Relationships between Hydrocolloid Coating and Mushroom Structure

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Agaricus bisporus edible mushrooms were coated with different gum-based coatings, including alginate and alginate−ergosterol, with or without emulsifier. The structures of the different parts of the mushroom tissue were studied by SEM. Various penetration modes of coating and cross-linking solutions into the tissue were examined. The techniques of ICP and X-ray analysis were used to quantify the presence and depth of penetration of minerals into the mushroom tissue before and after coating. Critical surface tension of the "solid" upper surface of the mushroom was estimated using the Zisman plot. The alginate-based coating solutions were tested for their surface tension and contact angles on the mushroom tissue. Reduction in the surface tension and the contact angles of the coating solutions lead to better wettability of the surface. Coated mushrooms were found to have a better appearance, a better color, and an advantage in weight in comparison with the uncoated ones. The alginate−ergosterol−Tween coating combination was most suitable for maintaining the size and shape of the coated mushroom.

Keywords: *Coating; hydrocolloid; mushroom; penetration; surface tension*

INTRODUCTION

Mushrooms are highly perishable and tend to rapidly lose quality attributes such as color and crisp texture. The reduction in mushroom quality after harvesting is determined by metabolic changes (such as browning) and microbiological deterioration (Burton, 1989). Mushrooms lack a protective epidermal structure to prevent excessive moisture loss and therefore suffer from a very high transpiration rate (San Antonio and Flegg, 1964).

Many methods have been proposed to extend the shelf life of fresh edible mushrooms. They include the overwrapping of mushroom pouches with poly(vinyl chloride) (PVC) stretchable films (Gormley and MacCanna, 1967), special highly permeable PVC films to buffer the high CO_2 production in the case of temperature abuse (Lopez-Briones et al., 1993), packaging mushrooms in pretreatment gaseous atmosphere at 18 °C in various styrene plastic films (Nichols and Hammond, 1973), controlled atmosphere packaging (CAP) or modified atmosphere packaging (MAP) of mushrooms (Beit-Halachmy and Mannheim, 1992; Lopez-Briones et al., 1993), and a combination of modified atmosphere with the addition of calcium hypochlorite to reduce microbial counts (Kuyper et al., 1993).

The reduction in the quality of fresh mushrooms during storage is in part a consequence of the mushrooms unique structure. Cultivated mushrooms of the type *Agaricus bisporus* contain approximately 90% water on a wet basis. Therefore, they are subject to rapid spoilage through the activity of the enzyme polyphenol oxidase, which mushrooms contain in substantial quantities (Yapar et al., 1990).

Many recent reports on coating of fresh produce by edible films can be found in the literature (Krochta and de Mulder-Johnson, 1997). Suitable coating depends on adjustment of the coating solutions to the structure of the coated object, considering the parameters of viscosity, porosity, surface tension, wettability, and roughness, among many others (Hershko and Nussinovitch, 1998a,b). Although a lot of information is available on edible coating in general (Kester and Fennema, 1986; Guilbert 1986; Nussinovitch and Hershko, 1996; Nussinovitch, 1997), less information is available on mushroom coating specifically.

A new approach to extend the shelf life and preserve the texture of fresh mushrooms by hydrocolloid coating was proposed by Nussinovitch and Kampf (1993). Calcium−alginate films, applied immediately after harvest to each piece of produce and dried to enrobe the mushroom, reduced transpiration and maintained a modulated atmosphere around each coated mushroom. Such coating is also advantageous in achieving better color and maintaining a lower rate of weight loss in comparison to uncoated mushrooms.

To develop new coatings for fruits and vegetables, information on the surface properties of the coated object should be derived using methods developed many years ago by Young (1805) and Zisman (1964). These ideas were applied to food by Mack (1935), who measured contact angles on apple surfaces. In a report from 1966, Hall also measured contact angles on pieces of apple-peel surface and discussed briefly the wettability of plant and fruit surfaces. These reports and later reports by Hagenmaier and Baker (1993), Banks, (1984b), Cisneros-Zevallos and Krochta (1997), and Hershko et al. (1998) paved the way to further progress in this complicated area.

The objectives of this study were to microscopically explore the differences in the structures of various parts of mushroom tissue in order to relate them to the possible penetration of the mushroom tissue by solutions and minerals during the coating process. Other objec-

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tives included estimating the critical surface tension of the mushroom's outer surface and measuring the surface tensions of the coating solutions and their contact angles on these mushroom surfaces. Such measurements could lead to a better understanding of the relationships between coating solutions and coated object.

MATERIALS AND METHODS

Eighty fruit bodies of fresh mushrooms, *Agaricus bisporus* (20 fruit bodies for each treatment and for control), were chosen and brought to the lab within 1 h of collection from a growing house. The mushrooms were not rinsed before coating, because they arrived clean after harvest. Controls were treated similarly to the coated specimens except for coating. Mushrooms weighing about 20 g each were coated with a thin film of food-grade alginate MW 70 000−80 000, 61% mannuronic acid and 39% guluronic acid contents (Kelgin, LV, Nutrasweet Kelco Co., a unit of Monsanto Co., San Diego, CA). The mushrooms were immersed in a 2% (w/w) sodium alginate solution after dialysis. Residual alginate was then allowed to drip off before immersing the mushroom in a 2% (w/w) solution of $CaCl_2$ (or $BaCl_2$ for X-ray analysis) (Frutarom Laboratory Chemicals, Haifa, Israel) for about 30 s to induce a spontaneous cross-linking reaction and to achieve a thin layer of gel coating the mushroom. Separate experiments were performed in which 0.2% (w/v) ergosterol (5,7,22-ergostatrien-3β-ol: 3β-hydroxy-5,7,22-ergostatriene), $C_{28}H_{44}O$ MW 396.7 (Sigma Chemicals Co., St. Louis, MO), was incorporated into the alginate solution after ergosterol had been dissolved in 5 mL of warm ethanol (~60 °C). Coating solutions were homogenized using an Ultraturex T-25 (Janke and Kunkel), at 13 500 rpm. Different compositions of the coating gum solutions included alginate with the addition of 0.25% Tween 80 (ethoxylated 20-sorbitan monooleate, Riedel de Haen, Seelze, Germany) with or without 0.2% ergosterol. Coated mushrooms and films peeled off the mushrooms were visually inspected under a strong light source using a binocular, to determine the completeness of the coating. Coated and uncoated mushrooms were stored in a controlled chamber at a temperature of 2−3 °C and relative humidity of ~70% for 2 weeks. After 2 weeks, the humidity was reduced to 40−50% for 6 more days for verification of longer storage. Weight loss was monitored by periodic weighing. The results are the average of at least 20 weighings at an accuracy of ±0.1 g.

Color Measurements. Color was analyzed and monitored over time using the Minolta Chroma meter (CR-100, Tokyo, Japan), and the results are reported as the L^*, a^*, b^* indice modes of CIE. Before measurements were taken, the instrument was calibrated with a white standard plate provided by the manufacturer.

Area Measurements. Perpendicular projection of the mushroom cap was monitored by periodic measurements using a DT area meter (Delta T-Devices, Burwell Cambridge, England), attached to a video camera (Sony, Tokyo, Japan) and a monitor. The determined areas are the average of at least 20 measurements at an accuracy of ±0.1 cm^2.

Surface Tension. All the above-listed gum−solution combinations, including alginate solution after dialysis at a final concentration of 2% as is or with the addition of either 0.2% ergosterol or 0.25% Tween 80 or with the addition of both, were ultrafiltrated by Amicon to remove rarely observed solid particles prior to measuring the surface tension. Dialysis of alginate solutions against doubly distilled water was performed using sodium acetate sleeves (Medicell Int Ltd., London, England), with a molecular cut off of 10 000 daltons (Pohl, 1990). The surface tension was measured with the Wilhelmy platinum plate (Lauda tensiometer). Each sample was equilibrated for a minimum of 24 h, until a constant value was obtained. The surface tension readings were within ±1 dyn/cm. To ascertain that the low viscous hydrocolloid-based solutions would not affect the results obtained by the Lauda tensiometer plate, the measurement of glycerol (known to have a viscosity of 850 cP at 25 °C) was carried out under the same conditions and the results were identical to those cited in the literature (64.9 dyn/cm), indicating that a viscosity of < 850 cP does not affect the surface tension measurements with this technique (Garti and Reichman, 1994). Care was taken to adjust the solution concentration so that viscosity of < 850 cP would be certain.

Zisman Plot. Estimation of the critical surface tension (γ_c) of the mushroom cap surface was obtained using contact-angle measurements followed by extrapolation from the Zisman plot (Adamson, 1976). This plot was obtained by plotting the cosine of the contact angle of pure liquids and binary mixtures on the solid surface to be studied versus the surface tension of the liquids (Zisman, 1964). To perform the measurement of the contact angle, a syringe was filled with 5 μL of a particular test solution, after it had been cleaned a few times with the solution, and a drop was placed on top of the specimen. The contact angles (±5°) were measured by a goniometer (NRL C.A. Model 100, Rame Hart Inc.) (Bikerman, 1970). All measurements were conducted in an atmosphere of ~60% relative humidity (RH) and 23 °C 10 s after placing each drop on the tested mushroom cap surface followed by a second measurement 60 s later (Bikerman, 1970). The studied liquids included water, glycerol, ethylene glycol (Frutarom Laboratory Chemicals, Haifa, Israel), formamide (Sigma), ethylene glycol E-200, ethylene glycol P-E-15-20 (Dow Chemical, Dow Benelux, N. V. Terneuzen, The Netherlands), and binary mixtures of water:ethanol in concentrations of 90:10, 60:40, and 20:80, respectively (Kaelble, 1967).

Electron Microscopy. To study the tissue structures of the mushrooms, electron microscopy of air-dried mushroom-peel specimens was performed after coating them with gold (thickness 150−200 Å), using a Polaron 5150 sputter coater (Polaron Equipment Limited, Holywall Industrial Estate Watford, Hertfordshire, England) (Forini et al., 1991). Coated and uncoated tissues were observed in a Joel 35C scanning electron microscope (SEM) (Tokyo, Japan). Tissues of air-dried mushrooms were also microscopically studied under low vacuum conditions (10^{-3} Torr). In such cases, no gold coating was needed. The working distance was 20 mm taken at 25 kV.

Detection of Coating Residues on Mushroom Peel by Electron Microprobe Analysis (EMPA). Aurothioglucose (ATG) (5% w/w; Sigma) was dissolved in the coating solution as a tracer prior to the application to mushroom (Banks, 1984a,b; Hershko et al., 1996). Coating was performed as described for 2% alginate. After air-drying, the coating films were peeled from the mushroom surface. The mushroom−tissue specimens were then mounted on plastic stubs and examined using a low vacuum SEM fitted with a backscattered electron detector (BSED) and an energy disperse X-ray spectrometer (XDXS) (Tracer 5500 with Si (LI) window). The intensity of the BSED signal is a function of the mean atomic number of the excited site; therefore, a brighter region in the image modulated by the BSED would indicate the presence of heavier atoms (regions with high gold concentration can be detected). The different elements in the sample (heavier than B) could be unequivocally identified by their characteristic X-ray radiation; thus, the presence of the peaks at ranges of 9.5−9.8, 2.02−2.08, 7.8−8.1, 8.3−8.6, and 11.2−11.6 keV in the X-ray spectrum is a fingerprint of gold.

X-ray analysis was also used to detect barium cations on the cap surface and on the velum surface, both up to a depth of 1 mm. Peaks at a range of 4.3−4.5 keV in the X-ray spectrum are a fingerprint of barium. The results are reported as a percentage of the total amount of dominant minerals in our specimens.

Mineral Analysis by ICP-Atomic Emission Spectrometer. Inductively coupled plasma (ICP of Spectro Analytical Instruments) atomic emission spectrometry (Rezaaiyan and Nikdel, 1990) was used to determine the mineral content of the gum-coating solution after dialysis and the different parts of the mushrooms including the cap, stipe, gills, and outer layer at a thickness of 1 mm. The outer layer of the cap surface and the cap were also examined after the coating film

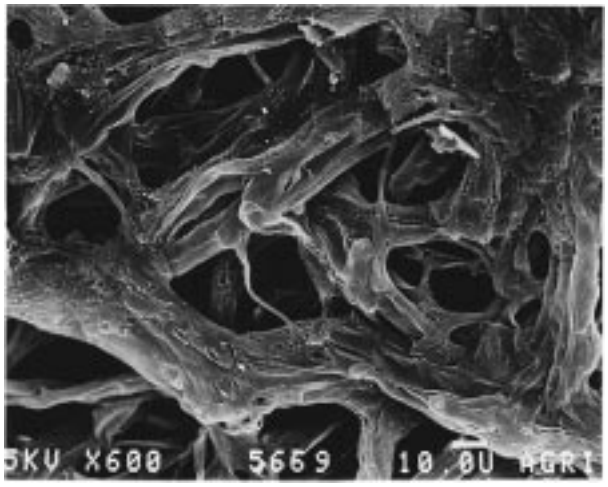

Figure 1. Top view of the surface of *Agaricus bisporus* obtained by low vacuum SEM.

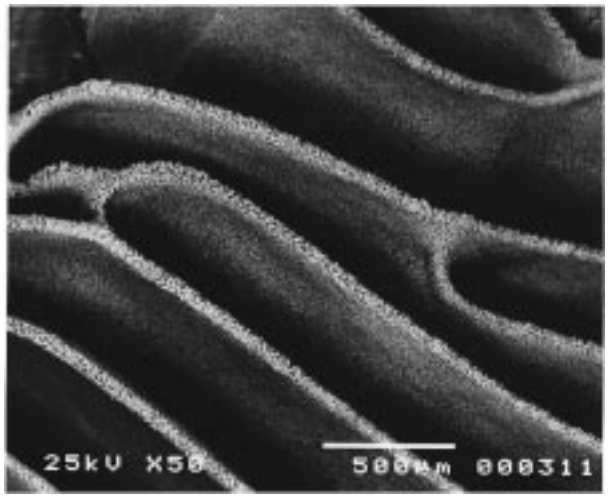

Figure 2. Cross section of *Agaricus bisporus* from the direction of the gills, obtained by low vacuum SEM.

was removed. Films were composed of calcium alginate either with or without ATG (see EMPA analysis).

Predetermined weights of the above-mentioned specimens were digested with 5 mL of concentrated HNO_3 in a microwave oven (Matusiewicz et al., 1991) at 500 W for 5 min and ventilated for 5 more min to reduce the temperature and pressure and to avoid losses. All work was performed in pressurized closed vessels. The total volume was adjusted to 10 mL and flushed as an aerosol into the plasma. Stationary plasma was produced by flushing a quartz tube with argon. The emission intensity at each wavelength was proportional to the concentration of the tested element. Emitted light was collected by optical fibers and analyzed. The results are given in parts per million (ppm). The residues of water in the tested specimens were determined routinely by drying the specimens at 105 °C overnight. The moisture content was calculated from the weight of the dry specimens and expressed as the percentage of original tissues.

RESULTS AND DISCUSSION

The success of coating a fresh vegetable with hydrocolloid-based films depends, among many factors, on the properties and structure of the vegetable surface. *A. bisporus* fruit bodies were chosen as the experimental model for this study since there is almost no information on the relationship between a spongy specimen and a coating system. Since most of the studies regarding edible coatings were performed on nonspongy materials, studying mushrooms seems advantageous. In addition, the mushroom tissue is very sensitive and prone to rapid moisture loss and browning. Therefore, it is an interesting model which provides us with relevant, rapid results. The *A. bisporus* fruit bodies has a unique structure in comparison with other fruits and vegetables. In general, it is possible to look at this mushroom as a spongy material. However, the structure of such a sponge and its porosity vary if the specimen is observed from the direction of its pileus (cap) or from its velum (veil) and lamellae (gills). From the top (pileus), electron micrographs revealed a structure composed of open formidable voids (Figure 1). The size of these voids varied between 100 and 480 μm^2. Such a structure is the outcome of branched interconnected hypha filaments of the fungi. A microscopic study of specimens taken from beneath the cap shows the different textures of the velum and gills. Figure 2 shows the structure of the repeatable elongated gills, which have a cellular structure with unique voids

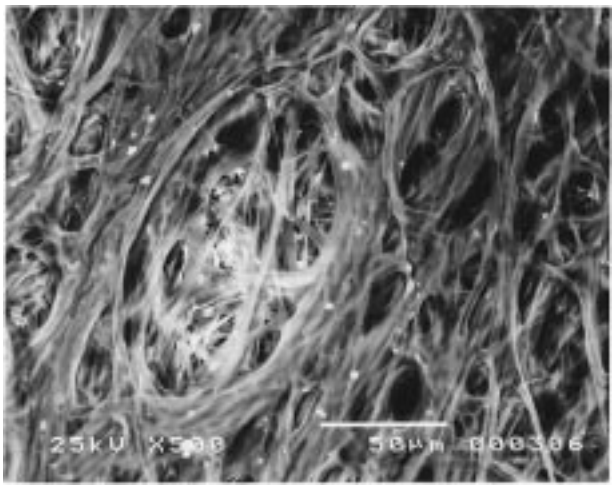

Figure 3. Top view of the velum obtained by low vacuum SEM.

(Alexander, 1978; Jennings and Lysek, 1997). The velum (Figure 3) is composed of a tissue different from the cap and gills. The differences are a consequence of the mushroom's age, stage of maturity, and conditions of storage. Thus, different patterns of liquid penetration into the tissue can be hypothesized (see below). The hyphal membrane is composed of hemicellulose, cutin, and glycogen. The membrane contains hydrophobic materials and sterols (Young, 1995; Newell, 1992). Evidence of the presence of a hydrophobic protein, namely, hydrophobin, which forms hydrophobic rodlet layers on the outer surface of the fruiting body was reported recently (Lugones et al., 1996). This layer may be solely responsible for the nonwettability of the surface of the fruit body. Most of the protein was found within the cap's outer tissue and none in the gills. Another report mentioned the accumulation of hydrophobin in the outer layer of the mushroom cap during fruit-body development (de Groot et al., 1996). If these hydrophobic layers found at the surface of the mushroom reach a condition of intimate contact with the wetting coating gum solution which includes hydrophobic components, a better chemical similarity can be achieved. In other papers (de Bruyne, 1939; Nussinovitch et al., 1994; Nussinovitch and Hershko, 1996), the chemical hydrophobic similarity between the gum-coating solution and the surface to be coated proved to

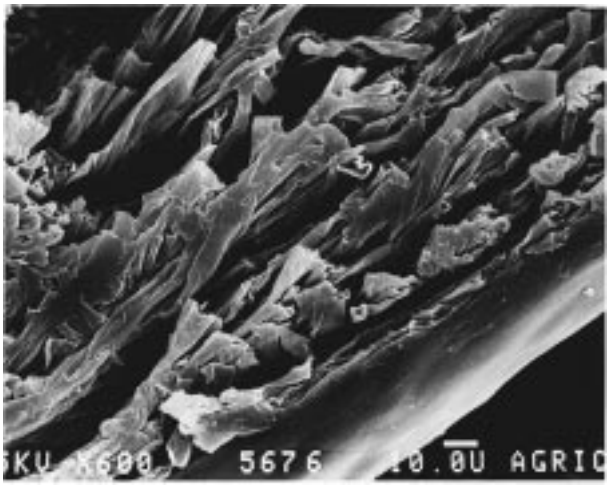

Figure 4. Cross section of alginate-coated mushroom. Coating on the right side.

be most important for the success and adhesion of the coating. For coating purposes, a predetermined insight into the properties of the surface to be coated and the gum used is desirable. Estimation of the critical surface tension of the mushroom's upper surface was therefore performed. As observed, the mushroom tissue is a surface consisting of many voids. Contact-angle measurements were conducted for no less than 10 s; this followed reports by Bikerman (1970) and Allan (1959). Furthermore, measuring the contact angles on a porous surface at a contact time of 0.02−2 s was also reported (Auslander et al., 1997). We observed that the coating solutions were not absorbed into the mushroom even after a few minutes.

Measurements taken after 60 s reveal the same results for contact-angle values; hence, it can be concluded that equilibrium was achieved. It is also logical that biological specimens be examined rapidly to avoid changes in the tested surface due to tissue drying. The cosine of the contact angles obtained previously was plotted versus the surface tension values of the tested solutions. We followed the acknowledged contributions of Kaelble (1967) and Auslander et al. (1977) in which it was concluded that it is possible to properly estimate the critical surface tension of a solid surface by using a nonhomologous series of liquids. Moreover, from preliminary experiments, we concluded that it is not possible to measure contact angles for nonpolar liquids, since they spread spontaneously and are absorbed by the mushroom surface. Linear regression of these data was performed in order to extrapolate the critical surface tension from such a Zisman plot. A critical surface tension of 22.1 ± 2.5 dyn/cm was found (Figure 4). This value is small in comparison with some natural solid surfaces. For example, natural films composed of amylopectin, amylose, starch, and casein were found to have surface tensions of 35.0, 37.0, 40.0, and 43.0 dyn/cm, respectively (Barton, 1983). The critical surface tension of garlic skin was found to be even lower, 18.3 dyn/cm (Hershko and Nussinovitch, 1998b). For wax-coated grapefruit and orange peels, the critical surface tension was approximately 23.0 dyn/cm (Hagenmaier and Baker, 1993), and values of 25.0−28.0 were reported for the leaf surfaces of soybean, corn, and wheat (McKay et al., 1985). Water has a surface tension of 72.8 dyn/cm. Coating gum solutions which are water-based, containing about 98% water, therefore have a high surface tension value. If the low surface tension value found for the mushroom surface (22.1 dyn/m) is also considered, the detection of a suitable water-based coating gum solution can be complicated. The surface tension of the coating solution (γ_{LV}) can be adjusted to the solid's surface tension by lowering its value. The surface-tension values of the specific combinations of solutions used for coating are listed in Table 1. The dissolution of gum in distilled water usually decreases the surface tension of the water and leads to a decrease in the surface tension of the solution. Since efficient coating involves good compatibility between the liquid and solid surface tensions (Wu, 1973), our aim was to reduce the surface tensions of the coating solutions, to adjust them to the lower surface tension of the fruit or vegetable surface, and to improve wettability (Wu, 1973). A 29% decrease in the surface tensions of water was observed after dissolution of 2% alginate in it (Table 1). Alginate, as other available hydrocolloids, has the potential of lowering the surface tension of solutions usually designated for use as coating agents (Gaonkar, 1991). Incorporation of ergosterol, Tween, or both into the alginate solution yielded a further reduction of about 7.8−15.8% in surface tension (Table 1). The contact angles of hydrocolloid solutions on the mushroom cap surface as determined by a goniometer are also listed in Table 1. The addition of ergosterol, Tween, or both yielded a reduction of ∼27% in the contact angles in comparison to alginate alone. No significant differences in contact angles of solutions containing components other than alginate were observed. Thus, all of these combinations contribute to a better spreading on the mushroom tissue. It is known from the literature (Wu, 1979) that there is some difference between the solid surface tension and the critical one. However, in this case, if the interfacial tension is calculated taking into account the value of the critical surface tension, small negative values would be detected. Such small deviations toward negative values were experimentally reported for much more homogeneous systems (Schoff, 1992). In addition, if higher surface tension values are to be evaluated, as in the case of most reports (Wu, 1979), then the calculated interfacial tension would have higher, positive, and more common values. The decrease in surface tension and contact angles resulting from the addition of ergosterol, Tween, or both to the alginate solution could hypothetically yield a better wetting of the coated surface (Mittal, 1977). Elimination of dewetting (Schoff, 1992) is presumably much more important from a commercial point of view, especially when all our tested combinations adhered to the mushroom surface and later dried to achieve fixation of the coating. Microscopic observation shows that the coating is in contact with the mushroom tissue (Figure 5). Adhesion strengths cannot be experimentally stud-

Table 1. Surface Tensions and Contact Angles of Coating Solutions[a]

solution	surface tension, dyn/cm	contact angle, deg
2% alginate	51.5	55 a
2% alginate + 0.2% ergosterol	28.4	40 b
2% alginate + 0.25 mL of Tween 80	32.5	42 b
2% alginate + 0.2% ergosterol + 0.25 mL of Tween 80	31.8	41 b

[a] Each result is the mean of five determinations. Different letters within a column indicate significant differences at $P < 0.05$.

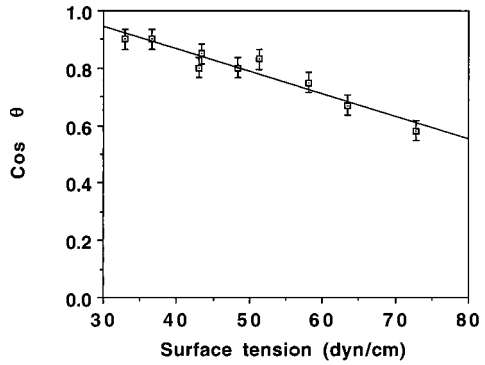

Figure 5. Zisman plot for mushroom's outer layer.

ied in our case, since the dry film involved is very thin and closely glued to the mushroom tissue; therefore, very delicate equipment, which is not commercially available, should be produced, especially for such future tasks.

To estimate what role the gum solution plays during the coating process, a further understanding of the ultrastructure and surface properties of the mushroom is necessary. It has been previously hypothesized that the mushroom cap is covered with a monolayer of water molecules (Fockens and Meffert, 1972). The thin layer of water encourages the introduction of the gum-coating solution into the mushroom. Ergosterol is present in the hyphal membrane (Goulston et al., 1975; Newell, 1992), constituting the major surfaces with which the hydrocolloid solution comes in contact when the coating process takes place. As the mushroom is immersed in the bath (see Materials and Methods), and after a short delay, the low viscous gum solution penetrates the spongy texture and enters the open voids. Since the velum has a unique structure that presumably eliminates penetration of liquids, most of the penetration, if not all, occurs from the side of the pileus. If the gum or another liquid succeeds in penetrating the velum when the latter is damaged or opened, then easier penetration of the gills is possible due to the larger sizes of the voids (Figure 3). The low viscous gum solution slides along the hyphal tubing, wetting it and adhering to its surfaces.

The amount of gum solution that penetrates into the mushroom tissue depends mainly on the gum's physical and chemical properties. Its viscosity, surface tension, flow pattern, hydrophobic−hydrophilic nature, osmotic pressure, and other factors related to these properties are important in trying to explain the complexity of a "simple" coating of a porous vegetable by a gum solution.

From previous studies (Hershko et al., 1996), it is well-known that it is easier to follow traces of migrating minerals within the coated object than to detect absorbed hydrocolloids within the vegetable tissue. For example, the simple microanalysis of alginate residues by NaOH (Ewart and Chapman, 1952) is not possible here, because the alginate and the mushroom tissue become yellow after this reaction. Another study by Banks (1984a) hints that ATG has a migrating capacity presumably similar to that of the gum in which it is immersed for vegetable coating. We followed this approach for an X-ray analysis and ICP techniques and observed the penetration of the gold compound into the mushroom tissue in order to obtain an estimation of the presence of alginate within the mushroom tissue after the coating. The mineral contents of the coating solution and the mushroom's cap (pileus), stipe, gills, and the outer layer of the mushroom's cap were analyzed by ICP. The outer layer of the mushroom was also studied after manually removing the alginate coating or the alginate−gold films. The dominant mineral concentration ranges in the cap, stipe, outer layer (1 mm thick), and gills were 37 300−63 600, 2 872−4 400, and 10 770−17 810 ppm for K, S, and P, respectively, for the four above-listed parts of the mushroom (Table 2). The total mineral content was more or less the same within the cap, stipe, and gills and lower in the velum. The mushroom's outer layer, which had been coated and manually peeled, contained a few minerals which can also be found in the mushroom before coating. The greatest differences in quantities were observed for Na and Ca. It is assumed that the excess in sodium concentration is related to its presence as a part of the alginate salt. The origin of Ca is presumably from the cross-linking salt solution. Thus, a possible passage by diffusion of minerals after the coating process is evidenced.

Two different modes to achieve cross-linking of the gum via coating film production were examined. In the first, the mushroom was immersed in calcium solution before immersion in the alginate solution. In the second, the fruit body of the mushroom was first introduced into the gum solution and then into the cross-linking bath. It is not surprising to find, in general, a high mineral content within the mushroom tissue when immersion in the mineral bath preceded immersion in the gum solution. After removing the coating gelled film and the outer layer (i.e., an outer specimen that was taken from a depth of 1 mm under the mushroom surface), trying to detect mineral traces in this layer as well as in the remaining mushroom, it was observed that the remaining mushroom tissue (cap) contained a higher level of minerals than the uncoated commodity. From these data, it can be concluded that minerals from the gum-coating solution penetrated the mushroom tissue. When ATG was introduced either into the alginate or into the cross-linking solution, its residues were found on the surface from which the coating had been peeled for both modes of application mentioned above. As a result of ATG addition either to the gum or to cross-linking solution in the two modes of immersion, four combinations by which gold can penetrate the mushroom tissue are possible. The concentration of gold detected by the ICP was higher when ATG was introduced into the alginate solution than when it was introduced into the calcium solution. In other words, a value of 827 ppm was obtained when the specimen had been immersed in alginate solution before immersion in calcium solution and a value of 496 ppm was obtained when the specimen had been immersed in calcium solution first. When ATG was introduced into the cross-linking solution, the detected values of gold were 75 ppm for immersion in alginate solution prior to immersion in calcium solution and 273 ppm for immersion in calcium solution first. These findings are possibly due to the fact that direct contact between the liquid (either gum or cross-linking solution containing ATG) and mushroom tissue achieves a higher gold content within the mushroom tissue. It is not clear, however, why the addition of ATG to alginate solution always yielded a higher gold concentration. X-ray analysis was applied to examine whether coating residues had penetrated into or had been absorbed by the outer layer of the "skin" during wetting and gelation

Table 2. Element Content in Alginate Solution and Different Parts of the Mushroom (Outer Layer Peeled from Coating Film and Cap under the Peeled Surface) Calculated According to ICP Results and Given in ppm

mineral	alginate[a]	cap	peel	stipe	gills	cap[b]	peel[c]	peel[d]
Ca	37.1	301.0	531.0	308.7	336.3	918.2	6 250.0	4 929.2
Ti	0	0.4	1.4	1.1	1.0	1.0	2.8	0.6
Cu	0	45.7	44.4	54.7	82.8	49.6	41	51.9
Mg	2.7	1 136.0	1 177.0	1 383.0	2 391.0	1 185.6	936.8	989.0
Fe	0.2	40.3	5.5	32.7	42.4	47.3	65.9	61.8
Si	0.2	29.4	26.9	31.4	7.1	12.0	22.8	16.5
Ni	0.7	0	0	0	0	0	0	7.0
Cd		0.9	0.8	0	0.63	0	0	1.5
Pb	0.1	11.8	18.3	42.6	27.2	8.7	47.7	75.6
Zn	0.3	80.4	83.7	95.9	152.7	10.8	103.1	128.6
Ag	0	0.5	0.2	0.51	0	0	0	1.1
V	0	0	0.5	0	0	0.4	3.3	2.5
Cr	0	1.1	0.7	1.39	1.4	1.3	4.1	2.8
Mn	0	4.4	5.4	6.03	10.0	4.7	2.6	2.9
K	2.8	63 600.0	51 300.0	56 200.0	37 340.0	58 578.3	55 902.4	51 131.1
Na	419.6	909.0	855.0	961.0	613.0	6 888.1	2 238.2	1 741.9
Ba	0.2	0.1	0.2	0.2	0.2	0.7	0	0.4
Sr	0.5	1.1	1.5	1.3	1.4	1.3	2.6	2.9
Md	0	1.5	1.1	1.2	2.2	1.9	4.4	0
Se	0	6.8	5.5	5.5	7.8	7.6	18.3	1.5
As		0.9	0	0	1.6	3.8	0	0
Sn		0.8	0.5	1.1	0.6	0.2	0	0
B		3.0	1.3	0.5	3.0	0	0	0
S	4.5	2 872	3 482.0	3 147.0	4 400.0	3 566.2	3 555.5	3 698.1
P	0.1	10 770.0	11 670.0	11 340.0	17 810.0	13 958	10 069.4	10 287.1
Al	0.3	16.9	37.4	17.7	2.9	12.6	30.2	32.6
Bi		11.5	10.7	2.7	15.9	11.9	12.4	10.8
Sb		51.2	48.3	30.5	37.4	32.4	33.5	30.8
Rb		14.3	10.9	15.8	18.0	14.6	13.8	15.0
Zr		700	750.0	817.0	1983.0	670.0	760.0	720.0
total	469.3	80 611.0	70 070.1	74 499.2	65 288.5	85 987.2	80 120.8	73 943.2

[a] Alginate solution after dialysis at final concentration of 2%. [b] Cap after manually removing the alginate coating. [c] Outer layer at thickness of 1 mm taken after removal of coating which was first dipped in 2% $CaCl_2$. [d] Outer layer at thickness of 1 mm taken after removal of coating which was first dipped in 2% alginate.

followed by drying. An outer layer with a thickness of 2 mm cut from the mushroom surface was tested for its main minerals, namely, potassium, chlorine, phosphorus, sulfur, calcium, and sodium; these were detected at concentrations of 58.4%, 16.0%, 15.2%, 8.5%, 1.4%, and 0.4%, respectively. No gold residues were detected in the precoated mushroom. Other minerals could be found only at levels <0.1% and were not detected by the X-ray. Their presence is evidenced by the ICP results.

Separate determinations of deeper tissue resulted in finding similar proportions of minerals within the mushroom, with no gold residues at all. After coating the mushroom with alginate−gold solution and cross-linking it with $CaCl_2$ and after the coating had been carefully removed from the surface followed by microscopic observation showing no film left, electron microprobe analysis was used to detect gold residues. Gold was clearly detected at a depth of 1.4 mm but not at deeper regions. An X-ray spectrum of the mushroom's outer layer (Figure 6) revealed the presence of gold represented by the lines between 9.5 and 9.9 keV. The dominant minerals detected on the surface by this analysis were K, Cl, P, S, Ca, Na, and Au at levels of 42.31%, 23.28%, 10.07%, 6.87%, 3.65%, 5.24%, and 8.58%, respectively. Detection of minerals at a depth of 1.4 mm showed the same minerals at percentages of 50.27%, 24.17%, 7.91%, 4.71%, 3.22%, 2.95%, and 6.77%, respectively.

To follow the possible penetration paths of gold into the mushroom, we examine both the outer and the inner surfaces of the cap and velum. The mushrooms were first immersed in an alginate−gold solution and then in the $CaCl_2$ solution. After about 1 h, the gelled film

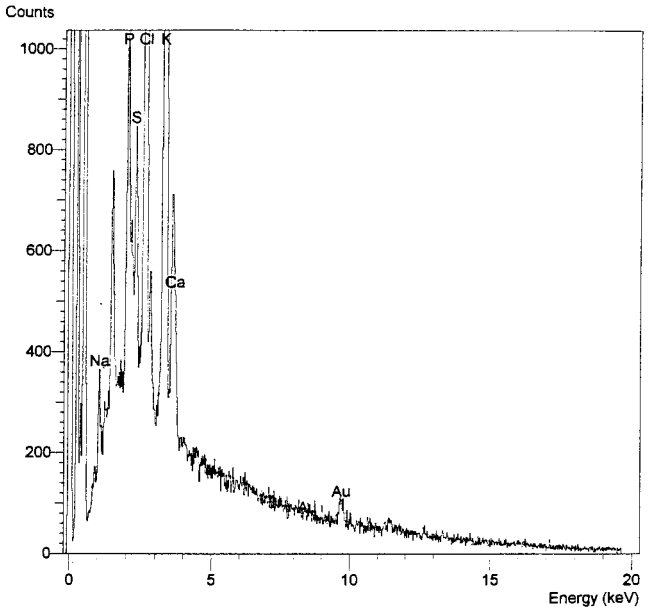

Figure 6. X-ray spectrum of the surface of the outer layer of the mushroom cap. Au peaks in the range of 9.4−9.9 keV.

was peeled and the tissues were observed by X-ray analysis. Each specimen was taken from a depth of 2 mm. Both external surfaces, of the cap and of the velum, contained the same concentration of about 500 ppm Au, decreasing by some 11% for the inner side of the cap. In the inner side of the velum, no gold was detected. This is partial evidence for the different nature of the velum as a barrier to diffusion, which can delay or prevent gold penetration. Noting Banks' ap-

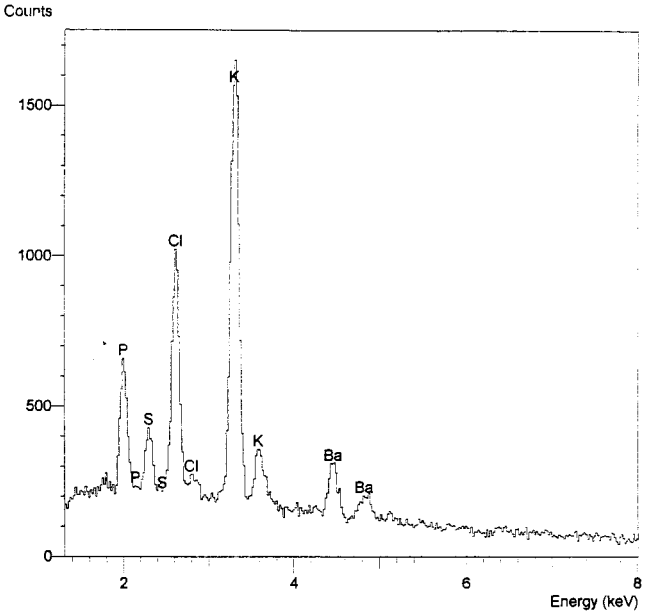

Figure 7. X-ray spectrum of the surface of the outer layer of mushroom cap. Ba peaks in the range of 4.4−4.6 keV.

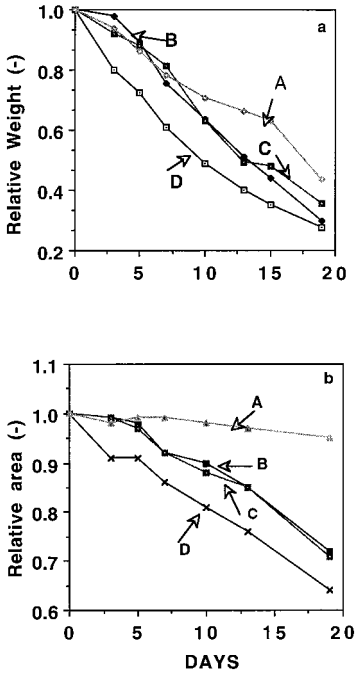

Figure 8. (a) Relative weight loss and (b) relative area loss of coated and uncoated mushrooms during 19 days of storage. A, alginate + ergosterol + Tween; B, alginate; C, alginate + ergosterol; D, control.

proach (1984a), minerals within the gum solution that either cannot be found or are in minor quantities within the vegetable tissue are an indicator of gum penetration (Hershko et al., 1996), although additional quantitative tools are required to study these effects. Alginate can be found where gold is detected. The cross-linking reaction by the calcium cations of the possibly penetrated alginate solution creates a thick three-dimensional layer of calcium alginate gel within the structure of the coated mushroom. When this inner three-dimensional matrix is formed, mushrooms are more resistant to drying processes. In fact, it was observed that such coated mushrooms preserved their initial volume in a better manner than the untreated ones, since the three-dimensional gel casing that was formed slowed the contraction of the mushroom tissue and, therefore, its regular collapse was somehow avoided.

To obtain an estimation of the presence of minerals originating from penetration of the cross-linking solution into the mushroom surface, detection of barium residues (used as a cross-linking agent instead of calcium) after coating was performed. Natural mushroom cap contains about 0.0024 ppm barium, which is insignificant in comparison to the calcium concentration and is less prone to natural fluctuations than calcium. Except for this fact, barium was chosen because it yields the strongest gel by alginate cross-linking (Stokke et al., 1993). The created barium gelled film was peeled, and X-ray analysis was used to detect barium residues on the surface. Barium peaks were observed at the range of 4.3−4.6 keV, and its concentration was about 4.9−6.0% of the total mineral content (Figure 7). There are several reports in the literature regarding storage conditions for fresh mushrooms. These include RHs ranging between 80% and 95% at 2−4 °C (Lopez-Briones et al., 1993; Beit-Halachmy and Mannheim, 1992) and 40−50% at 18 °C (Nichols and Hammond, 1973). It was reported that mushrooms that were contained within a sealed package such as stretchable PVC, polyethylene, or oriented polypropylene (Lopez-Briones et al., 1993) at RH 95% suffer several problems: undesired color (browning) and texture changes, condensation of water vapor on mushroom surface and packages expansion of the pileus, and microbial contamination, which leads to decreases in quality and shelf life (Kuyper et al., 1993; Lopez-Briones et al., 1993). In our experiments, we stored mushrooms in ca. 70% RH in order to accelerate water evaporation from these fruit bodies and, thus, to detect the advantages of the gum-dried coating. Evaporation of water from the coating gelled film enrobing the mushroom continued during storage. Gradually, the rate of water evaporation from the produce slowed.

We used low RH conditions in a way parallel to other experiments done by Roy et al. (1995a,b) to reduce the content of condensed water on the mushroom surface and to improve its visual appearance (color). The alginate sterol coating contributed to the quality of the fresh coated mushrooms, especially in these fields where disadvantages of storing in high humidities were noticed.

As mentioned in the Materials and Methods section, coated and uncoated mushrooms were stored at 2−3 °C and 70% RH. Uncoated mushrooms lost about 20.1%, 27.4%, and 39.2% of their original weight during the 3, 5, and 7 days of storage, respectively (Figure 8a). Mushrooms coated with 2% alginate lost about 2.2%, 10.8%, and 24.3% during their 3, 5, and 7 days of storage, respectively.

To change the hydrophobicity of the coating and to obtain a chemical similarity between the coating solution and the outer mushroom tissue, as has been proposed before (Nussinovitch and Hershko, 1996), ergosterol was incorporated into the coating solution. Of course, other hydrophobic compounds can be used as well, however ergosterol is commercially extracted and therefore available. During the entire experimental period (19 days), alginate- and alginate−ergosterol-coated mushrooms kept an almost constant advantage of 20% in weight over uncoated mushrooms. Alginate−ergosterol−Tween behaved similarly for about 7 days and later improved its performance, reaching an advantage of up to 48% in weight. This is not surprising,

Table 3. *L of Coated and Uncoated Mushrooms during 19 days of Storage**

	*L** value at							
solution	0 days	3 days	5 days	7 days	10 days	13 days	15 days	19 days
control	87.32 ± 2.87 a	87.15 ± 1.90 a	85.75 ± 1.98 a	76.13 ± 3.50 a	68.22 ± 4.00 a	63.69 ± 3.01 a	63.01 ± 3.22 a	63.38 ± 2.82 a
alginate	88.13 ± 2.76 a	84.59 ± 5.40 a	86.47 ± 2.85 a	82.58 ± 6.27 a	80.63 ± 6.52 bc	77.33 ± 6.55 bc	75.60 ± 7.38 b	74.72 ± 6.89 b
alginate + ergosterol	86.85 ± 2.98 a	84.90 ± 2.56 a	84.15 ± 3.32 a	80.10 ± 7.29 ab	74.95 ± 7.29 ab	72.80 ± 7.69 b	69.36 ± 7.15 b	66.68 ± 5.32 ab
alginate + ergosterol + Tween	87.29 ± 1.68 a	84.74 ± 2.58 a	83.82 ± 5.41 a	84.81 ± 2.96 b	82.94 ± 5.11 c	82.42 ± 3.35 c	83.74 ± 1.93 c	80.42 ± 6.40 c

[a] Results are the average of at least 20 determination ± SD. [b] Different letters within a column indicate significant differences at $P < 0.05$.

because the addition of the hydrophobic compound to the film probably resulted in reduced permeability to water vapor (Martin-Polo et al., 1992). In comparison to a previous study (Nussinovitch and Kampf, 1993), the weight advantage of 2% alginate-coated mushrooms after 6 days of storage at 4 °C was less than 2%. Therefore, the addition of ergosterol is of great importance. The emulsifier, apart from its possible contribution to better spredability of the coating solution on the mushroom cap, can also act as a mediator to the naturally hydrophobic surface of the mushroom (Lugones et al., 1996). After harvest, mushrooms are sold for a few days before a major decrease in their appearance occurs. After 3 days, alginate-coated mushrooms lost ~2.2% moisture in comparison to 1.8% moisture loss (Nichols and Hammond, 1973) from mushrooms stored at 2 °C and 85−95% RH for 5 days. After 5 days, weight loss increased in comparison to the results achieved for the mushrooms stored at 95% RH. Since fresh mushrooms lose their cap shape as a result of contraction, the changes in the cap's projection area during the experimental period were measured. The advantage of coating in this aspect is shown in Figure 8b. Uncoated mushrooms lost between ~7.5% and 35.7% of their cap-projection area during 3−19 days after harvest, respectively. Alginate-coated mushrooms lost between 2.0% and 28% of their cap-projection area during 3−19 days after harvest, respectively. During the first 3 days of storage, alginate−ergosterol-coated mushrooms lost 0.004% of their initial projection area. After 19 days of storage, the uncoated mushrooms lost about 83% of their initial weight, and a 36% reduction in their cap-projection area was observed. Alginate−ergosterol−Tween coating was found to be most efficient in comparison to the other coatings; thus, a reduction of 64% in weight and 5% in cap-projection area were observed. These factors are important for fresh and semidried mushroom commercialization. It is important to note that water loss as was done in this study is time and money consuming; however, such treatment results in a quality semidried product. Such a product may be the result of drying of the three-dimensional alginate−gel structure created (as discussed) at a depth of 1−1.4 mm under the mushroom's outer layer. Dried, partially collapsed gel would create rigid porous structures (Rasis et al., 1997), which in turn would help the mushroom maintain a shape resembling that of the whole fresh product. Since slices of dried mushroom are very dark, i.e., have an *L** value of 45 (Yapar et al., 1990), there may be demand for whiter and whole semidried mushrooms if such products are available. The color of mushrooms is an important factor in their salability. Therefore, studying color changes is most important for commercial purposes. Numerical *L** (lightness−darkness) values with time are shown in Table 3 (the higher the *L** value, the lighter the color). At room temperature, bruised or cut mushrooms lose their white color due to undesirable discoloration caused by the enzyme polyphenol oxidase (Gray, 1970). The advantages of the coated versus the uncoated mushroom can be seen in Table 3. During the first 5 days, no significant changes in *L** values for the studied mushrooms were observed. After 7 days, the uncoated mushrooms' *L** value decreased by 12.8% and the contribution of the coating to a better appearance could be observed for the first time. The most efficient coating presented a minimal reduction of 2.8% in *L** value during the same period. The alginate−ergosterol−Tween coating had the highest *L** values and, as such, was the best of the four tested treatments. As expected, the uncoated commodity showed the most pronounced browning. A higher *L** value and a better appearance during the period of 7−19 days were observed for all the coatings in comparison to the uncoated commodity. After 19 days and for the uncoated mushrooms, the *L** value was reduced by ~27.4% versus 7.8% for the alginate−ergosterol−Tween coating (Table 3). Changes in hue (*a**) and chroma (*b**) were also monitored. The *a** value of the uncoated mushrooms increased by ~80.8% with time. The *a** value of the alginate−ergosterol−Tween-coated mushrooms increased by 27.6% during the same time. For uncoated mushrooms, *b** increased by 42.6%, whereas for the efficient coating (alginate−sterol−Tween), an increase of 29.3% was detected. Since mushrooms are whitish, changes in the *L** value seem to be most closely related to our perception of their color and, consequently, to their marketing qualities. For *a** and *b**, all the presented values are within the range of gray color; thus, the changes that occurred also influenced the mushroom toward darker tones.

CONCLUSIONS

The use of hydrocolloid solutions for coating mushrooms is beneficial for the extension of the shelf life of these commodities. The coating results in a better appearance and color for the coated mushrooms. The dry gum coating also preserves the mushroom's cap-projection area so that it remains closer to the dimensions of the fresh produce. Furthermore, the coating decreases weight losses for prolonged periods in comparison to regular commercial storage. Fine adjustment of the coating solution, study of the delicate structure of the coated commodity, and application of methods adapted from the fields of adhesion, synthetic color, coating, and the glues industry will help the biologists, food technologists, growers, and researchers to better understand and control the coating of fresh produce and other food products.

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