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Characterization and effect of edible coatings on minimally processed garlic quality

Robson Maia Geraldine [a], Nilda de Fátima Ferreira Soares [b,*], Diego Alvarenga Botrel [b], Letícia de Almeida Gonçalves [a]

[a] *Escola de Agronomia e Engenharia de Alimentos, Universidade Federal de Goiás, GO, Brazil*
[b] *Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Viçosa, MG, Brazil*

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Abstract

The main benefits of edible active coatings are their edible characteristics, biodegradability and increase in food safety. In this study the physical properties of the agar-agar based (1%) coatings incorporated with 0.2% chitosan and 0.2% acetic acid, as well as their effects on coating of minimally processed garlic cloves were evaluated. Moisture loss of coated garlic cloves was, on average, three times lower when compared to the control samples (no coated garlic cloves). There was a marked increase in color difference values (ΔE^*) for control cloves compared to the other treatments. Filamentous fungis and aerobic mesophilic were inhibited on garlic cloves coating incorporated with acetic acid + chitosan antimicrobial compounds. During 6 days-storage, at 25 °C, the filamentous fungis and yeasts count was maintained between in 10^2 and 10^3 CFU g^{-1} for the coated garlic cloves and around 10^6 CFU g^{-1} for the control. The coatings provided significant reduction ($p < .05$) in clove respiration. Coated garlic cloves, had a respiration rate ($\approx$30 mg CO_2 h^{-1} kg^{-1}) halved compared to the non-coated garlic cloves. Water vapor transmission was lower for the films added with chitosan. These films showed no visible color difference, possibly because of the reduced thickness, since chitosan films tend to have a more intense shade.

Keywords: Agar; Edible coating; Chitosan; Garlic; Minimal processing

1. Introduction

Functional edible active coatings may contribute to prolong minimally processed food shelf life, working as barrier to gases, water vapor, solutes and guaranteeing microbiological safety (Park, 1999; Weng, Chen, & Chen, 1999). Spoilage and pathogenic microorganisms usually grow on food product surfaces. The incorporation of antimicrobial agents into packaging flexible films (coatings) is, therefore, an alternative to this problem (Weng et al., 1999). In addition, the potential of edible coatings for aroma retention and as an oxygen barrier makes them of interest for food and packaging technologies (Miller & Krochta, 1997).

Although the use of films and edible coatings in food quality preservation is not a recent concept, researches in this field have recently been intensified. The factors that contribute to the renewed interest include the consumer's demand for high quality food, environmental concerns in relation to the accumulation of non-biodegradable packaging and opportunities to create new markets for the production of films from renewable resources (Gennadios, Hanna, & Kurth, 1997; Rosa, Franco, & Calil, 2001). Edibility, biodegradability and increased food safety are the main benefits of active edible films. Their environmental friendly aspects make them alternatives in packaging systems, without the ecological costs of the synthetic non-biodegradable materials. In the future, they will be able to replace partially or totally conventional synthetic packaging (Krochta & Mulder-Johnston, 1997).

* Corresponding author.
E-mail address: nfsoares@ufv.br (N.F.F. Soares).

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Freile-Pelegrin et al. (2007) investigated the biodegradation behavior and functionality of agar. Accelerated weathering exposure of agar films suggests that outdoor climate parameters play a significant role in the degradation process. Both photodegradation and fluctuations in ambient temperature and humidity lead to deterioration in morphological, structural and mechanical agar film properties during early stages (30–45 days) of exposure, due to the decrease in molecular size and number of sulfate groups. These changes alter agar crystallinity, leading to formation of micro-fractures and polymer embrittlement. These chemical and morphological conditions promote microbial and fungal attacks.

Some plant organs, such as garlic cloves, are wrapped in a natural packaging (peel). This barrier regulates the transport of oxygen, carbon dioxide, moisture and, also, reduces the loss of flavor and aroma (Miller & Krochta, 1997). Minimally processed garlic loses this barrier and, therefore, the maintenance of the appropriate atmosphere for the product must be established by other means, if the shelf life is to be maintained. In this manner, not only O_2 and CO_2 concentrations, but also the moisture loss must be taken into consideration (Soares, Geraldine, Puschmann, & Teles, 2002). The interest in the quality and microbiological safety of minimally processed garlic, besides the reduction of non-biodegradable packaging waste, promotes the use of some properties of agar-agar based edible coatings incorporated with natural additives such as chitosan and acetic acid.

Plasticizing agents can be essential to overcome the brittleness of the biopolymeric films, by reducing the intermolecular forces, thus improving the mechanical properties. Glycerol is shown to improve film flexibility, reduce film puncture strength, elasticity and water vapor barrier properties of wheat gluten films (Gontard, Guilbert, & Cuq, 1993). The presence of a polyol as a plasticizer in chitosan/gelatin blends was found to impart an enhanced mobility to the polymer blend and mechanical strength and higher gas/water permeation rates proportional to the total plasticizer content (Arvanitoyannis, Nakayama, & Aiba, 1998). Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, and Aiba (1997), studied the physical properties of chitosan-poly(vinylalcohol) blends plasticized with sorbitol and sucrose and concluded that the tensile strength decreased proportionally to the plasticizer content whereas the percentage elongation increased considerably, particularly in the case of sorbitol. Addition of fatty acids did not influence significantly the mechanical properties of chitosan films (Srinivasa, Ramesh, & Tharanathan, in press).

Chitin is an abundant naturally occurring biopolymer and is found in the exoskeleton of crustaceans, in fungal cell walls and in other biological materials (Andrady & Xu, 1997). It is mainly poly(β-(1-4)-2-acetamido-D-glucose), which is structurally identical to cellulose except that a secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by an acetamide group. Chitosan is derived from chitin by deacetylation in an alkaline media. Therefore, chitosan is a copolymer consisting of β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino-D-glucose units with the latter usually exceeding 80%. Chitosans are described in terms of degree of deacetylation and average molecular weight and their importance resides in their antimicrobial properties in conjunction with their cationicity and their film-forming properties (Muzzarelli, 1996).

The objectives of the present work were to evaluate some physical properties of edible active agar-agar based coatings, incorporated with chitosan and acetic acid, as well as their effects on the coating of minimally processed garlic, in relation to its physiological and microbiological characteristics. Thickness, gramature, water vapor transmission and color characteristics of films were tested. Respiration rate, mass loss, color alteration and microbial count were evaluated in the final product.

2. Materials and methods

2.1. Edible coatings

All coatings were produced with polymeric agar-agar pure powder (Biobras) at a concentration of 1% (w/v) in distilled water, and melted in the microwave (Intelloware, LG). Glacial acetic acid and chitosan (87% deacetylation, Polymar) were used as additives. Three types of edible coatings were produced: without additives (RC1), incorporated with 0.2% of acetic acid (RC2) and incorporated with 0.2% of chitosan (from 1% chitosan and 1% acetic acid distilled water solution) (RC3).

2.2. Edible coating application on garlic cloves

Approximately 10 kg of Chonan Garlic, from the local market, were thrashed, peeled and sanitized, according to methodology described by Soares et al. (2002), stored in poly(ethylene terephthalate (PET) boxes (around 150 g of garlic per box) and coated with edible film. The edible coating, still liquid, was placed inside the boxes (15% of garlic mass) over the cloves. Boxes were kept at 25 °C for 9 days.

2.3. Final product analysis

2.3.1. Respiration rates

Garlic clove respiration rate was estimated by carbon dioxide gas quantification, with infrared gas analyzer (IRGA, model LCA 2), in an open system consisted of an acrylic tube (200 mL) coupled to an air change pump. The respiration rate was estimated in milligrams (mg) of CO_2 per kilogram (Kg) of fresh material per hour (h). Whole bulbs and cloves recently thrashed, the peel and the cloves coated with RC1, RC2 and RC3 were analyzed.

2.3.2. Color

Superficial color alterations were monitored with a colorimeter (HunterLab, model Miniscan XE). CIELab

parameters (a^* indicates the chromaticity in the green (−) to red (+) axis; b^* indicates the chromaticity in the blue (−) to yellow (+) axis and L^* indicates variation from black (0) to white (100)) were used to calculate the color difference (ΔE^*) (Gennadios, Weller, Hanna, & Froning, 1996):

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$$

where, ΔL^*, Δa^* and Δb^* represents the differences between the color parameters of the sample and the color parameters of the white standard.

The parameters were taken in the first day and every 3 days throughout the storage period. Analyses were carried out on damaged (cut) and non-damaged surfaces.

2.3.3. Mass loss

Mass variation of garlic cloves coated was determined by weighing the cloves, in a semi-analytical balance (Gehaka, model BG400) with 0.001 g readability. The analysis was performed on the processing day and every 3 days thereafter.

2.3.4. Microbiological analysis

The microbiological evaluation of the minimally processed garlic was based on counts of aerobic mesophilic, filamentous fungi and yeasts on the processing day, and every 3 days thereafter. Analyses were carried out with 25 g samples of plant material, aseptically weighed and manually homogenized with 225 ml of 0.1% peptoned water (Merck). Following, appropriate decimal dilutions were done to obtain plate counts between 25 and 250 colonies. Counting of mesophilic aerobics was carried out on standard plate count agar (PCA, Merck), after incubation at 35 ± 1 °C for 48 h. Counting of mould and yeasts was carried out on potato dextrose agar (BDA, Merck) incubated at 25 °C, for 4 days (Silva, Junqueira, & Silveira, 1997). Samples were evaluated after processing and every 3 days throughout storage. All microbiological analyses were done in duplicate. Results corresponded to the average counts and were expressed in Log CFU g^{-1}.

2.4. Coating analysis

2.4.1. Film production

In order to analyze color, thickness, gramature (mass per unit area) and water vapor transmission, the coating was produced as film from the coating solutions mentioned in Section 2.1. Approximately 20 g of coating solution was poured onto glass plate (14 cm of diameter), previously covered with polyethylene terephthalate (PET) film. Plates were left standing until the coating solution solidified. Later, they were taken to an oven (40 °C and 20% RH) for 12 h. Finally, the films were removed from the PET surface and analyzed.

2.4.2. Color

Film color was determined using a colorimeter (HunterLab, model Miniscan XE). The CIELab parameters were used to calculate the Theta (θ) angle (Francis, 1998) and color difference (ΔE^*) (Gennadios et al., 1996):

$$\theta = \arctan(b^*/a^*)$$

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$$

2.4.3. Thickness, gramature and water vapor transmission

A bench micrometer (Tumico, St. James, MN) was used to measure film thickness. Data was obtained from the average of ten random measurements per each film.

Gramature was determined by dividing film weight in grams (semi-analytical balance: Gehaka, model BG400; readability: 0.001 g) by its area in square meters.

Water vapor transmission (WVT) of films was determined gravimetrically using a modified ASTM E96-95 procedure. The test films were sealed to a glass dish containing silica gel with perfect sealing between the dish and films. The dishes were placed in a constant temperature desiccator with distilled water. Dishes were weighted at every 6 h on a semi-analytical balance (Gehaka, model BG400), 0.001 g readability. The WVT was calculated from the slope (w/t) of a linear regression of weight loss versus time.

$$\mathrm{WVT} = \frac{w}{t \cdot A}$$

where A is the permeating area (58.76 cm^2).

During all WVT measurements, the environment inside the desiccator had a constant temperature at 22±1 °C and 99% of relative humidity. Sample thicknesses for all measurements were in the range of 12–15 μm.

3. Results and discussion

The effect of the edible coatings on moisture loss from minimally processed garlic is shown in Fig. 1. Results showed lower moisture loss for coated cloves, independently of coating type, compared to the control. After 9 days of storage, the uncoated cloves had lost approximately 1% of their initial mass. No significant differences were observed ($p \geqslant .05$) among the different coatings, for

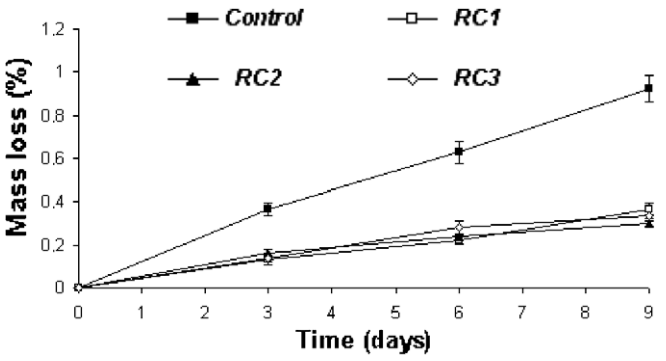

Fig. 1. Mass loss in minimally processed garlic without coating (Control) and coated with agar-agar (RC1), agar-agar and acetic acid (RC2), agar-agar and chitosan (RC3), during storage at 25 °C. Bars represent the mean standard error.

all times, and the moisture loss was approximately three times lower than the control. Development of roots occurred in cloves coated only with agar-agar (Fig. 2).

Reduction of the moisture loss of minimally processed garlic coated with edible film helped maintain post-processing quality and freshness during storage, with cloves remaining turgid longer. Several authors have reported the effects of edible coatings on the reduction of moisture loss in intact or minimally processed vegetables (Baldwin, Nisperos-Carriedo, & Baker, 1995; Jiang & Li, 2001; Li & Barth, 1998; McHugh & Krochta, 1994). According to Sarantópoulos, Alves, Oliveira, and Gomes (1996), moisture loss in food with high water activity can cause undesirable alterations, compromising product appearance. This was confirmed by the control treatment, where the higher moisture loss reduced the visual quality of cloves. Root formation in garlic cloves with RC1 might be associated with water availability in the coating. The absence of roots in coatings with acetic acid and chitosan indicates that these additives somehow affected root primordium formation.

Alginate and gellan and carrageenan films also extended the shelf life of garlic, demonstrating the substantial advantages of using hydrocolloid coatings. Gellan created stronger and more brittle coatings. Addition of β-sitosterol to the gum solution prior to gelation improved adhesion of the film to the garlic. Alginate and gellan films created a modulated atmosphere around the coated garlic. Both coatings were transparent and improve the stability of the coated produce (Nussinovitch & Hershko, 1992).

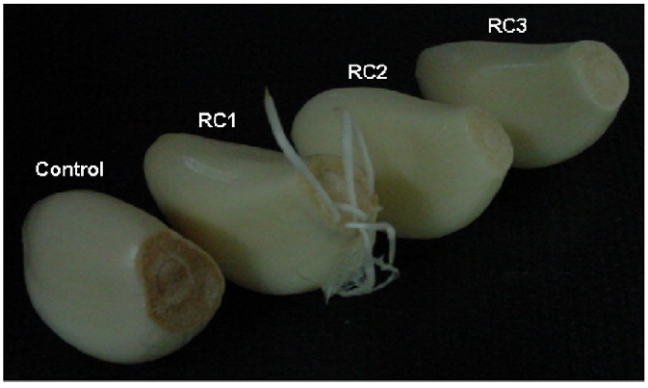

Fig. 2. Garlic cloves without coatings (Control) and coated with agar-agar (RC1), agar-agar and acetic acid (RC2) and agar-agar and chitosan (RC3), after 9 days at 25 °C.

Color differences determined on damaged and non-damaged clove surfaces compared to the white standard are showed in the Fig. 3. There was a marked increase in ΔE^* values of control cloves compared to the other treatments, for both damaged and non-damaged surfaces. Color variation on damaged surfaces was, however, considerably higher than on non-damaged ones. The color difference among cloves with different coatings was not significant ($p < .05$) and remained statistically unaltered throughout the storage period for non-damaged surfaces. Control cloves showed a significant increase in color difference 3 days after processing. Color difference of cloves treated with different films was also non-significant for damaged surfaces, however, it increased rapidly with time. The greatest color alteration was found on damaged surfaces of the control treatment.

The increase in ΔE^* of the control treatment, throughout the storage period, was probably due to clove exposure to ambient air (≈20% O_2), causing rapid browning, which may be related to polyphenoloxidase enzyme activity. This enzyme oxidizes phenolic compounds in the presence of O_2, causing browning in tissues (Mayer & Harel, 1979; Vámos-Vigyázó, 1981). Coating has probably reduced the O_2 concentration around the clove tissues, making it unavailable for the browning reactions. According to Vámos-Vigyázó (1981) tissue browning can be reduced not only by the inactivation of the enzyme, but also by eliminating the O_2 needed for the reaction.

Pen and Jiang (2003) found that surface discoloration of fresh-cut Chinese water chestnut, appeared after 3 days of storage at 4 °C and became more serious after 6 days, when quality decreased markedly. Treatment with a chitosan coating delayed the development of the discoloration.

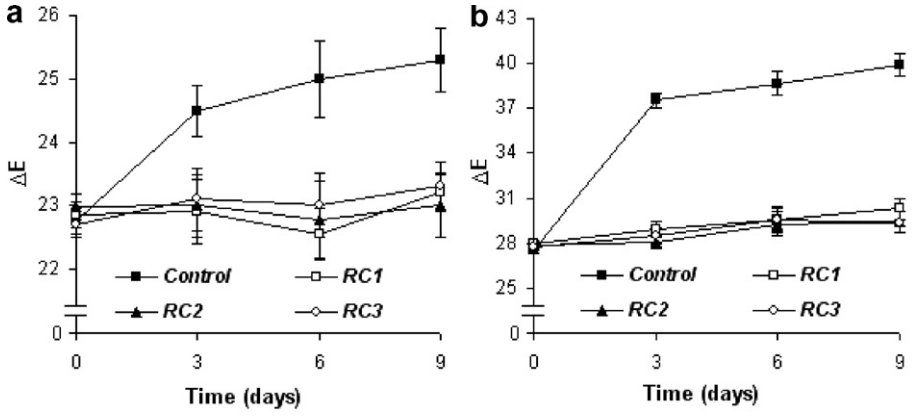

Fig. 3. Color difference (ΔE^*) on non-damaged (a) and damaged (b) surface of minimally processed garlic without coating (Control) and coated with agar-agar (RC1), agar-agar and acetic acid (RC2), agar-agar and chitosan (RC3), during storage at 25 °C. Bars represent mean standard error.

Application of the chitosan coating also inhibited disease development in fresh-cut Chinese water chestnut. The use of chitosan coating in combination with low temperature storage was effective for control of overgrowth of spoilage organisms.

The results of fungi and mesophilic aerobic counts showed the effectiveness of acetic acid and chitosan present as antimicrobial agents in the active coatings (Fig. 4). The fungi count was stable during 6 days-storage (between 10^2 and 10^3 CFU g^{-1}) with the active coating on garlic cloves, while rapid microbial growth took place in the control and coating without additive (around 10^6 CFU g^{-1} on the sixth day). In these last two treatments, visible colonies of filamentous fungi were seen on the garlic surface on the third day. The mesophilic count also showed inhibition of microbial growth in garlic with active coating compared to the other treatments. However, on the ninth day, the mesophilic count (between 10^7 and 10^8 CFU g^{-1}) was statistically the same ($p > .05$) between the garlic treated the with active the coating and control, but this was no observed difference for the fungi count. There was a tendency for greater microbial growth, in both fungis and mesophilic counts, for coatings without additive, reaching values of 10^9 CFU g^{-1}, 9 days after processing.

A aqueous based of coating was not enough to maintain microbiological quality. It was necessary to make it actively functional by the addition of acetic acid and chitosan to inhibit microorganism development. Coating without an antimicrobial can contribute to product deterioration, compared to the control, possibly by providing more water, which favored microbial growth. According to Ahvenainen (1996), low acidity (pH 5.8–6.0) and high moisture of minimally processed vegetables, along with tissue wounding during processing, are ideal growth conditions for most microorganisms. In garlic, this was characterized by the presence of visible fungis colonies over the cut surfaces of control cloves and by the fast mesophilic growth on moistened surfaces of cloves coated with RC1, with counts around 10^9 CFU g^{-1}, after 9 days of storage at 25 °C. In minimally processed products, the usual mesophilic bacteria count is around 10^3–10^9 CFU g^{-1}. This value can be around 10^3–10^6 CFU g^{-1}, soon after processing (Nguyen-the & Carlin, 1994; Zagory, 1999).

The antimicrobial action of chitosan has been reported by others authors. The decay of strawberries was reduced significantly when inoculated-berries were coated with chitosan. The early sign of mold development in the strawberries appeared after 8 days-storage at 13 °C. After 21 days at 13 °C, the percentages of decayed-berries in chitosan coated (1.0% and 1.5% w/v) were 11 and 10, respectively, while in the control it was 52%. Chitosan has the capacity to inhibit growth of several fungi, to induce chitinase, and to elicit phytoalexins in the host tissue. Thus, the control of decay in strawberries could be attribute to either the fungistatic property of chitosan per se or to its ability to induce defence enzymes (i.e. chitanase and β-1,3-glucanase) and phytoalexins in plants or a combination (El Ghaouth, Arul, Ponnampalam, & Boulet, 1991).

The lowest respiration rate was found in whole bulbs (Fig. 5), increasing as the cloves were thrashed and peeled.

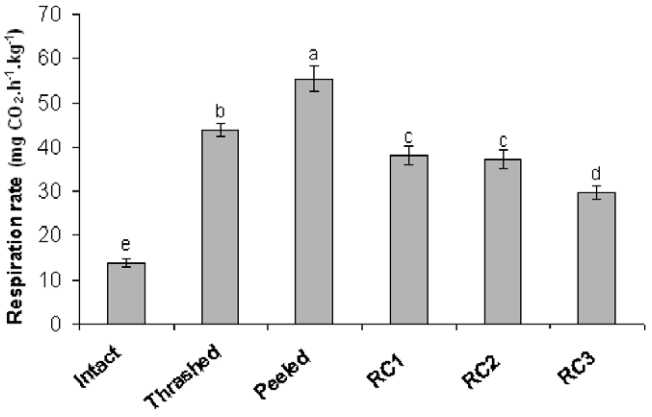

Fig. 5. Garlic respiration rates in different conditions: bulb (whole), cloves (thrashed), peeled, peeled and coated with agar-agar (RC1), agar-agar and acetic acid (RC2), agar-agar and chitosan (RC3), at 25 °C. Bars represent the mean standard error. Means followed by the same letter were not significantly different ($p \geq .05$) from each other by the Tukey test.

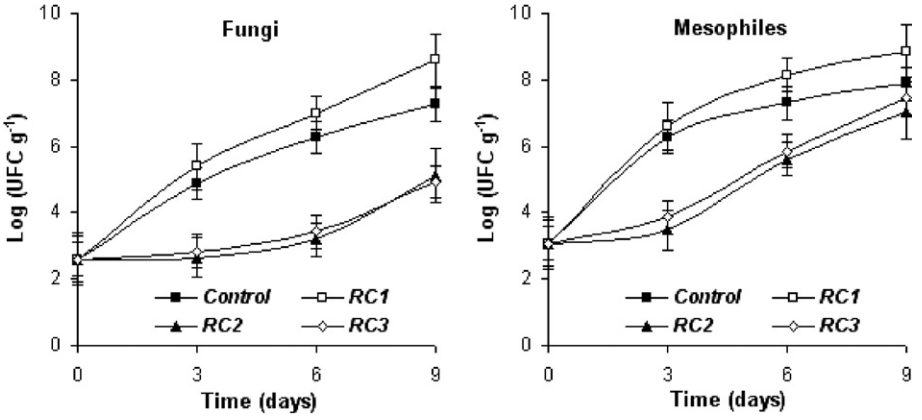

Fig. 4. Filamentous fungi and yeasts and mesophilic aerobic counts of minimally processed garlic without coating (Control) and coated with agar-agar (RC1), agar-agar and acetic acid (RC2), agar-agar and chitosan (RC3), during storage at 25 °C. Bars represent mean and standard errors.

The difference between the whole bulb respiration (≈14 mg CO_2 h^{-1} kg^{-1}) and peeled cloves (≈55 mg CO_2 h^{-1} kg^{-1}) considerable. However, the coatings provided a significant reduction in clove respiration. In cloves covered with RC3 coating this reduction reached approximately half (≈30 mg CO_2 h^{-1} kg^{-1}) of that of the peeled cloves.

The increase in garlic respiration rate, from intact to minimally processed, demonstrates the effect of processing on product respiration. Similar results were reported by Geraldine (2000) who found that manual peeling caused a large increase in garlic respiration compared to thrashed cloves. The peel acts as a barrier regulating oxygen and carbon dioxide flow, and consequently reducing respiration rate (Miller & Krochta, 1997). Yet physical damage or wounding caused during processing can affect respiration, as reported by Watada, Ko, and Minott (1996) in several fruits and vegetables. Garlic cloves coated have their respiration reduced, suggesting that coating acted as a gas barrier between the product and external atmosphere. The capacity of edible coatings to modify gas transport is important to maintain the quality of vegetables, as they mantain an active metabolism during storage (Guilbert, Gontard, & Gorris, 1996). However, coatings or films should allow gas modification around the vegetable to enable ingress of O_2 (for respiration) and consequently escape of excess CO_2 (Gorris & Peppelenbos, 1992). El Ghaouth, Ponnampalam, Castaigne, and Arul (1992) studied the effect of chitosan on respiration and ethylene production of tomatoes. After 10 days of storage at 20 °C, the control fruit exhibited a significantly higher respiration rate than the treated fruit. Fruit coated had a significantly lower rate of ethylene production than the control fruit. Chitosan coating decreased the O_2 and raised the CO_2 levels within the tomato fruit, with a greater effect at the higher coating concentration. Some edible coatings, such as wheat gluten and soybean protein base, are very effective oxygen barriers at low relative humidity (Brandenburg, Weller, & Testin, 1993; Gennadios, Weller, & Testin, 1993).

Table 1 shows some physical properties of the three produced edible coatings. All determined properties were not significantly different ($p < .05$) between the films with agar-agar and agar-agar with acetic acid. Thickness, gramature and water vapor transmission of films with agar-agar and chitosan were significantly different from the other films.

Table 1
Thickness, gramature and water vapor transmission of the three types of films/coatings

Coatings	Thickness (μm)	Gramature (g/m^2)	Water vapor transmission ($g\ h^{-1}\ m^{-2}$)
Agar-agar	12.0 a	11.42 a	3.049×10^{-3} a
Agar-agar and acetic acid	12.2 a	11.60 a	2.988×10^{-3} a
Agar-agar and chitosan	14.8 b	14.07 b	2.476×10^{-3} b

Values followed by the same letter (in the same column) were not significantly different from each other ($p \geq .05$) by theTukey test.

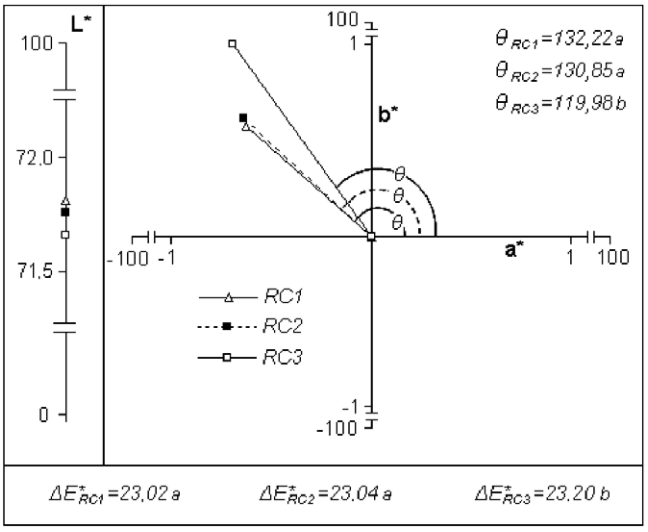

Fig. 6. Hunter's Diagram showing L^*, a^*, b^* values and their derivations in color shade (θ) and color difference (ΔE^*) of films/coatings for agar-agar (RC1), agar-agar and acetic acid (RC2), agar-agar and chitosan (RC3). Values followed by the same letter were not significantly different from each other ($p \geq .05$) by the Tukey test.

The differences in thickness, gramature and water vapor transmission of chitosan coating, compared with the others, were basically caused by the highest polysaccharide concentration in the film. After drying, the coating added with 0.2% chitosan presents approximately 20% more mass in its final structure. The values of WVT demonstrate that the developed films have a high permeability. Polysaccharide coatings are hydrophilic, and, therefore, usually present higher water vapor transmission (Hagenmaier & Shaw, 1990, 1991).

Hunter's Diagram shows the means of L^*, a^* and b^* parameters for each analyzed film and their derivations of color saturation (Theta, θ) and color difference (ΔE^*) (Fig. 6). Results demonstrate that the acetic acid did not affect color parameters of the agar-agar base film. However, these parameters were affected by chitosan, changing saturation and values of color difference.

The small difference in the values of ΔE^* between RC3 and the other edible coatings, although significant ($p < .05$), was not enough to allow its visual perception. The reduced thickness of these films has probably contributed to such characteristic, because the chromaticity values in a^* and chromaticity in b^* tend to have a more intense shade in chitosan films, with a yellowish color, compared to the others.

4. Conclusions

This study showed that the active coatings evaluated can guarantee the quality of minimally processed garlic for an extended period, especially with respect to microbiological aspects. In addition, the coating ensured lower color variation, moisture loss and respiration rate, prolonging shelf life. In this context, the use of these and other actively func-

tional edible coatings is an excellent complement to minimal processing.

Acknowledgments

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