materials will be derived from renewable and biodegradable resources. However, natural polymeric materials vary in their rate of degradation, and some proteins, for example, cannot presently be classified as degradable because of standard definitions (Lin and Zhao, 2007). The use of coatings creates a modified atmosphere surrounding the commodity similar to that achieved by controlled or modified atmosphere storage conditions. The application of edible coatings has been widely studied for horticultural products, as fruits and vegetables, while hardly explored for dairy products. The modified atmosphere created by edible coatings can protect the food from the moment it is applied, till its final retail destination and in the home of the consumer (Diab et al., 2001; Durango et al., 2006; Ribeiro et al., 2007). Many works have focused on the use of polysaccharides-based coatings to extend and improve the shelf-life of fruits and vegetables (Diab et al., 2001; Durango et al., 2006; Ribeiro et al., 2007), being few the works that use polysaccharide-based coatings to extend the shelf-life of cheese. Kampf and Nussinovitch (2000) have used hydrocolloid films based on k-carrageenan, alginate and gellan to coat a semi-hard cheese, having reported a reduction of weight loss of the coated cheeses. Also Duan et al. (2007) showed that chitosan–lysozyme films and coatings can be applied in Mozzarella cheese packaging to control the post processing microbial contamination, improving the microbial safety of cheese products.

Cheese is the generic name for a group of fermented milk-based food products, being at the same time the most diverse group of dairy products. Cheese is, in contrast with other dairy products, biologically and biochemically dynamic and, consequently, inherently

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unstable (Fox and McSweeney, 2004). During the maturation and storage process different reactions take place, influencing texture, flavour, and all the other chemical and physical properties of cheese (Pantaleão et al., 2007). The cheese protection by coating with synthetic films for moisture regulation and protection against contamination is a well-known procedure being cellophane, cellophane–polyethylene, saran, parakote, pliofilm, cryovac and aluminium foil some of the used coatings; aqueous dispersions of butyl rubber and a copolymer of vinyl and vinylidene chloride are also used (Kampf and Nussinovitch, 2000). "Regional" cheese, used in the present work, is a semi-hard cheese sold with an acetate polyvinyl coating with the addition of a commercial antifungal agent.

In semi-hard cheeses, the factor that most affects cheese stability is the water activity (a_w), which depends mainly on moisture and salt contents. During cheese ripening, a_w decreases until the surface is in equilibrium with the surrounding atmosphere, thus influencing the microbiological and chemical evolution of the cheese (Saurel et al., 2004). Additional environmental factors must be considered when selecting a material for cheese coating (e.g. light, relative humidity, temperature, O_2 and CO_2 concentration). All these factors affect not only cheese's physical characteristics but also its flavour during storage. In fact, many different compounds contribute to cheese flavour and most of them are formed during cheese ripening (Robertson, 2006). During cheese storage biological reactions keep on occurring due to microorganisms and enzyme activity, being the cheese quality influenced by the oxygen and temperature of storage, and gas exchanges with the environment. Cheese releases CO_2 and simultaneously consumes O_2 during its life cycle being required the control of the gas exchange to maintain the cheese quality and increase its shelf-life.

The present work evaluates, at different temperatures, the use of previously optimized chitosan and galactomannan coatings (Cerqueira et al., 2009a) to decrease the O_2 consumption and the CO_2 production rates of the cheese and to improve the shelf-life of "Regional" cheese.

2. Materials and methods

2.1. Raw material

Edible coating solutions were prepared with: chitosan with a degree of deacetylation of 90% approximately (Aqua Premier Co., Thailand); galactomannan from *Gleditsia triacanthos* seeds (collected in the Botanic Garden, Porto, Portugal, in 2006), extracted as described in Cerqueira et al. (2009b); corn oil (Sovena, Portugal); glycerol 87% (Panreac, Spain) and sorbitol 97% (Acros Organics, Belgium); Tween 80 (Acros Organics, Belgium); lactic acid (Merck, Germany) and distilled water. All these ingredients are food grade.

A commercial semi-hard cheese was obtained from Queijo Saloio S.A. (Portugal), being the samples stored at 4 °C until further use (approx. 5 days). "Regional" cheese is a full fat cheese produced with a mixture of caprine, bovine and ovine pasteurized milk; after being coated with synthetic coating (polyvinyl acetate) and antibiotic (natamycin), it is submitted to a short (aprox. 15 days) ripening period at low temperatures (5 °C and 12 °C in different stages of the ripening process). It requires storage conditions of 0–22 °C during retail. The cheese physicochemical composition is: moisture 46% (w/w), fat 25% (w/w), protein 18.4% (w/w), total ash 3.58% (w/w), chlorides 1.54% (w/w), pH 4.8 and total acidity 1.40 ($g_{lactic\ acid}$/100 g_{cheese}) (Pantaleão et al., 2007).

2.2. Coating preparation

Previous work (Cerqueira et al., 2009a) showed that polysaccharide edible coatings can be optimized having in consideration the surface, permeability and colour properties. This methodology has been applied in order to optimize coating solutions for "Regional" cheese; the optimum composition has been determined both for a solution of chitosan (0.5% chitosan and 2.0% glycerol/sorbitol) and for a solution of galactomannan (1.5% galactomannan, 2.0% of glycerol and 0.5% of corn oil (w/v)). The coating solutions were stored at 4 °C until further use.

2.3. Coating application

The cheeses were coated with the solutions (of chitosan and galactomannan) by gently brushing their surface until all of it was covered, being the residual coating allowed to drip off. Then the cheeses were left during 4 h at 4 °C (92% RH) until the coating was dry. For gas exchange measurements the whole cheese, with approximately 200 g, was used while for the monitorization of shelf-life parameters the cheeses were sliced in 30 g pieces, being the coating applied as described before. Different sample sizes were used due to the experimental setup used for gas exchange measurements, where a cheese with approximately 200 g was needed to obtain the desired free container volume/total volume ratio.

2.4. O_2 and CO_2 exchange rates measurements

The closed system method was used for measurement of the gas exchange rate of the cheese. Several air-tight cylindrical containers of 0.14 m height were fabricated using 4 mm thick acrylic tube of 0.14 m diameter. The top and bottom of each container were covered with lids of the same material having the same thickness, being the top lid fitted with a septum for gas sampling. The acrylic container was considered impermeable to the gases (based on the supplyer's information). A whole intact cheese sample was placed in each container after equilibrating to the desired temperature. The change in gas composition (O_2 and CO_2 concentrations) inside each container was monitored with a gas analyser (Dansensor, Checkmate 9900, Denmark) during 7 days.

The O_2 consumption and CO_2 production rates were determined applying Eqs. (1) and (2) (Salvador et al., 2002), developed for a closed system impermeable to gases.

$$R_{O_2} = -\left(\frac{dy_{O_2}}{dt}\right) \cdot \left(\frac{V_f}{w}\right) \quad (1)$$

$$R_{CO_2} = -\left(\frac{dy_{CO_2}}{dt}\right) \cdot \left(\frac{V_f}{w}\right) \quad (2)$$

where, R_{O_2} is the O_2 consumption rate, mL[O_2] kg^{-1} h^{-1}, R_{CO_2} is the CO_2 production rate, mL[CO_2] kg^{-1} h^{-1}, w (kg) is the weight of the cheese, V_f (mL) is the free volume of the container, calculated by:

$$V_f = V_p - \frac{w}{\rho_{ch}} \quad (3)$$

where, V_p (mL) is the total volume of the container, w (kg) is the weight of the cheese and ρ_{ch} is the true density of the cheese, in this case 1.095×10^{-3} kg mL^{-1}, obtained experimentally following the method described by (Owolarafe et al., 2007). The graph of O_2 consumed vs. time or CO_2 produced vs. time was used to calculate the slopes corresponding to the derivatives, dy_{O_2}/dt (or dy_{CO_2}/dt), that are directly proportional to the values of R_{O_2} and R_{CO_2} (see Eqs. (1) and (2)).

2.5. Temperature effects on the kinetics of O_2 consumption and CO_2 production

The Arrhenius equation (Eq. (4)) was applied to study the effect of temperature on chemical reaction rates.

$$x = x_{ref} \cdot e^{\left(\frac{-E_{ax}}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)} \tag{4}$$

where x stands for a generic parameter, x_{ref} is the value of the generic parameter x at the arbitrarily chosen reference temperature, T_{ref} is the reference temperature (285 K), T is temperature, in K, E_{ax} is the activation energy for the parameter x, in kJ mol^{-1}, and R is the universal gas constant, 8.314×10^{-3} kJ mol^{-1} K^{-1}.

2.6. Cheese analysis

The weight loss was evaluated with a precision balance. The cheese was individually weighed at the beginning of the experiment and during the storage period, being the weight loss calculated by:

$$\Delta w = \frac{I_w - f_{iw}}{I_w} \cdot 100 \tag{5}$$

where I_w is the initial weight and f_{iw} is the weight at the time i.

The moisture content was determined by measuring weight loss at 105 °C for 24 h (IDF, 1982).

Colour determination was done using a Minolta colorimeter (CR 300; Minolta, Japan), where the changes in the surface colour of cheese samples were measured by the Hunter total colour difference (ΔE) expressed as L (whiteness or brightness/darkness), a (redness/greenness) and b (yellowness/blueness). The Hunter total colour difference (ΔE) was calculated by:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \tag{6}$$

where L_0, a_0 and b_0 are the initial values, obtained before packaging, and L, a, b are the values measured during the experiment.

The texture profile analysis (*TPA*) of cheese samples was performed with a TA.XT PlusTexture analyser (Stable Micro Systems, UK) with a load cell of 5 kg and a 5 mm cylindrical plunger at a constant penetration speed of 2 mm min^{-1} (TPA) (three penetrations per cheese).

The pH value of each sample was recorded using a digital pH meter (3310, Jenway) equipped with a glass electrode that was inserted directly into the cheese sample for the measurement.

Microbiological analyses were performed by the determination of the total mesophilic count and total mould/yeast growth; in order to do this, cheese samples (20 g) were transferred aseptically to a stomacher bag, containing 180 mL of ringer solution (Merck, Germany) and the mixture was homogenized in a stomacher for 60 s. At each sampling day, two samples were analysed per treatment. Appropriate dilutions of the sample homogenates were prepared in sterile ringer solution and inoculated in duplicate in selective media: Plate Count Agar (PCA) (Merck, Germany) was used for the total mesophilic count and Potato Dextrose Agar (PDA) (Le Pont de Claix, France) was used to evaluate total mould/yeast count, being the spread plate technique used in both cases. PCA plates were incubated at 35 °C for 3 days and PDA plates were incubated at 25 °C for 5 days.

2.7. Design of experiments

The studied variables affecting gas exchange rate and chemical and microbiological analyses of cheese were temperature and the coating itself. Table 1 shows the settings (levels) used for each variable. For the gas exchange rate analyses a 3^2 design was applied,

Table 1
Factors and levels used to measure R_{O_2}, R_{CO_2} and shelf-life in "Regional" cheese.

Experimental setup	Factors	Levels		
R_{O_2} and R_{CO_2}	Coating	NO	CH	GT
	Temperature (°C)	4	12	20
Shelf-life	Coating	NO	–	GT
	Temperature (°C)	4	–	20

where the temperature (4, 12 and 20 °C) and the coating (without coating (NO), with chitosan (CH) and with galactomannan (GT) coating) were the independent variables. Three different temperatures within the storage temperature range provided by the manufacturer were used with the objective of evaluating the influence of the temperature in the cheese gas exchange rate analyses and in the shelf-life parameters.

For the cheese chemical and microbiological analyses a 2^2 design was applied, being the temperature (4 and 20 °C) and the coating (NO and GT coating) the independent variables. The relative humidity (HR) to the three used temperatures was 92% HR to 4 °C, 78% HR to 12 °C and 65% to 20 °C. Experimentally determined quality parameters, during storage under different conditions, were analysed using Statistica 7.0 (Statsoft, Inc.). Pareto analysis was used to determine the environmental factors that were most significant in the changes of quality parameters and sensory characteristics of cheese during storage. The Tukey test ($\alpha = 0.05$) was used to determine any significance of differences between specific means (SigmaStat, trial version, 2003, USA).

3. Results and discussion

3.1. O_2 and CO_2 exchange rates

To understand how coating and temperature influence the gases exchange rates, the cheese was coated using chitosan and galactomannan, and the values of R_{O_2} and R_{CO_2} were measured at 4, 12 and 20 °C. R_{O_2} ranged between 0.335 and 0.195 mL kg^{-1} h^{-1}; 0.540 and 0.375 mL kg^{-1} h^{-1}; and 1.45 and 0.635 mL kg^{-1} h^{-1} at 4, 12 and 20 °C, respectively, and R_{CO_2} varied between 0.265 and 0.125 mL kg^{-1} h^{-1}; 0.425 and 0.200 mL kg^{-1} h^{-1} and 2.250 and 0.900 mL kg^{-1} h^{-1} at 4, 12 and 20 °C, respectively.

Fig. 1 shows an increase of R_{O_2} with the increase of the temperature. The highest values were obtained for cheese samples with-

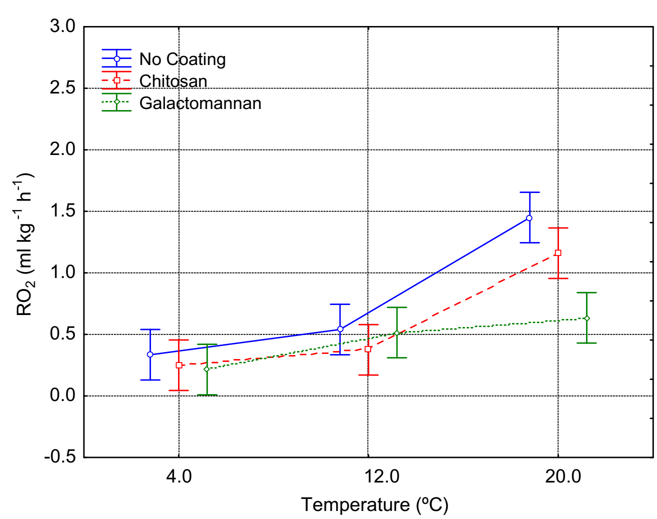

Fig. 1. Interaction charts showing the effect of the temperature and coating in R_{O_2}.

out coating stored at 20 °C. The R_{O_2} values of uncoated cheese and of cheese with chitosan coating, have a great increase (ca. 1.7-fold and 2.1-fold, respectively) when temperature is increased from 12 to 20 °C. However, from 12 to 20 °C, a smaller increase (ca. 0.2-fold) is observed in the R_{O_2} of the cheese coated with galactomannan coating, with a value ca. 80% lower that the values of the other samples. Pareto analysis at 95% significant level was used to quantify the effects of temperature and type of coating on R_{O_2} (Fig. 2), showing that temperature, type of coating, interaction of both and the quadratic effect of the temperature affect significantly the values of R_{O_2}.

Fig. 3 shows that the increase of R_{CO_2} with the increase of the temperature is attenuated due to the presence of the coating. As for R_{O_2}, it was also the galactomannan coating the one presenting the lower increase in CO_2 production when the temperature was increased from 12 °C to 20 °C. Pareto analysis (Fig. 4) showed that temperature, quadratic effect of temperature, type of coating and interaction of temperature and type of coating affect significantly the values of R_{CO_2}. As in the case of R_{O_2}, also in R_{CO_2} the quadratic effect of the type of coating was negligible.

O_2 consumption and CO_2 production are clearly temperature-dependent phenomena, and, with lower significance, type of

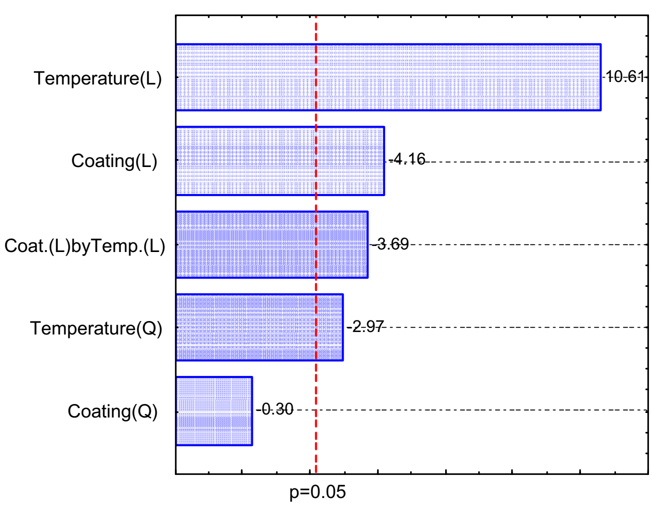

Fig. 2. Pareto charts showing the effect of the temperature and coating in R_{O_2}.

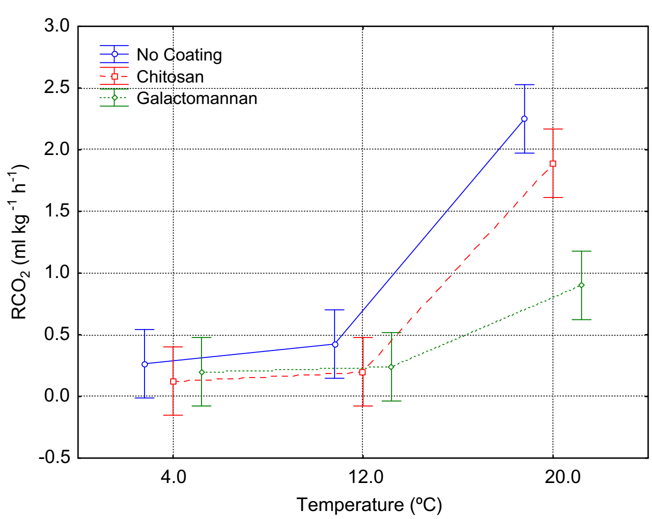

Fig. 3. Interaction chart showing the effect of the temperature and coating in R_{CO_2}.

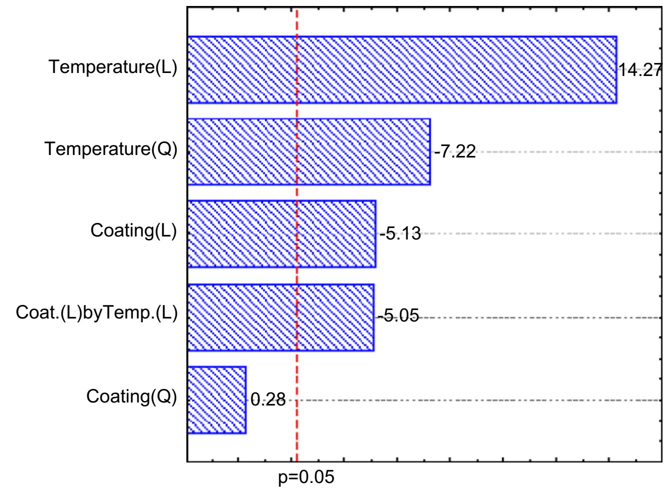

Fig. 4. Pareto charts showing the effect of the temperature and coating in R_{CO_2}.

coating-dependent phenomena. The very significant effect of temperature on cheese gas exchange was already observed by Fedio et al. (1994) and Vivier et al. (1996), and has been attributed mainly to the growth of the microflora in cheese, that leads to increased values of O_2 consumption and CO_2 production (Robertson, 2006). The decrease of gas exchanges in the presence of coating are essentially due to the barrier properties of the polysaccharide coatings. The higher influence of the galactomannan coating in the values of R_{O_2} can be explained by its lower oxygen permeability (O_2P) (0.94×10^{-15} g Pa^{-1} s^{-1} m^{-1}), when compared with the value of O_2P for the chitosan coating (2.26×10^{-15} g Pa^{-1} s^{-1} m^{-1}) (Cerqueira et al., 2009a). The values of R_{O_2} obtained with the present work were found to be very similar to those reported by other researchers, ranging between 1 and 2 mL kg^{-1} h^{-1} for Swiss cheese (Fedio et al., 1994) and Feta cheese (Vivier et al., 1996). Previous work, using a "Regional" cheese without ripening, showed values of R_{O_2} ranging between 13.65 and 8.33 mL kg^{-1} h^{-1} and of R_{CO_2} ranging between 14.52 and 9.27 mL kg^{-1} h^{-1} for uncoated and coated cheese, respectively (Cerqueira et al., 2009a). In this work only one temperature (21.86 °C) was tested and unripened cheese was used, while in the present work three different temperatures were studied in a cheese already submitted to a ripening period, which allowed to characterize the temperature dependence of the gas consumption/production rates. In that previous work shelf-life parameters were not monitored. The differences observed in the values of R_{O_2} and R_{CO_2} reported in that work and in the present work are due to differences in respiration caused by changes in the microbial load. These changes are presumably attributed to the fact that in the former work the cheese was analyzed without ripening, while in this study the cheese used was previously submitted to a ripening period.

Different models describing the influence of temperature in the gas exchange rate have been reported in the literature, being the most common the Arrhenius equation which has been widely used to evaluate the effect of temperature on the gas exchange rate of different products (Ratti et al., 1996; Bhande et al., 2008; Andrich et al., 1998; Fedio et al., 1994). The Arrhenius model has also been used to describe the dependence of temperature of other very complex reactions including bacterial growth and metabolism, and microbial death (Labuza et al., 1992).

In this work the temperature influence in R_{O_2} and R_{CO_2} was well described by an Arrhenius equation ($R^2 > 0.85$), and the activation energy for gas production and consumption in "Regional" cheese was calculated from the slope of the linear plot log x vs. $1/T$ (Eq. (4)). Fig. 5 shows the Arrhenius plots for some of the studied cases.

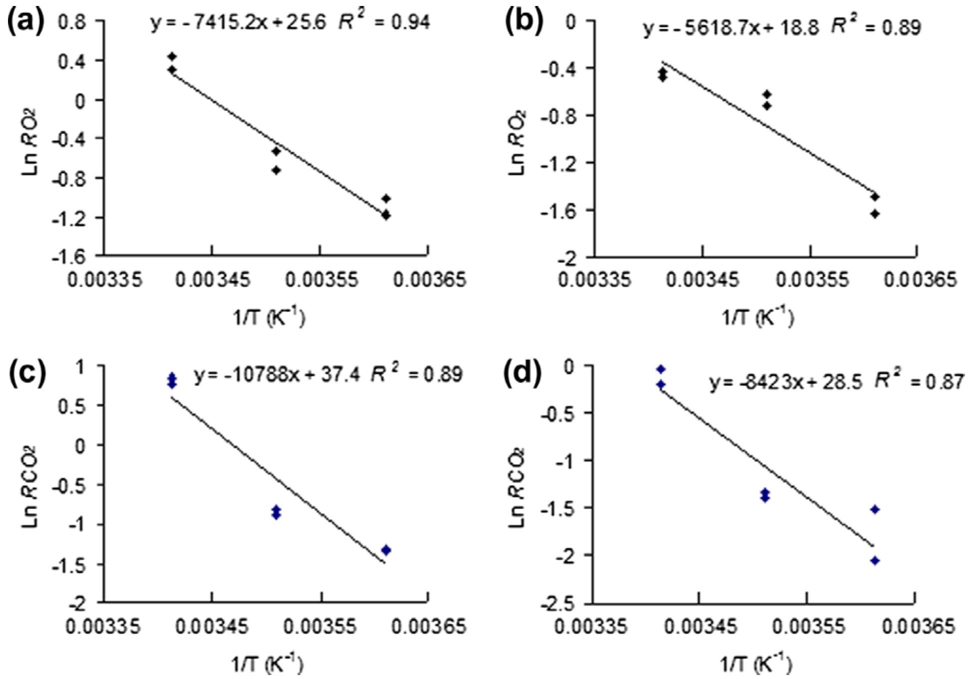

Fig. 5. Arrhenius plots for R_{O_2} without coating (a) and with GT coating (b), and R_{CO_2} without coating (c) and with GT coatings (d).

The parameters of the obtained linear plot are shown in Table 2. Cheese coated with the galactomannan solution has the lower values of activation energy, while cheese coated with the chitosan solution presents the higher values of activation energy. Despite the obtained lower values of R_{O_2} and R_{CO_2} for the cheese coated with chitosan coating at all the measured temperatures when compared with uncoated cheese, higher values of E_a are observed as consequence of a more significant effect of temperature on R_{O_2} and R_{CO_2}. This increase can be explained by the lactic acid, used in chitosan coating formulation, that decreases the surface pH of the cheese thus favoring the increase of the yeast and moulds growth in cheese (McSweeney, 2007). All cheese samples tested have E_a values lower than the values reported by Fedio et al. (1994) that report values above 116.94 kJ mol^{-1}.

3.2. Chemical and microbiological analyses

In order to better understand the gas exchange rates phenomenon that happens in "Regional" cheese, chemical and microbiological analyses were performed comparing the uncoated cheese and the cheese coated with galactomannan coating. This coating was chosen in detriment of the chitosan coating due its better performance in terms of the gas exchange rates.

Weight loss analyses provided information on how the moisture loss during the storage period was influenced by the temperature and the presence of coating and if those factors can change the moisture loss profile of the cheese. Fig. 6a shows the weight loss in the coated and uncoated cheeses for two temperatures (4 °C and 20 °C). In all cases the weight loss increased during the storage time, being the increases higher for the cheeses without coating ($p < 0.05$). Pareto charts (Fig. 7a) show that the presence of the coating is the only significant factor in the weight loss. Temperature does not seem to affect the moisture loss of the cheese during the 21 days of the experiment. These results showed that only the coating can retard water loss. These results are in agreement with those of Kampf and Nussinovitch (2000) where alginate, gellan and k-carrageenan, used as coating materials on semi-hard cheeses, were shown to present a lower weight loss (approximately 2.0%) when compared with uncoated cheese samples.

The values for moisture loss after 21 days ranged between 23.4% and 19.6% for uncoated and coated cheeses, respectively. These values are in agreement with weight loss results, and Fig. 6 shows that the moisture content decreases in all cases, being higher for the samples without coating stored at 20 °C. At the end of 21 days of storage the cheese samples have moisture content values between 15.3% and 13.0% for coated cheeses at 4 °C and 20 °C, respectively, and 12.8% and 11.1% for uncoated cheeses samples at 4 °C and 20 °C, respectively.

Pareto charts (Fig. 7b) show that temperature and the presence of coating are factors affecting significantly the moisture content. Pantaleão et al. (2007) showed that the decrease of the water content of "Regional" cheese using *Humidipak* and Microperforated packaging systems can be less pronounced, with higher values of moisture content after 21 days of storage. These differences can be explained by the cheese surface area of the sample used in our work, lower than the used in Pantaleão et al. (2007). The ration between the surface area and the weight of the samples used in the experimental setup influences the cheese performance, being the cheeses with higher surface area less influenced by the water loss (Pantaleão et al., 2007). Both weight loss and moisture loss are related to the water loss of the cheese that depends on the kinetics of water permeation through the coating used (Robertson, 2006). The galactomannan coating used in this work, with a water vapour permeability value of 3.24×10^{-11} g m^{-1} s^{-1} Pa^{-1} (Cerqueira et al., 2009a), decreased the water loss of the cheese.

Table 2
Arrhenius equation parameters for R_{O_2} and R_{CO_2}.

Cheese	O_2 consumption			CO_2 production		
	Slope	R^2	E_a (kJ mol^{-1})	Slope	R^2	E_a (kJ mol^{-1})
NO	−7415.2	0.94	−61.6499	−10788	0.90	−89.6914
CH	−7863.1	0.92	−65.3738	−13898	0.85	−115.548
GT	−5618.7	0.90	−46.7139	−8423	0.87	−70.0288

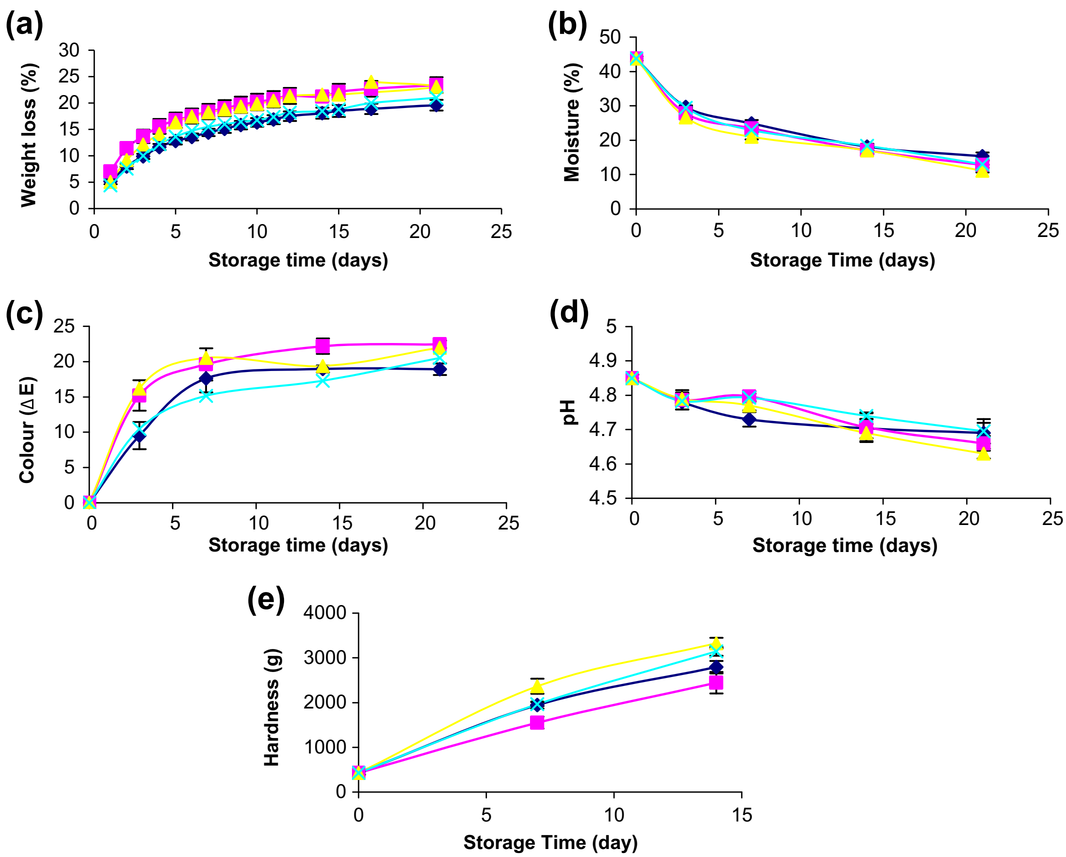

Fig. 6. Average and standard deviation throughout storage time for cheese without coating ■ and with coating ◆ at 4 °C, and without coating ▲ and with coating × at 20 °C in terms of: weight loss (a), moisture (b), colour difference (*ΔE*) (c), pH (d) and hardness (e).

The presence of the coating decreased the moisture loss of the cheese in 2.5% and the weight loss in 3.8% at 4 °C, while at 20 °C the moisture loss and the weight loss decreased 1.9% and 3.1%, respectively.

Colour analysis, based in the colour difference (*ΔE*), showed that the uncoated cheese at 20 °C has higher values of *ΔE*. Fig. 6c shows that the differences between uncoated and coated cheese during the storage time become more and more significant ($p < 0.05$) during the first 3 days. The Pareto chart (Fig. 7c) shows that the presence of coating, the temperature and the interaction between the presence of coating and the temperature are significant factors affecting the colour difference. The cheese storage using a coating and the decrease of the temperature can be used to decrease colour changes, with clear advantages in terms of the marketing of the cheese. The influence of temperature in colour change was also reported by Metzger et al. (2000) where the whiteness parameter (*L*-value) of Mozzarela cheese increased with the cheese heating. Colour change can be essentially attributed to cheese oxidation, that is lower in cheeses with coating due to the protection from the oxygen and light oxidation provided by the coating's O_2P and opacity (Cerqueira et al., 2009a).

After 14 days of storage, the pH became regular in cheese samples, without statistically significance between samples ($p > 0.05$) (Fig. 6d). pH presented values ranging between 4.85 and 4.63 and Pareto charts showed that no factor was significantly affecting pH variation after 21 days (results not shown). Fig. 6e presents the hardness values for cheese samples during the storage time, showing that coated cheeses stored at 4 °C have the lower hardness values. The Pareto chart (Fig. 7d) shows that cheese hardness (checked in days 0, 7 and 14), has the temperature as the most important factor; being the interactions between the temperature and the presence of a coating also significant effects (Fig. 7d). The hardness values measured in the present work were higher than those reported in Pantaleão et al. (2007); this is possibly due to the higher water loss measured in our cheese samples and also to the different cylindrical plunger used in this work, which makes the comparison somewhat tricky.

Microbiological analyses of cheese samples (Tables 3 and 4) presented a smaller increase of the CFU/g in coated cheese. In Table 3, the total mesophilic bacteria count showed a great increase for all samples in day 7, being a decrease observed between the days 14 and 21. Similar results were observed for the total moulds/yeast counts. These results are in line with the values of R_{O_2} and R_{CO_2} of the cheese (measured during 7 days), where at 4 °C no statistical difference ($p > 0.05$) is found between R_{O_2} and R_{CO_2} of the uncoated and galactomannan coated cheese, this not being the case at 20 °C temperature at which a statistical difference ($p < 0.05$) was observed (see Figs. 1 and 3).

The presence of coating decreases significantly ($p < 0.05$) the growth of mesophilic aerobic bacteria in days 7 and 14 for cheese samples stored at 20 °C. A similar result is observable when counting the total moulds/yeasts (Table 4) in day 7 and day 21 at 20 °C. The Pareto chart (relative to the values obtained in the day 21, only), showed that total mesophilic bacteria numbers are influenced by the temperature, only. The smaller increase in the microbial counts on cheese coated with galactomannan coating can be explained by the reduced gas permeability, as confirmed by the values R_{O_2} and R_{CO_2}, of this coating that decreases the oxygen transfer rate into the cheese, that becomes less available for the growth of mesophilic bacteria, moulds and yeast. The counts obtained for

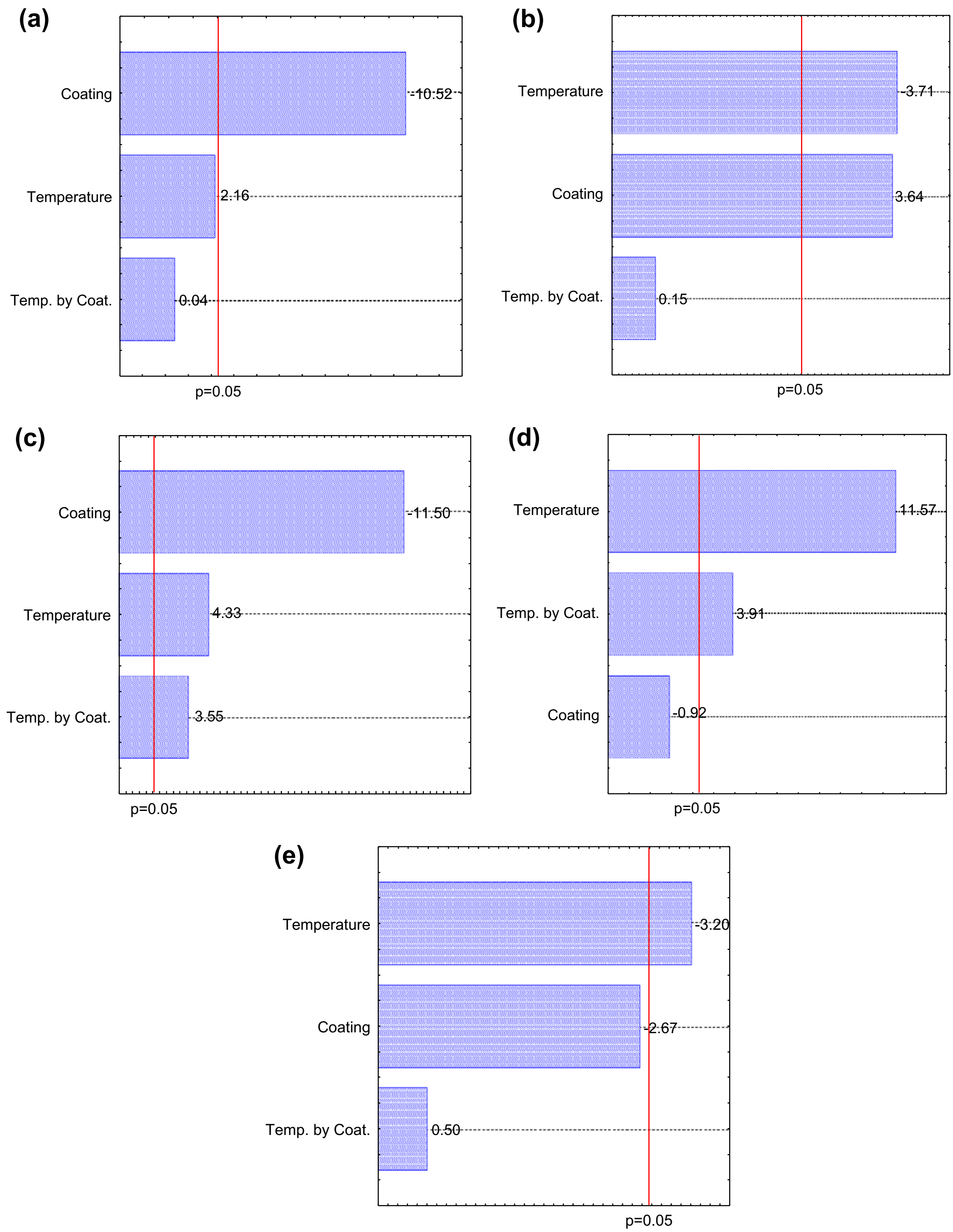

Fig. 7. Pareto charts of the effects obtained from the fractional factorial design in the last (21st) day of analysis for: weight loss (a), moisture (b), colour difference (ΔE) (c), hardness (d) and total mesophilic count (e).

mesophilic and mould/yeast ranged between 5.3–7.0 and 5.6–6.9 log(CFU g^{-1}), respectively, and are in agreement with other works with mozzarella, Turkish white and Cheddar cheeses (Duan et al., 2007; Öner et al., 2006; Suppakul et al., 2008).

Although galactomannan coatings have been proved to improve cheese shelf-life, further studies have to be made evaluating their suitability to incorporate natural preservatives in order to prevent post contamination. Some further improvements may

Table 3
Total microbial count log(CFU g^{-1}) of cheese samples as a function of the day of storage.

Coating	Temperature	Day			
		0	7	14	21
NO	4 °C	5.3 ± 0.2[a]	6.7 ± 0.4[ab]	5.9 ± 0.5[a]	6.5 ± 0.3[a]
	20 °C	5.3 ± 0.2[a]	7.0 ± 0.1[a]	6.7 ± 0.2[b]	5.9 ± 0.1[bc]
GT	4 °C	5.3 ± 0.2[a]	6.6 ± 0.3[ab]	5.2 ± 0.4[a]	6.1 ± 0.1[ab]
	20 °C	5.3 ± 0.2[a]	6.4 ± 0.2[b]	5.7 ± 0.2[a]	5.7 ± 0.2[c]

[a–c] Different superscript letters in the same column indicate a statistically significant difference (Tukey test $p < 0.05$).

Table 4
Total moulds/yeast count log(CFU g^{-1}) of cheese samples as a function of the day of storage.

Coating	Temperature	Day			
		0	7	14	21
NO	4 °C	5.6 ± 0.0[a]	6.6 ± 0.0[a]	5.9 ± 0.2[a]	5.9 ± 0.2[ab]
	20 °C	5.6 ± 0.0[a]	6.9 ± 0.1[b]	6.0 ± 0.3[a]	5.8 ± 0.0[a]
GT	4 °C	5.6 ± 0.0[a]	6.6 ± 0.0[a]	5.6 ± 0.2[a]	6.1 ± 0.1[b]
	20 °C	5.6 ± 0.0[a]	6.0 ± 0.2[c]	–	5.4 ± 0.2[a]

[a–c] Different superscript letters in the same column indicate a statistically significant difference (Tukey test $p < 0.05$).

be made while applying coatings on cheese samples, such as the use of electrostatic coating (Amefia et al., 2006), layer-by-layer methodologies (Weiss et al., 2006) or using simultaneous the combination of active coating with modified atmosphere packaging (Del Nobile et al., 2009).

4. Conclusion

Cheese samples without coating presented the higher values of R_{O_2} and R_{CO_2} for all temperatures analyzed in the study. Temperature had an important effect on R_{O_2} and R_{CO_2}, and the cheese with galactomannan coating presented the lower value of E_a, thus showing the positive influence of the galactomannan coating in the decrease of the respiration rates in cheese. Based in the obtained results for R_{O_2} and R_{CO_2}, the galactomannan coating providing the lowest gas exchange rates was applied on cheese samples and chemical and microbiological analyses were performed. The coatings significantly improved the performance of the system in terms of the reduction of water loss and decrease of both the colour change and microbial counts. The temperature has been evaluated showing significant influence in moisture loss, colour, hardness and total mesophilic bacterial count. Galactomannan coatings can be used to improve cheese shelf-life, however some further improvements may be made on the coating application methods in order to have a more uniform distribution of the coating on the cheese surface.

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