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Gellan and alginate vegetable coatings

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Gellan and alginate hydrocolloid coatings of garlic bulbs (*Allium sativum*) were studied. Coatings served as a barrier to moisture loss. Incorporation of ingredients that can be found naturally in garlic skin, or are chemically similar to these, into the gum solution before coating, improved adhesion of the film to the surface of the coated commodity. Adhesion strengths were about three times higher than those recorded for a film made of gum and crosslinking agent alone. Electron microscopy technique revealed the structure of the garlic skin and hydrocolloid coating. Distances between the film and the vegetable were measured using image processing, and they could sometimes be reduced by varying the film composition. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Many polysaccharide-based coatings have been developed for fruit and nut products. Low-methoxy pectin (LMP) coatings were used for nuts and dried dates (Swenson *et al.*, 1953). A powdered formulation of hydroxypropyl starch was used for prunes (Jokay *et al.*, 1967). Amylose starch and plasticizer were used to coat raisins and dates (Moore & Robinson, 1968). A laminated coating composed of an initial layer of an amylose ester of fatty acid and a layer of soy or zein protein was used to coat freeze-dried peas, carrots and apple slices (Cole, 1965). Pieces of freshly cut fruits and vegetables were coated with dust of CMC and starch to prevent loss of juice and to preserve texture (Mason, 1969). Water loss and browning of cut apple slices were inhibited by chitosan and lauric acid coatings (Pennisi, 1992). Information about synthetic polymers and derivatives of natural polymers used in coatings can be found in reports and patents from the late 1960s and the early 1970s (Danials, 1973; Shillington & Liggett, 1970; Mulder, 1969; Delong & Shepherd, 1972; Rosenfield, 1968).

Carrageenan-based coatings—Soageena and Soafil (edible polysaccharides)—were developed by Mitsubishi International Corp, (IFT, 1991), but aside from their designation for fresh produce, no information on them is available (Baldwin, 1994). Other carrageenan coatings have been used to retard moisture loss from coated food (Torres *et al.*, 1985). Protein, lipid and composite films have been studied extensively (e.g. Kumins, 1965; Guilbert, 1986; Deasy, 1984; Roth & Loncin, 1985; Kamper & Fennema, 1984; Schonherr, 1982; Hagenmaier & Shaw, 1990). Alginate-based coatings have been used to coat meat (Allen *et al.*, 1963; Earle, 1968; Williams *et al.*, 1978; Wanstedt *et al.*, 1981; King, 1983; Glicksman, 1983). Other sodium alginate-based and gellan coatings have been investigated (Nussinovitch & Kampf, 1992, 1993; Nussinovitch, 1994; Nussinovitch *et al.*, 1994; Hershko & Nussinovitch, 1995, 1996a, b).

The aim of this study was: to check the possibilities of extending the shelf-life of garlic by using gellan and alginate coatings, to follow changes in weight, appearance, and adhesion strength of the film to the coated vegetable skin, to get information on the nature and structure of the garlic skin, and to generally test the advantages and disadvantages of hydrocolloid coatings used for long periods.

MATERIALS AND METHODS

Fresh and dry garlic heads (about 60–70 g each) were coated with a film of food-grade alginate, M_w 70 000–80 000, 61% mannuronic acid and 39% guluronic acid contents (Kelgin LV, Kelco Division of Merck & Co., USA) (Kelco, 1976; King, 1983; Guilbert, 1986). Garlic bulbs were immersed in a 2% (w/w) sodium alginate solution. Residual alginate was then allowed to drip off, before immersing the garlic in a 2% (w/w) solution of calcium chloride (Frutarom Laboratory Chemicals, Haifa, Israel) for about 30 s to induce a spontaneous crosslinking reaction. Small portions of the film surface were inspected under a light microscope. The coated

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garlic were stored at room temperature (20°C). Weight loss, and mechanical and color properties were monitored throughout the experiments. Weight loss was monitored by periodical weighing. Results are the average of at least 20 weighings, at an accuracy of ±0.1 g.

Other coatings were performed using food-grade gellan (Kelcogel, Kelco Division of Merck & Co., USA) and κ-carrageenan (Sigma Chemical Co., St Louis, MO, USA). Garlic heads were immersed in a bath (50°C) of 1 or 2% (w/w) gellan solution, previously dissolved in distilled water, at about 90°C. Residual gellan was then allowed to drip off, before immersing the garlic bulbs in a 0.1% (w/w) solution of $MgCl_2$ (Merck) for about 30 s to induce gelation. Following gelation, bulbs were dried with warm air (~40°C) until a moisture content of about 15% remained in the film. κ-carrageenan was dissolved in distilled water (at about 90°C), cooled to about 50°C and used to coat the garlic bulbs by introducing them into a bath. Later the garlic bulbs were immersed in a 1% (w/w) KCl (Sigma) solution to induce gelation. Separate experiments were performed when β-sitosterol (24 β-Ethylcholesterol, $C_{29}H_{50}O$), (Sigma Chemical Co., St Louis, MO, USA) was incorporated into the gellan or carrageenan solutions after it was dissolved in 5 ml of warm ethanol (~60°C). The garlic bulbs were coated with the gellan-sitosterol or carrageenan-sitosterol solutions and coatings gelled after dipping in the $MgCl_2$ or KCl baths. They were then dried as previously described. Weight loss was also determined, as described above.

Gellan and alginate films produced for an evaluation of their mechanical properties, the films were obtained by the casting technique, which consists of cooling the gel solution to a temperature above the setting temperature of the gel, (50°C), before pouring into vertical electrophoresis cells. This system is used in many laboratories to produce films with almost constant thickness. The gel solution was poured into a 'sandwich' prepared from glass plates assembled with screw clamps and aligned using the casting stand's alignment slot and an alignment card. After the gels were cast, they were left to equilibrate for 36 h under high humidity at room temperature before preparation of the specimen with a dumbell-shaped cutter. The casting technique enabled us to get gellan films with the same composition as those used to coat the garlic. However, this technique cannot be used to create uniform alginate films composed of only sodium alginate and calcium salt. It was because we wanted to study the influence of added sitosterol on the properties of this type of film that we used the above-described alginate solution with the addition of 1% (w/w) $CaHPO_4$ (Riedel-deHaen, Seelze, Germany) and 1% (w/w) sodium hexa meta phosphate (SHMP), (BDH Laboratory Supplies, Poole, England), to achieve uniform films via casting which most closely approximated the sodium alginate-calcium salt film. Gelation in this case was induced by the addition of 1% (w/w) glucono-δ-lactone (GDL-Sigma). A test-section length of 60 mm and a high ratio of test length to end length (about 5.0 mm) of the dumbell specimen enabled us to neglect the effect of the end pieces (Lelievre *et al.*, 1992; Hershko & Nussinovitch, 1995). The stress and strain at failure was measured by the Instron Universal Testing Machine (UTM), (model 1100, Canton, Mass. USA). They represented the true values in the test sector of the gel. The Instron was interfaced with a 486-compatible IBM personal computer via a card. A special program developed by the Instron Corporation and modified in our laboratory enabled conversion of the Instron's voltage vs. time measurements into digitized force-deformation, force-time, stress-strain or stress-time files, using any desired definition of stress and strain. Specimen thickness was measured with a micrometer. The tip of the specimen was mounted on the Instron UTM, set in tension mode. Polypropylene cloth-like material produced by Shiltex Kvuzat Shiller, Israel (thickness of a single strand: 0.625 mm, and weight/unit length: 0.012 kg/m) was inserted into the standard upper and lower tensile grips to increase friction and allow a satisfactory grip during the tension experiment.

All film specimens for tension tests were deformed at a constant deformation rate of 25 mm/min. Tests were conducted immediately after gel specimens were cut from the film layer. The breaking force and deformation at failure were read directly from the force-deformation files of the computer interfaced with the UTM. Each specimen was visually inspected during the test, and if failure developed at or near the grips, the test was aborted and its data discarded. For each film concentration, at least six specimens were examined, taken from duplicate gel batches.

Peel testing, i.e. the force necessary to peel the coating, was used to estimate the adhesion of the film to the garlic. The coating was peeled at 90° from the substrate, and the adhesion strength was estimated by the force per unit width necessary to peel the coating (Croll, 1983).

Electron microscopy of dried garlic skin specimens was performed after coating with gold (thickness 150–200 μ) using a Polaron 5100 sputter coater (Forni *et al.*, 1991). The chemically-fixed tissue and coatings were observed in a Jeol 35C SEM.

Electron micrographs were scanned by a scanner (Avision, SCSI Model-680, USA) and analyzed on a Macintosh IIsi computer using the public domain NIH Image Program (written by Wayne Rasband at the U.S. National Institute of Health, Springfield, VA).

To determine the concentrations of accumulated carbon dioxide, 1-ml gas samples were taken from under the coated garlic skin at ~1 mm beneath the skin, about 2 weeks after coating, using gas chromatography (GC model 580, Gow-Mac Instrumentation Co., Bridgewater, NJ, USA, equipped with a Porapak Q column and TCD detector). Results were expressed as $\%CO_2$ in the sample. The operating conditions were: a column temperature of 35°C for 7 min, increased to 225°C at 32°C/min, the injector temperature was 100°C, detector temperature 240°C, filament temperature 280°C, carrier gas (He) flow rate 30 ml min $^{-1}$.

Water vapor transmission rate through dried alginate, aliginate and sitosterol, gellan and gellan with sitosterol was determined by ASTM E96-93 (Standard Test Methods for water vapor transmission of materials).

DATA PRESENTATION

For film tension tests, the force deformation curves of the specimens were transformed into corrected ('true') stress, σc, and Hencky's ('true') strain, ε_H, by the following transformations (Hershko *et al.*, 1994):

$$\sigma c = F(L_0 + \Delta L)/(A_0 L_0) \quad (1)$$

where F is the force, A_0 and L_0 the initial cross-sectional area and length of the specimen, respectively, and ΔL the absolute deformation, and

$$\varepsilon_H = \ln((L_0 + \Delta L)/L_0). \quad (2)$$

Adhesion strength (Croll, 1983) was derived from the peeling of hydrocolloid films at 90°. These films adhered to the garlic skin by

$$F/b \quad (3)$$

where **F** is the peeling force and **b** is the width of the coating.

RESULTS AND DISCUSSION

Garlic was chosen as a model for coating studies owing to its complex structure. It is a perennial plant with a compound bulb composed of several partial bulbs (cloves) enclosed by a common membrane. The fresh or dried bulb is marketed and our coating experiments focused on this part accordingly. Garlic is biologically interesting because of its antibiotic activity in the gastrointestinal tract. It is a weak vermifuge and is used to prevent arteriosclerosis, but its hypotensive action is probably only slight (Fluck *et al.*, 1941). Additional and even more important reasons for choosing garlic as a model for study are: it is less sensitive to heat (thus warm solutions of gellan or carrageenan can be used, increasing chances of achieving better wettability); the character of its skin is unique (sterols can be found on its outer epidermis); its spear head opens with time thus the cloves are separated, and coating can therefore be very useful to its salability. Alginate was used to coat garlic heads for the previously mentioned reasons. Gellan was used for garlic coating because it gives a transparent, glossy appearance to the garlic, it does not substantially alter the price per unit weight of the produce, the film can be easily removed and is biodegradable. Biodegradability could become even more important if other fruits or vegetables having a peel which is discarded (such as mango, papaya, banana, etc.) are coated for shelf-life extension.

Films were peeled off the garlic and inspected visually under a strong light source and/or binoculars, and small surfaces were inspected under a light microscope to detect the rare hole or incompleteness. It is well known that weight decreases during the storage of fruits and vegetables are due mainly to transpiration. To avoid evaporation of water, the storage atmosphere needs to be saturated with water vapor. For cold storage of fresh vegetable produce, 80–100% relative humidity is regarded as optimal. In practice, however, a lower relative humidity frequently prevails, partly because of technical shortcomings, partly to prevent the growth of microorganisms, particularly fungi. Weight loss values in % per day of unpacked vegetables have been previously published (Gorini *et al.*, 1990). In cold storage (0–2°C), at relative humidities of 76–90% and storage times of 7–21 days, cauliflower loses 0.4% of its weight per day, french beans 0.1–0.3%, green peas 0.2–0.4%. Under room temperature conditions (16–24°C), a relative humidity of 65–80% and storage times of 2–14 days, cauliflower loses 1.0–2.0% of its weight per day, lettuce 5.0%, spinach 1.5–3.5%, french beans 1.0–2.0%, green peas 1.0–2.0% and ripe tomatoes 0.1–0.3%.

An additional aim of the garlic-coating project was to include at least one natural compound isolated from its surface or one very similar or identical to compounds naturally present on its surface, within the gum solution. Such an inclusion might contribute to better surface adhesion, resulting in a tighter coating which could extend garlic's shelf-life (This point is detailed further on.)

Weight loss of freshly harvested garlic was about 17.5% after storage for about 23 days at ambient temperatures and a relative humidity of about 70% (Fig. 1). Alginate-coated and alginate-sitosterol-coated garlic heads lost 15.8 and 14.2% moisture, respectively, within the same period. It should be noted that it took at least 24 h to obtain the 'fresh' produce after its collection and cleaning in the fields, and that moisture was lost at this stage, before the experiment was begun. Coating reduced the rate of moisture loss. The best coating was achieved when a natural compound was added to the gum solution and before gelation around the garlic head.

The idea of adding a natural compound to the gum solution was based on the hypothesis that chemical similarity can contribute to better adhesion or better compatibility between the produce and the coating and thus to a more successful coating. Since the cuticles of higher plants carry a partial or continuous covering of amorphous epicuticular wax, it was therefore interesting

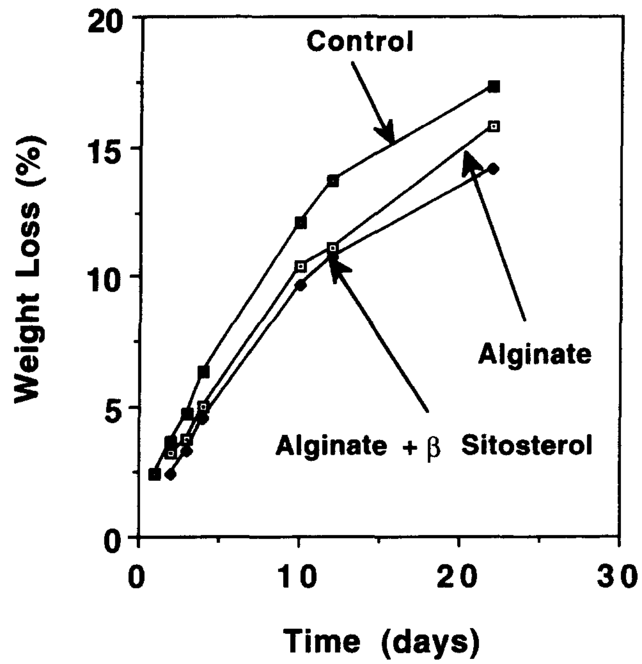

Fig. 1. Weight loss of uncoated and alginate- and alginate-β-sitosterol-coated fresh garlic bulbs.

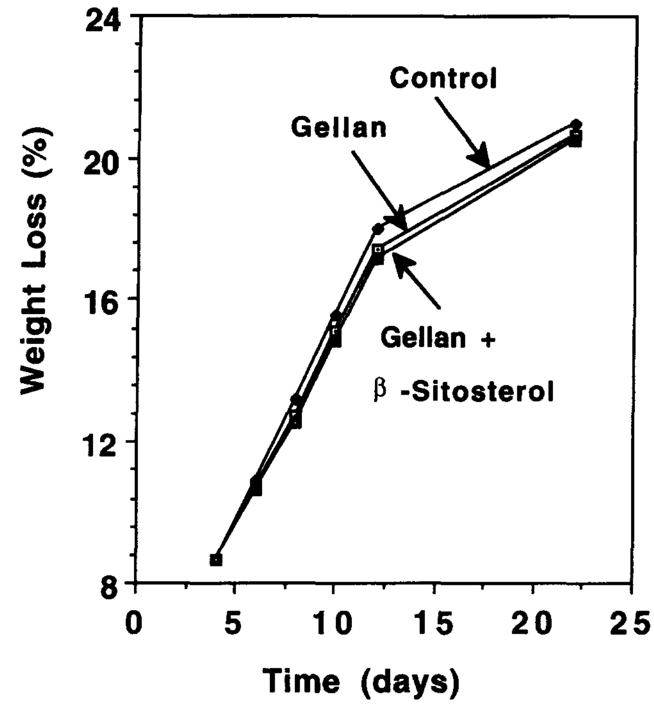

Fig. 2. Weight loss of uncoated and gellan- and gellan-β-sitosterol-coated fresh garlic bulbs.

to check the latter's influence on different properties of the film. The morphology of epicuticular wax is known to differ between species and organs, and during the life cycle of a plant (von Wettsein-Knowles, 1979; Baker, 1982). In addition to epicuticular wax deposits, there are other waxes of different composition, more intimately associated with cutin and suberin (Baker, 1982). Waxes are composed of complex mixtures of several classes of long-chain aliphatic compounds. The great variety of cyclic, branched, and long-chain aliphatic constituents of plant-surface waxes has been reviewed (Tulloch, 1976; Kolattukudy, 1980a; Kolattukudy *et al.*, 1981; Baker, 1982). Wax may contain over 50 distinct compounds, and typically one or a few alkanes in the C_{21} to C_{37} range or primary alcohols in the C_{22} to C_{32} range predominate (Martin & Juniper, 1970; Tulloch, 1976; Kolattukudy, 1980b; Baker, 1982). β-Sitosterol, the ingredient we chose to add, is regarded in the chemical literature as a steroid. The term steroid is generally applied to compounds containing a hydrogenated cyclopentanophenanthrene carbon skeleton. Most of these compounds are alcohols, and sometimes the name sterol is used for the whole class. However, sterol is better reserved for the substances that are actually alcohols (Roberts & Caserio, 1965).

The differences in percent weight moisture loss between treatments were at most 3.3%. Since garlic is an expensive crop and 1% less weight loss translates to 10 kg per ton, use of an appropriate coating is economically justified. With the gellan coating (Fig. 2), fresh garlic suffered more moisture loss than with alginate coating. Although the hydrocolloid coating contributed to slowing the rate of moisture loss, less significant differences were observed between coated and uncoated garlic.

After about a month, garlic dries and is sold as 'dry garlic' for the rest of the year. Decreases in percent weight loss were noted when β-sitosterol was used in combination with gellan on dry garlic which was coated 45 days post harvest (Fig. 3). It is important to note

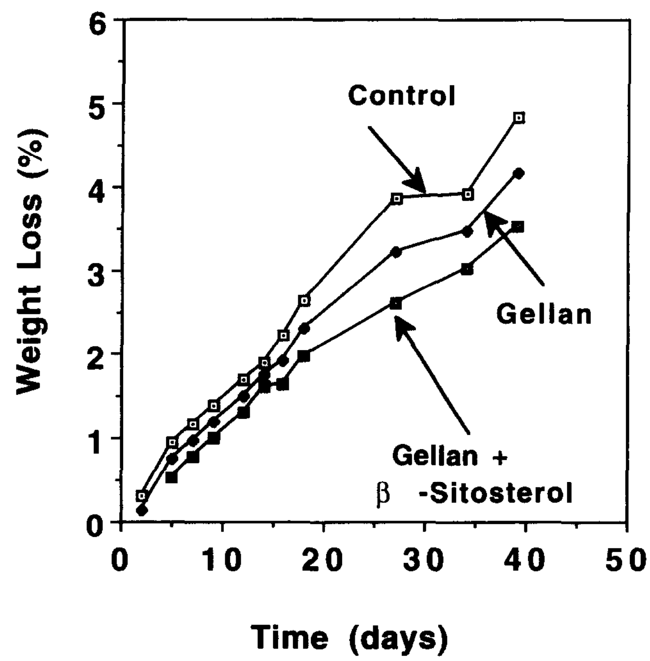

Fig. 3. Weight loss of uncoated and gellan- and gellan-β-sitosterol-coated garlic bulbs. Coating was performed 45 days postharvest.

Table 1. Transfer of water vapor through films*

Film Type	β-Sitosterol	Rate (g/ d m^2)
Alginate	–	551
"	+	497
Gellan	–	540
"	+	450

*Each result is the average of 10 determinations

Table 3. Accumulation of carbon dioxide beneath garlic skin

Film type	Addition of β-Sitosterol (% W/W)	$CaCl_2$ (% w/w)	$MgCl_2$ (% w/w)	CO_2 (%v/v)
Alginate	—	2	—	0.20 (0.15)
"	0.2	2	—	0.23
Gellan	—	—	0.2	0.10 (0.08)
"	0.2	-	0.2	0.17

*Each result is the average of seven determinations
In parentheses % CO_2 in garlic without coating

that the rate of water loss at this stage of coating is slower than when garlic heads are coated immediately after harvest.

For gellan, alginate and κ-carrageenan, addition of the natural compound β-sitosterol to the gum solution before coating always gave better results regarding percent weight loss of either fresh or dried garlic bulbs (Figs 1–4). The breadth of this experiment, which included totally different hydrocolloids in terms of chemical structures and gelation mechanisms, enabled us to conclude the generality of the improvement in coating and its dependence on the nature of the added natural wax ingredient, i.e. on the latter's chemical similarity to ingredients found in the garlic's inherent wax. Percent weight loss could be related to the rate of water vapor transfer through the film (Table 1). From this table it is possible to see the advantage (decreased transfer of water vapor through the coatings) of the film including the natural compound vs. film alone. The reduction in water vapor transfer through the film was about one-tenth for the alginate-based coating and 20% in the case of gellan. In both cases, a gum concentration of 2% was used with the addition of 0.2% of β-sitosterol.

The effect of an added ingredient(s) on the film's mechanical properties was comprehensively studied. The addition of the steroid β-sitosterol to 2% alginate or gellan gums (Table 2) did not influence the stress at failure of alginate and gellan films, but had some effect on the strain at failure in the case of alginate, whereby the film became more brittle.

The coating of vegetables with a 2% hydrocolloid solution prior to gelation and drying influenced shelf-life. The addition of 0.2% β-sitosterol to the gum solutions, followed by drying and film achievement, increased the concentration of carbon dioxide under the garlic's skin, in comparison to hydrocolloid film alone or no film (Table 3). This achievement of a modulated atmosphere around each piece of fresh produce could explain the resultant decrease in weight loss of the coated garlic. It is also important to note that the concentrations of carbon dioxide were less than that which typically causes anaerobic respiration. The addition of sitosterol to the film's ingredients also improved adhesion of the film to the garlic skin. This was checked by a peeling test. The adhesion strengths values for alginate film was 2.2 mN/mm and 7.8 mN/mm after the addition of sitosterol. For gellan, an average value of 2.5 mN/mm was found as compared to 5.8 mN/mm after the addition of the natural compound. With either alginate or gellan, the maximal adhesion strength was found to be 3.5 times higher than in the case of no added natural compound. Other advantages of sitosterol addition could be concluded from image-processing studies. The distances between the skin and the garlic were found to decrease (from 8.5 to 4.2 μ on average) with the addition of β-sitosterol to gellan, resulting in better adhesion. In the case of alginate, no significant difference in distances were observed. Important information regarding salability of coated produce can be derived from its shape, color and esthetics of the coating. From the picture (Fig. 5) of garlic coated with gellan, the transparency of the film can be easily observed. Attention should be paid to gellan's tight packaging of the garlic and the advantage achieved by its not permitting the head to open into individual cloves. It should be noted that the same effort was needed to separate the coating from the garlic. Figure 6 shows the coated garlic skin under SEM. Garlic skin is mechanically supported by its vascular system (see tubings within the skin). It is easy

Table 2. Mechanical properties of alginate and gellan films produced by casting

Film type	Addition of β-Sitosterol (% w/w)	$CaCl_2$ (%w/w/)	$MgCl_2$ (%w/w)	σ (MPa)	ε_H (-)
Alginate	—	2	—	7.5^a	0.10^a
"	0.2	2	—	7.0^a	0.05^b
Gellan	—	—	0.2	25.1^b	0.06^b
"	0.2	—	0.2	20.9^b	0.04^b

*Each result is the average of 10 determinations

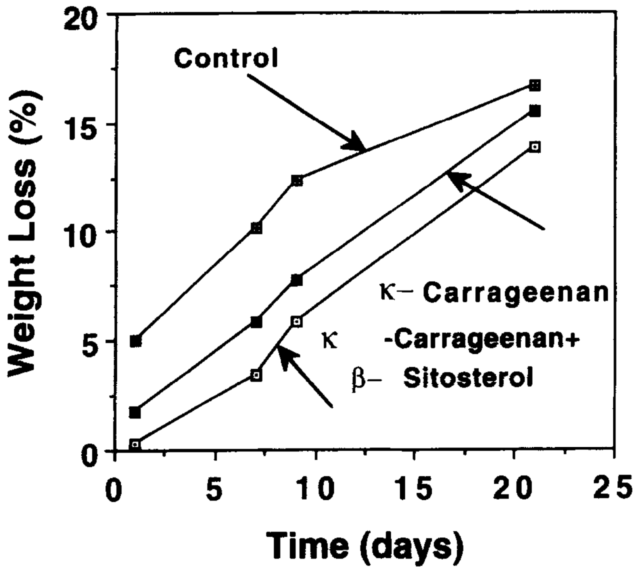

Fig. 4. Weight loss of uncoated and κ-carrageenan- and κ-carrageenan-β-sitosterol-coated garlic bulbs.

Fig. 5. Gellan- coated (right) vs. uncoated (left) garlic bulbs.

Fig. 6. SEM micrograph of garlic skin in cross-section.

to see the many sublayers which compose the skin. After observing many samples (Fig. 7), we concluded that each skin can be composed of a different number of sublayers, from a few to 12 or even more. The coating, whether it is alginate or gellan is transparent. It adheres to the outer skin (surface) and adapts to its shape whether it is concave or convex. This can be seen from Fig. 6 where the coating is glued on top of the skin and from Fig. 8, where the peeled coating adapts the shape of the garlic skin. Measured distances between the skin and the coating films (Fig. 9) were in the order of a few microns. No holes or defects were observed.

CONCLUSIONS

The substantial advantages of using hydrocolloid coatings are demonstrated. Alginate and gellan and carrageenan films extended the shelf-life of garlic. Gellan

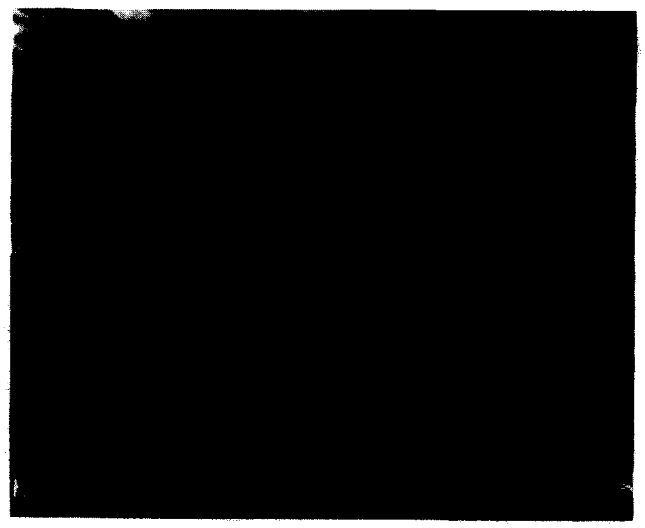

Fig. 7. The sublayer structure of a garlic skin cross-section.

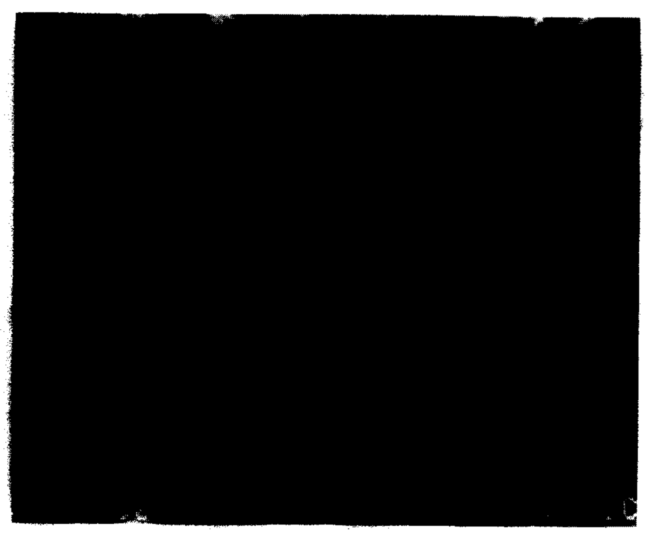

Fig. 8. Peeled coating from garlic head skin.

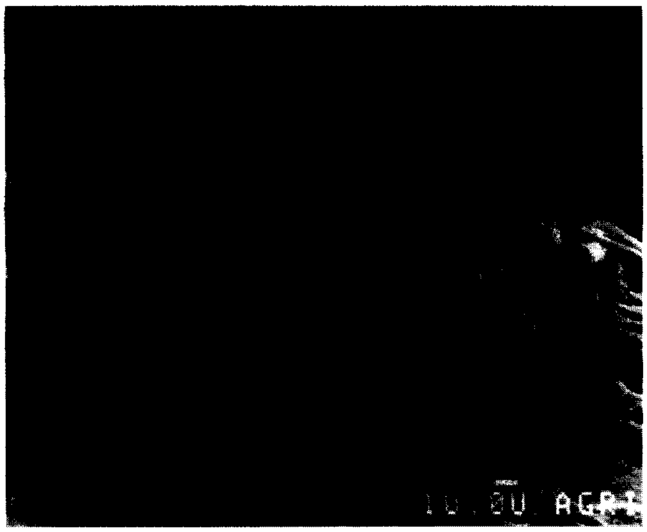

Fig. 9. Space between garlic (bottom) and gellan-sitosterol coating (top). From such electron micrographs, the average distance between coating and coated object was derived.

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 could improve the salability of the coated produce.

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