OPTIMIZATION OF AN ALGINATE BASED EDIBLE COATING WITH BEESWAX, NISIN AND EDTA TO MAXIMIZE SHELF LIFE OF FRESH MUSHROOMS (Agaricus bisporus)

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ABSTRACT

Fresh white button mushroom (*Agaricus Bisporus*) is a highly perishable crop with limited shelf life. Enzymatic browning, bacterial spoilage and high respiration rate are the main causes of quality deterioration. Calcium alginate coatings with lipids, antimicrobial, and chelating agents can delay quality deterioration and extend shelf life. The three objectives of this thesis were to evaluate the effect of three coatings: alginate (A), alginate containing beeswax (AB), and alginate containing beeswax, nisin and Na$_2$EDTA (ABN) on the quality of fresh white button mushrooms; to determine the effect of alginate, beeswax, nisin and Na$_2$EDTA concentration on the quality of fresh white button mushroom as measured by color change ($\Delta L^*$), weight loss (WL), degree of cap opening (MI) and bacterial growth (BG); and to optimize the coating composition that maximizes shelf life using response surface methodology.

As part of the first objective of this thesis, mushrooms were coated with A, AB and ABN and stored at 12°C/60%RH for 16 days. After 8 days of storage, mushrooms coated with ABN had significantly (p<0.05) lower $\Delta L^*$ as compared to uncoated control (C). Coatings AB and A had significantly (p<0.05) lower $\Delta L^*$ as compared to C after day 14 and 16 respectively. No significant difference (p>0.05) was found in the rate of WL compared to C. Mushrooms coated with A, AB and ABN had significantly (p<0.05) lower MI as compared to C. Mushrooms coated with ABN had significantly (p<0.05) lower BG during the early days of storage. Overall, shelf life of mushrooms coated with ABN was extended by 6 days. These results indicate that edible coatings can be used to extend shelf life of fresh mushrooms.

As part of the second objective of this thesis, mushrooms were coated with various concentrations of alginate, beeswax, nisin and Na$_2$EDTA as determined by a central composite experimental design, and stored at 12°C/60%RH for 16 days. Alginate significantly (p<0.01) reduced the rate of BG throughout storage. Beeswax significantly decreased $\Delta L^*$ (p<0.01) and BG (p<0.05) after 6 days in storage, but increased WL
(p<0.10). Na$_2$EDTA significantly reduced $\Delta L^*$ (p<0.05) and BG (p<0.01), while nisin significantly decreased $\Delta L^*$(p<0.10) and MI (p<0.01) during days 0-6. Nisin also increased WL significantly (p<0.05) throughout storage, but significant (p<0.01) interactions indicated that at high concentrations of Na$_2$EDTA and alginate, this effect was reduced.

As part of the third objective of this thesis, response surface methodology (RSM) was used to find the optimal concentration of each compound to maximize shelf life by reducing $\Delta L^*$, MI and BG. The optimal coating contained 2.49% alginate, 0.82% beeswax, 8.18mg/mL Na$_2$EDTA and 4000IU/mL nisin. In a validation study, $\Delta L^*$ and MI measurements of mushrooms with the optimal coating were compared to those of the uncoated control. The optimal coating significantly (p<0.05) reduced $\Delta L^*$ and MI, extending the shelf life 7 days past that of the control.
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To my family
Chapter 1

RATIONALE AND OBJECTIVES

1.1. Rationale

White button mushrooms (*Agaricus bisporus*) are highly perishable and their shelf life is limited when sold as fresh produce. Over 800 million pounds of white button mushrooms were produced in the United States between 2008-2009, from which 85% were sold fresh (USDA 2009).

In 2009, mushrooms ranked 13th among the 20 most consumed raw vegetables in the United States according to the Food and Drug Administration (CFR 2009). Shelf life extension technologies such as antimicrobial wash, modified atmosphere packaging, humidity controlled packaging, irradiation and edible coatings have been proposed to extend the shelf life of mushrooms (Burton and others 1987; Nussinovitch and Kampf 1993; Sapers and others 1994; Roy and others 1995a; b; 1996; Beelman and Duncan 1997; Gautam and others 1998; Barron and others 2002; Beelman and Demirci 2007). However, the coating of mushrooms with biopolymers as carriers of antimicrobial agents has not been explored.

The use of edible coatings has been considered as a potential approach in response to the increasing demand for fresh and minimally processed foods in the food retail industry (Lin and Zhao 2007). The crucial quality attributes in fresh produce are appearance, color, texture, flavor, nutritional value, and microbial safety; edible coatings have been proven to preserve these quality attributes by the regulation of moisture and aroma transfer, and oxygen and carbon dioxide equilibrium (Lin and Zhao 2007). The capability of edible coatings to carry functional ingredients, such as antimicrobials and
antioxidants, help to achieve microbial safety and color preservation of fresh produce (Cuppett 1994).

1.2. Hypothesis and objectives

The hypothesis of this study was that an alginate based edible coating containing beeswax, a lipid, nisin, an antimicrobial, and Na₂EDTA, a chelating agent, would extend the shelf life of fresh white button mushrooms (Agaricus bisporus).

The objectives of this study were:

- To evaluate the effect of three coatings: alginate, alginate containing beeswax, and alginate containing beeswax, nisin and Na₂EDTA on the quality of fresh white button mushrooms (Agaricus bisporus) as measured by color change, weight loss, degree of cap opening and microbial growth.

- To determine the effect of the concentration of alginate, beeswax, nisin and Na₂EDTA on the quality of fresh white button mushroom (Agaricus bisporus) as measured by color change, weight loss, degree of cap opening and bacterial growth.

- To optimize the coating composition that maximizes shelf life using response surface methodology.
References


Chapter 2

LITERATURE REVIEW ON SHELF LIFE EXTENSION OF FRESH MUSHROOMS (*Agaricus bisporus*)

2.1. Introduction

The white button mushroom (*Agaricus bisporus*) is a highly perishable crop with a limited shelf life when sold as fresh produce. Figure 2.1. shows sales of white button mushrooms in the United States over the past years. In 2009-2010 period 777 million pounds of white button mushrooms were produced, from which 86% were sold as fresh mushrooms (USDA 2010).

Figure 2.1. *Agaricus* mushrooms sales in the U.S.A. by type and percent of total

Source: (USDA 2010)
According to the Food and Drug Administration, mushrooms ranked 13th among the 20 most consumed raw vegetables in the United States (CFR 2009). Several technologies such as modified atmosphere packaging, humidity controlled packaging, irradiation and antimicrobial wash have been proposed to extend the shelf life of mushrooms (Burton and others 1987b; Sapers and others 1994; Roy and others 1995a; b; 1996; Beelman and Duncan 1997; Gautam and others 1998; Barron and others 2002; Beelman and Demirci 2007).

2.2. Quality parameters of fresh white button mushroom (*Agaricus bisporus*)

White button mushrooms are composed of a thread-like cell structure called hyphae which is compressed together to form fruiting bodies. As a living organism, the mushroom grows, respires and senesces and the rate at which these changes occur affects directly it’s quality and shelf-life (Nichols 1985). As a result, one of the main objectives in the research of mushroom’s post-harvest physiology is to change the rate of these physiological processes to improve the mushroom quality and extend shelf life (Nichols 1985). The main parameters that affect the quality of mushrooms are: color, stipe growth, sporophore development, firmness and water content. (Guthrie 1984)

2.2.1. Color

One of the most important quality factors in *Agaricus bisporus* is the degree of whiteness (Gormley 1975). The hyphae of white button mushrooms is colorless or translucent but appears white. However, as mushrooms age they suffer enzymatic browning (Nichols 1985). Enzymatic browning is an enzyme catalyzed oxidation reaction of phenolic substrates into quinones, which later undergo oxidation and polymerization resulting in dark pigments called melanins (Jolivet and others 1998). Mushrooms contain copper oxygenases, also called polyphenol oxidases (PPOs: laccases...
and tyrosinases) and peroxidases (Jolivet and others 1998). The main enzyme responsible for browning of the sporophore is tyrosinase, given that laccase has a very low activity in the fruiting body (Turner 1974). At the developing stage of the fruiting bodies, tyrosinase and the substrate are kept apart by membrane boundary layers in the cells. However, after harvest the mushroom processing and handling causes tissue damage which allows the enzymes and substrate to come in contact and start the reaction (Nichols 1985).

The color of white button mushrooms can also be altered by bacterial contamination, *Pseudomonas tolaasii, P. reactans* and *P. gingeri* have been identified as responsible for post-harvest discoloration (Wells and others 1996). The brown blotch disease caused by *P. tolaasii*, a biotype of *P. fluorescens* is the most studied (Jolivet and others 1998), this disease manifests itself as pale yellow spots that can readily turn rich brown (Gandy 1985), the spots may be 1-4 mm in diameter at the beginning, then turn darker and sunken, coalescing to cover the entire cap surface (Soler-Rivas and others 1999). *P. tolaasii* produces the lipodepsipeptide tolaasin, an extracellular toxin that has the ability to disrupt the membranes of fungal, bacterial, plant and animal cells by the formation of an ion channel (Brodey and others 1991). The membrane disruption allows tyrosinase to come in contact with phenolic substrates causing the oxidation reaction (Jolivet and others 1998). Tolaasin has been shown to activate tyrosinase, in a study where two susceptible strains and a resistant strain to bacterial blotch were infected with *P. tolaasii* or with tolaasin, the amount of tyrosinase level increased with the increase of bacterial concentration or toxin concentration in the susceptible strains, and remained unchanged in the resistant strain (Soler-Rivas and others 1997).

Six whiteness grades were defined by a panel discussion in a study, relating the panel description of whiteness with an L value as shown in Table 2.1. In this study the retailer acceptability and the consumer acceptability were defined as $L = 80$ and $L = 69-79$ respectively (Gormley 1975).
Table 2.1. Whiteness categories for mushrooms

<table>
<thead>
<tr>
<th>Category</th>
<th>Hunter L value</th>
<th>Panel description of whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;93</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>90-93</td>
<td>Very good</td>
</tr>
<tr>
<td>3</td>
<td>86-89</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>80-85</td>
<td>Reasonable</td>
</tr>
<tr>
<td>5</td>
<td>69-79</td>
<td>Poor</td>
</tr>
<tr>
<td>6</td>
<td>&lt;69</td>
<td>Very Poor</td>
</tr>
</tbody>
</table>

Source: (Gormley 1975)

2.2.2. Stipe growth

The stipe (stem) of the white button mushroom keeps growing after harvest. It is believed that chitin content increase is related to the post-harvest stipe elongation given that the level of chitin synthase is high during the maximum stipe elongation stage and it decreases at the end of elongation (Gooday and Deroussethall 1975) (Hammond 1979). It was earlier believed that the stipe elongation was due only to cell expansion and not to cell division. However, a study later proved that cell division occurs in the upper part of the stipe, below the veil, which is the most rapidly expanding region (Craig and others 1977).

A study showed that by trimming the stipe of mushrooms from 35 to 5mm from the cap, enzymatic browning and cap opening was reduced and thus shelf life was increased. Mannitol is the main substrate for after-harvest respiration. Given that mannitol content on the stipe is very high (28% of dry weight), trimming of the stipe reduced the respiratory substrate, and physiological changes were slowed down (Ajlouni and others 1992).
2.2.3. Sporophore development

Hammond and Nichols described the appearance of sporophore development in 7 stages according to cap growth, opening and gill exposure, being 1 the “pin”, 2 the “button” with no cap opening, and 7 the cap completely open and flat gill surface as shown in Figure 2.2 (Hammond and Nichols 1976).

![Figure 2.2. Appearance of sporophore development](source: Hammond and Nichols 1976)

Guthrie described the sporophore development as a post harvest maturity index, where the mushroom was assigned a score from 1 to 7 according to the cap opening and gill exposure as shown in Figure 2.3. (Guthrie 1984)
<table>
<thead>
<tr>
<th>Stage</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description of Sporophore</strong></td>
<td>Veil intact (tight)</td>
<td>Veil intact (stretched)</td>
<td>Veil partially broken (less than half)</td>
<td>Veil partially broken (greater than half)</td>
<td>Veil completely broken</td>
<td>Cap open, gills well exposed</td>
<td>Cap open, gill surface flat</td>
</tr>
</tbody>
</table>

**Figure 2.3** Appearance of sporophore development  
Source: (Hammond and Nichols 1976; Guthrie 1984)

**2.2.4. Texture and water content**

Unlike fruits and vegetables, mushrooms are not protected by an external epidermal structure, thus the transpiration rate from the fruiting body is very high and water loss is comparable to that of a free water surface (San Antonio and Flegg 1964). Weight loss as a result of desiccation is a problem for the mushroom retailer because it can cause toughening and shriveling of the cap loosing consumer acceptability. Moreover, weight loss causes economical loss when mushrooms are sold by weight (Gormley 1975).

Stiffness or resistance to deformation is in essence the same as firmness, a property used by consumers to evaluate the texture of mushrooms. Stiffness reduction during storage after harvest has been related to water loss and changes in hyphal density after cap opening. Stiffness reduction is more rapid at higher temperatures as is water loss. Overall stiffness is higher in the stipe than in the pileus, but after storage stipe presents higher drops in stiffness than the pileus (McGarry and Burton 1994).
2.2.5. Respiration rate

Live commodities such as fruits, vegetables and mushrooms undergo respiratory metabolism, the process where oxygen is combined with carbohydrates to form various compounds that end up as carbon dioxide and water. The rate of respiration is inversely related to the shelf life of the product, the higher the respiration rate, the lower the shelf life; Table 2.2 shows a classification for respiration rates of several commodities, mushrooms rank among the extremely high with a respiration rate higher than 60 mg CO₂ kg⁻¹ h⁻¹ (Saltveit 2004).

Table 2.2. Respiration rates of perishable commodities at 5°C

<table>
<thead>
<tr>
<th>Class</th>
<th>mg CO₂ kg⁻¹ h⁻¹</th>
<th>Commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Low</td>
<td>&lt; 5</td>
<td>Nuts, dates</td>
</tr>
<tr>
<td>Low</td>
<td>5 – 10</td>
<td>Apple, citrus, grape, kiwifruit, onion, potato</td>
</tr>
<tr>
<td>Moderate</td>
<td>10 – 20</td>
<td>Apricot, banana, cherry, peach, nectarine, pear, plum, fig, cabbage, carrot, lettuce, pepper, tomato</td>
</tr>
<tr>
<td>High</td>
<td>20 – 40</td>
<td>Strawberry, blackberry, raspberry, cauliflower, lima bean, avocado</td>
</tr>
<tr>
<td>Very High</td>
<td>40 – 60</td>
<td>Artichoke, snap bean, Brussels sprouts, cut flowers</td>
</tr>
<tr>
<td>Extremely High</td>
<td>&gt; 60</td>
<td>Asparagus, broccoli, mushroom, pea, spinach, sweet corn</td>
</tr>
</tbody>
</table>

Source: (Saltveit 2004)

Respiration is highly affected by temperature; an increase in temperature can cause exponential rises in respiration rates. Table 2.3 shows the respiration rate of mushrooms at several temperatures of storage (Adamicki 2004).

Table 2.3. Respiration rates of fresh mushrooms

<table>
<thead>
<tr>
<th>Temperature</th>
<th>mg CO₂ kg⁻¹ h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°C</td>
<td>28-44</td>
</tr>
<tr>
<td>5°C</td>
<td>70</td>
</tr>
<tr>
<td>10°C</td>
<td>97</td>
</tr>
<tr>
<td>15°C</td>
<td>–</td>
</tr>
<tr>
<td>20°C</td>
<td>240-288</td>
</tr>
</tbody>
</table>

Source: (Adamicki 2004)
2.3. Preservation methods for fresh mushrooms (*Agaricus bisporus*)

2.3.1. Mushroom wash

During the 1980’s sulfite treatments were banned from use on fresh produce, researchers have explored alternative washing treatments containing chemicals such as EDTA, hydrogen peroxide, chlorine dioxide, sodium erythorbate and calcium chloride (Guthrie 1984; McConnell 1991). Mushrooms washed with tap water have shown higher microbial populations and an increased rate of color deterioration indicated by lower reflectance values. However, when chlorine dioxide was added to the washing treatments at 50ppm bacterial growth was decreased and shelf life was improved. Chlorine dioxide antimicrobial action was increased by addition of calcium chloride (10mM) which also resulted in firmer mushrooms and reduced the rate of cap opening (Guthrie 1984). Wash treatment with a combination of 50ppm of chlorine dioxide, 0.1% sodium erythorbate and 0.05% calcium chloride has also shown to be effective in reducing microbial population and increasing shelf life (Guthrie 1984).

Mushrooms washed with solutions containing 1000ppm EDTA did not show a significant difference in whiteness compared to those treated with sodium sulfite after 1 day of storage. Moreover, whiteness was significantly higher in EDTA treated mushrooms after 3 days of storage. A combination wash of 1000ppm EDTA and 1% \( \text{H}_2\text{O}_2 \) resulted in mushrooms with significantly higher whiteness, reduced purple blotch defect and standard plate counts compared to other wash treatments tested in this study (McConnell 1991).

Washing treatment containing Na₂EDTA in combination with sodium erythorbate and cysteine has shown to reduce browning in mushrooms compared to unwashed control in whole and sliced mushrooms after 6 and 5 days of storage at 4°C respectively (Sapers and others 1994).
A two stage wash process (US Patent 5919507) alternative to sulfite has been developed; where mushrooms are subjected to a high pH wash (10.5-11) to reduce bacterial populations on mushroom surface, followed by neutralization buffer containing calcium chloride and enzymatic browning inhibitors such as erythorbic acid and EDTA. This method has been shown to increase quality of mushrooms compared to sulfite treated mushrooms on days 0, 3, 6 and 9 of storage, and also resulted in better quality than mushrooms washed with a combination of EDTA and hydrogen peroxide. This treatment was effective in reducing microbial counts (Beelman and Duncan 1997).

2.3.2. Temperature control

Control of storage temperature by refrigeration is the most common used technology to extend mushroom shelf life (Burton 1987). Studies have shown that the longer mushrooms are kept under refrigeration (1°C) the whiter the mushrooms at the point of removal and during further storage at room temperature (20°C). Delaying the time before mushrooms are stored under refrigeration had a negative effect on subsequent whiteness. After removal from refrigeration the rate of whiteness change was the same or even less than unrefrigerated mushrooms (Gormley 1975).

Vacuum cooling is a technique were cooling is achieved by a reduction in pressure which allows water to vaporize at a lower temperature, the heat required for water to evaporate is supplied by the product, thus heat is removed by evaporation. The general rule of thumb is that 1% w/w of the total water vapor removed results in 10°F reduction in the product temperature (Bernard 1974).

Mushrooms treated with vacuum cooling stored at 5°C did not show a significant difference from conventionally cooled mushrooms in 5 days of storage. However, when mushrooms were stored at 18°C after different periods of storage at 5°C less degree of browning was obtained in vacuum cooled mushrooms compared to conventionally cooled ones (p<0.05). The rate of weight loss of vacuum cooled mushrooms was significantly
greater than that of the conventionally cooled mushrooms (p<0.02), after 4.25 days of storage at 5°C vacuum cooled mushrooms lost 1.7% more water (Burton and others 1987a).

Mushrooms treated with vacuum cooling prior to storage at 4°C have shown significantly lower polyphenoloxidase activity, lipid oxidation and superoxide anion generation which is related to membrane lipid oxidation and senescence (P<0.05). Mushrooms treated with vacuum cooling also had significantly lower browning (P<0.05) up to day 7 of storage (Tao and others 2007).

2.3.3. Modified atmosphere packaging

When fresh produce is wrapped with plastic film, respiration of produce reduces oxygen and increases carbon dioxide concentration; this causes the atmosphere within the package to be modified retarding ripening and senescence of fresh commodities (Burton and others 1987b).

The effect of modified atmosphere packaging (MAP) of mushrooms after vacuum cooling was compared to hypobaric storage (20 - 30 kPa) and cold storage, all treatments stored at 4°C. It was found that mushrooms stored with MAP had significantly lower weight loss (p<0.05) compared to hypobaric and cold storage. Mushrooms stored with MAP also had significantly lower respiration rate and browning (p<0.05) compared to those at cold storage. (Tao and others 2006) In another study, MAP of mushrooms treated with vacuum cooling prior to storage resulted in significantly lower (p<0.05) superoxide anion generation, polyphenoloxidase activity and browning of the cap. This treatment also resulted in significantly higher (p<0.05) firmness (Tao and others 2007).

Development stage of the sporophore has been related to carbon dioxide concentrations in controlled atmosphere packaging, after 7 days mushrooms stored at 10°C under 4 different O₂ concentrations (5-20%) and 15% CO₂ did not have a broken
veil whatever the oxygen concentration. In this study higher concentrations of CO$_2$ resulted in slower opening of the cap (Briones and others 1992).

In a study where chitosan coated mushrooms were packaged in different films to achieve modified atmosphere: whole and sliced coated mushrooms packaged with polyolefin PD-941 film and sliced coated mushrooms packaged with PVC film had lower maturity index compared to uncoated control. The polyolefin PD-941 film provided a better barrier to moisture and oxygen resulting in lower weight loss and maturity index reduction, this film also improved color of sliced mushrooms without coating (Kim and others 2006).

2.3.4. Irradiation

Food is irradiated by exposure to radiant energy such as gamma rays, electron beams and x-rays. U.S. food regulations allow irradiation of fresh meat, poultry, wheat powder, white potatoes, many spices and seasonings, fresh shell eggs and fresh produce (USDA 2005). Low irradiation dose (1kGy) was approved for fruits and vegetables in 1986 for insect control and shelf life extension (Department of Health and Human Services 2005).

Gamma irradiation applied at rates of 4.5kGy/h and 32kGy/h with a total dose of 2kGy was shown to increase mushroom shelf life by 4 and 2 days respectively, as expressed by higher hue angle values obtained on the treated samples, which indicate less color change (Beaulieu and others 1999). Irradiated mushrooms and controls were stored at 15°C and 90% RH. Better preservation of cellular membranes was observed by electron microscopy on mushrooms treated with a rate of 4.5kGy/h, thus the lower shelf life extension with the rate of 32kGy/h was attributed to decompartmentation of vacuolar phenols and entry of oxygen into the cell, which resulted in more browning (Beaulieu and others 1999).
Mushrooms irradiated with a gamma ray dose of 2kGy and stored at 10°C, have shown a decrease in extent of cap opening, stipe elongation and weight loss after 11 days of storage. A 6 log reduction in the surface microbial load was achieved right after irradiation and after 10 days of storage irradiated mushrooms had 4 log CFU/g compared to 8 log CFU/g in the non irradiated control. Irradiated mushrooms had higher firmness and less browning which was also confirmed by reduced polyphenol oxidase activity (Gautam and others 1998).

Electron beam irradiation has shown to preserve quality of sliced mushrooms. Aerobic counts were reduced by 7 Log CFU/g in mushrooms irradiated with 1 - 5.2kGy. Recovery was observed after 9 and 12 days of storage at 4°C in mushrooms irradiated with 1kGy and 3.1kGy respectively, no recovery was observed after 21 days of storage in mushrooms irradiated with 5.2kGy. Irradiated mushrooms had significantly lower browning indicated by higher L*-values (p<0.05); after 10 days of storage at 4°C irradiated mushrooms had an L*value between 82-87 and non-irradiated control had an L*value of 69 (Koorapati and others 2004).

2.3.5. Edible coatings

Chitosan coating has shown negative effects on color on sliced and whole mushrooms indicated by lower L* values and higher ΔE* values. However, the coating treatment resulted in lower maturity index on both whole and sliced coated mushrooms packaged with polyolefin PD-941 film and sliced mushrooms packaged with PVC film. No significant differences in weight loss were found between coated and uncoated mushrooms (Kim and others 2006).

Alginate based edible coatings containing lipids and emulsifiers have been used to coat white button mushrooms, this will be covered in detail on Chapter 3 (see section 3.4.3).
2.4. Summary

Fresh *Agaricus* mushrooms sales have increased by 25% over the last 14 years. High perishability of fresh white button mushroom *Agaricus bisporus* is determined by enzymatic browning caused by mechanical and bacterial damage of the sporophore tissue, opening of the cap, stipe growth, firmness and water content. Temperature control during storage is the most common method used to extend mushroom shelf life. However, research has been carried out in this field and methods such as antimicrobial wash, modified atmosphere packaging, modified humidity packaging, irradiation and edible coatings have been proposed to preserve quality parameters and extend mushroom shelf life.

References


Burton KS. The quality and storage life of Agaricus bisporus; 1987; Braunschweig, Germany. p 287-93.


Chapter 3

LITERATURE REVIEW ON ALGINATE BASED EDIBLE COATINGS

3.1. Introduction

Alginates are the salts from alginic acid, an acidic polysaccharide that functions as a structural component in brown seaweeds (*Phaeophyceae*). Alginic acid is composed of (1,4)-β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (see Figure 3.1), which are arranged in homopolymeric regions of M and G blocks and scattered regions of alternating MG blocks (see Figure 3.2) (King 1983; Draget 2009).

![Figure 3.1. Alginate monomers](image1)

![Figure 3.2. Block arrangement](image2)
Commercial alginate is extracted from algae, mainly *Laminaria hyperborea, Macrocystis pyrifera, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica, Eclonia maxima, Lessonia nigrescens, Durvillea antartica* and *Sargassum* spp. Production of alginate from bacteria such as *Azotobacter vinelandii* and *Pseudomonas* has been explored but it is not yet economically feasible (Draget 2009).

Alginate has been applied in the food industry as stabilizers, thickeners, films and gels. A unique property of alginate is their ability to form an insoluble matrix when cross-linked with divalent cations such as Ca$^{+2}$ (King 1983). This property allows for alginate solutions to be applied as liquid coatings to enrobe foods and later become water insoluble by cross-linking (Kester and Fennema 1986).

Alginate can be cross-linked by diffusion setting or internal setting method:

*Diffusion setting:* is characterized by rapid gelling kinetics and occurs when cross-linking ions such as Ca$^{+2}$ diffuse from an outer source into an alginate solution. Commonly used as an immobilization technique (Draget 2009).

*Internal setting:* cross-linking ions are added to an alginate solution by controlled release, usually achieved by controlling pH, limiting solubility of cross-linking ion source or using chelating agents (Draget 2009).

Edible coatings can act as a supplement of packaging material for quality improvement and shelf life extension (Kester and Fennema 1986). The use of edible coatings has been considered as a potential approach in response to the increasing demand of fresh and minimally processed foods in the food retail industry (Lin and Zhao 2007). The crucial quality attributes in fresh produce are appearance, color, texture, flavor, nutritional value, and microbial safety (Lin and Zhao 2007). Edible coatings can be used to retard desiccation in fresh fruits. However, the biopolymer must meet a
certain gas permeability threshold to avoid anaerobic respiration which would cause rapid loss of quality (Kester and Fennema 1986).

Edible coatings have been shown to preserve quality attributes of fresh fruits and vegetables by the regulation of moisture and aroma transfer, oxygen and carbon dioxide equilibrium and oxidative reaction rates (Nisperos-Carriedo and others 1991; Lin and Zhao 2007; Rojas-Graü and others 2009). Edible coatings have also been applied in meat and dairy products to prevent moisture loss and lipid oxidation (Mehyar and others 2007; Conte and others 2009; Song and others 2010). The capability of edible coatings to carry functional ingredients, such as antimicrobials and antioxidants, help to achieve the microbial safety and color preservation of fresh produce and meat products (Cuppett 1994).

3.2. Physical properties of alginate based edible coatings

3.2.1. Film thickness

Thickness of edible coatings and films can be affected by viscosity, density draining time and solid concentration of the coating solution. Film thickness is related to other physical properties such as gas and water vapor permeability (Cisneros Zevallos and Krochta 2003; Lin and Zhao 2007; Vargas and others 2008).

Thickness of calcium alginate films has been shown to increase with concentration of the calcium chloride solution used to crosslink it. This has been attributed to a decrease in the degree of alginate solubilization as the concentration of calcium chloride increases (Pavlath and others 1999; Rhim 2004a). In other words, as the concentration of calcium chloride increases the rate of the cross-linking reaction is faster than the rate of solubilization of sodium alginate in the cross-linking solution. When
cross-linking by immersion was compared to cross-linking by mixing prior to casting the film, the later films showed significantly higher thickness (p<0.05) (Rhim 2004a).

### 3.2.2. Tensile strength

Tensile strength (Pa) is the maximum stress applied to a film before breaking during tensile testing and is calculated by:

\[
TS = \frac{F_{\text{max}}}{A}
\]

**Equation 3.1**

Where \(F_{\text{max}}\) (N) is the maximum load applied before braking and \(A\) (m\(^2\)) is the cross-sectional area of the film (thickness x width) (Gennadios and others 1994; Rhim 2004b).

Tensile strength of sodium alginate films has been shown to increase with increasing concentrations of calcium chloride cross-linking solution, and cross-linking time; addition of glycerin as a plasticizer to the films has shown to reduce tensile strength (Pavlath and others 1999).

Tensile strength of sodium alginate films has been shown to increase when films are cross-linked with calcium chloride by immersion compared to films cross-linked by mixing prior to casting. Moreover, no significant differences have been found between the later type of film and sodium alginate films (p>0.05) (Rhim 2004a).

Relative humidity has been shown to decrease tensile strength of calcium alginate films with and without plasticizers. At relative humidities between 76-85% higher tensile strength was observed in non-plasticized films, followed by films plasticized with glycerol and the lowest tensile strength was found in films plasticized with sorbitol and
fructose, this was attributed to the size of the plasticizer molecules, higher molecular weight plasticizer provided lower tensile strength (Olivas and Barbosa-Cánovas 2008).

Increasing concentrations of garlic oil from 0 to 0.4% (v/v) has shown to significantly decrease tensile strength of 1% calcium alginate films (p<0.05), the authors attributed this effect to the interference of garlic oil with the ionic interaction between alginate and calcium in the film network (Pranoto and others 2005).

3.2.3. Elongation at break

Elongation at break represents the ability of the film to stretch before rupture occurs and is calculated by

\[ E(\%) = \frac{L_f - L_o}{L_o} \times 100\% \]  \hspace{1cm} \text{Equation 3.2}

Where \( L_f \) is the final length right before rupture and \( L_o \) is the original length of the film (Gennadios and others 1994; Rhim 2004b).

Relative humidity has been shown to increase % elongation of calcium alginate films with and without plasticizers. At relative humidities between 76-85% higher % elongation was observed in films plasticized with glycerol, followed by fructose and sorbitol plasticized films and the lowest % elongation was found in non-plasticized films, this was attributed to the size of the plasticizer molecules, higher molecular weight plasticizer provided lower % elongation of the films (Olivas and Barbosa-Cánovas 2008).

Elongation at break of sodium alginate films has been shown to decrease when films are cross-linked with calcium chloride by immersion compared to films cross-linked by mixing prior to casting. Elongation at break also decreases with increasing calcium chloride concentrations in both cross-linking methods (Rhim 2004a).
Increasing concentrations of garlic oil from 0 to 0.3% (v/v) did not show significant differences in elongation at break of calcium alginate films (p>0.05). However, when the concentration was increased to 0.4% elongation at break was reduced almost by half (p<0.05) (Pranoto and others 2005).

3.2.4. Barrier properties

Barrier properties of edible coatings against water vapor and gases can be described by permeability, transmission rate and resistance (Greener Donhowe and Fennema 1994).

Permeability: describes steady state diffusion and solubility of a permeate through a nonporous barrier without significant imperfections, combining Fick’s first law of diffusion and Henry’s law of solubility:

$$P = \frac{\Delta m}{\Delta t} \cdot e \cdot \frac{\Delta p}{A}$$

Equation 3.3

where $\Delta m/\Delta t$ is the weight of moisture loss per unit time (g/day), $e$ is film thickness (m), $\Delta p$ is the partial pressure difference between the two sides of the film (Pa), and $A$ is the film area exposed to moisture transfer ($m^2$) (Greener Donhowe and Fennema 1994; Hambleton and others 2009).

Transmission rate: obtained from the expression for permeability excluding film thickness and partial pressure gradient, therefore it should only be used to compare films with the same composition and thickness:

$$P = \frac{\Delta m}{\Delta t} \cdot \frac{1}{A}$$

Equation 3.4

where $\Delta m/\Delta t$ is the weight of mass loss per unit time (g/day), and $A$ is the film area exposed to mass transfer ($m^2$). (Greener Donhowe and Fennema 1994).
Resistance: describes the ability of a heterogeneous material to work as a barrier to permeate. Resistance to water vapor can be obtained by:

\[
WVR = \frac{\left(\frac{a_w \cdot \%RH}{100}\right) \cdot P_{wv}}{R \cdot T} \cdot \frac{A_s}{J}
\]

Equation 3.5

Where \(a_w\) is the water activity of the samples, \%RH is the relative humidity, \(P_{wv}\) is the saturated vapor pressure (Pa), \(A_s\) is the film area exposed to moisture transfer (m\(^2\)), \(R\) is the gas constant (\(R_{water} = 461.52\) Pa m\(^3\)/K kg), \(T\) is the absolute temperature (K) and \(J\) is the slope of weight loss curve in stationary conditions (kg/day) (Greener Donhowe and Fennema 1994; Vargas and others 2008).

3.2.4.1. Water vapor permeability and resistance

Alginates with higher proportion of guluronic:manuronic acid have been shown to provide films with higher water vapor permeability (Olivas and Barbosa-Cánovas 2008). Water vapor permeability of sodium alginate films has been shown to decrease when films are cross-linked with calcium chloride by immersion compared to films cross-linked by mixing prior to casting. Moreover, no significant differences have been found between the later type of film and sodium alginate films (p>0.05) (Rhim 2004a).

Cross-linking time with calcium chloride has shown to decrease water vapor permeability reaching a minimum after 3 minutes of reaction; further reaction time was shown to increase water vapor permeability back. This was attributed to the effect of unsolubilization of the film by crosslinkage with calcium chloride, which would reduce water vapor permeability at the beginning of the reaction, after a certain period of time that alginate was in contact with the aqueous cross-linking solution, the solubilization of alginate would increase water vapor permeability back (Olivas and Barbosa-Cánovas 2008).
Water vapor permeability of sodium and calcium alginate films with and without plasticizer has been shown to be increased by increasing relative humidity (Olivas and Barbosa-Cánovas 2008; Hambleton and others 2009), this effect has been attributed to the plasticizing effect that water molecules confer to hydrophilic films which promotes molecule mobility and increases permeability (Gontard and others 1996; Hambleton and others 2009). Addition of several plasticizers in calcium alginate films has been studied, no significant differences in water vapor permeability were found between films plasticized with glycerol and films without plasticizer. However, water vapor permeability was decreased by adding fructose or sorbitol to the films (Olivas and Barbosa-Cánovas 2008).

Increasing concentrations of garlic oil from 0 to 0.3% (v/v) did not show significant differences (p>0.05) in water vapor permeability of calcium alginate films (range 18.73-23.42 g mm/m² day kPa). However, when the concentration was increased to 0.4% water vapor permeability significantly increased to 30.89 g mm/m² day kPa (p<0.05). The authors explained this effect with the hydrophobicity of garlic oil which would extend intermolecular interactions of the alginate matrix, allowing moisture to pass through the film (Pranoto and others 2005).

Water vapor resistance was measured in 2% calcium alginate films with several concentrations of glycerol (1-3%), palmitic acid (0.1-0.9%), glycerol monostearate (0.1-0.9%) and β-cyclodextrine (0.1-0.9%). Several combinations indicated by an orthogonal experimental design were tested, but none of the ingredients incorporated in the coating were shown to have a significant effect on the water vapor resistance of the coating (Fan and others).
3.2.4.2. Water solubility and moisture content

Water solubility of sodium alginate films cross-linked with calcium chloride by dipping has been shown to increase with temperature. However, no significant differences have been found in solubility with increasing concentrations of calcium chloride from 2-5%. Swelling ratio of films cross-linked by dipping was shown to decrease with increasing soaking temperature (Rhim 2004a).

No significant differences have been found in moisture content of plasticized and non-plasticized calcium-alginate films when relative humidity is lower than 76%. However, when relative humidity is increased from 85-98% a steep increase in moisture content of plasticized films has been observed, moisture content of non-plasticized films also increased but it was significantly lower than that of plasticized films (p<0.05) (Olivas and Barbosa-Cánovas 2008).

Moisture content of sodium alginate films has been shown to decrease when films are cross-linked with calcium chloride by immersion compared to films cross-linked by mixing prior to casting. Moreover, films cross-linked by both methods had lower moisture contents compared to sodium alginate films (Rhim 2004a).

3.2.4.3. Oxygen permeability

Sodium alginate films have shown to have significantly lower oxygen permeability (5.66 cm$^3$µm/m$^2$dkPa) compared to pectin (19.49 cm$^3$µm/m$^2$dkPa) and methylcellulose (268.43 cm$^3$µm/m$^2$dkPa) (Fontes and others 2011).

Alginate-apple puree based films have been shown to be better oxygen barriers compared to pectin-apple puree based films, alginate based films had half the permeability of the pectin based films (10.20 ± 0.91 and 22.64 ± 1.28 cm$^3$µm/m$^2$dkPa)
respectively), this difference was attributed to the lower oxygen permeability of alginate compared to that of pectin (Rojas Graü and others 2007).

3.3. Alginate based composite coatings – lipid incorporation

Composite coatings combine lipids and hydrocolloids to obtain the combined advantage of water vapor barrier provided by lipids and improved mechanical properties provided by hydrocolloids (polysaccharides or proteins) (Greener Donhowe and Fennema 1994; Lin and Zhao 2007; Vargas and others 2008).

Environmental scanning electron microscopy (ESEM) has shown incorporation of emulsified beeswax in an alginate coating to be well dispersed on the surface of the film. Lipid migration towards the surface of the film has been observed after drying and attributed to density difference. Lipid aggregation has been found in sodium alginate films by laser light scattering granulometry, this aggregation was attributed to the lipid migration to the surface observed with ESEM (Hambleton and others 2009).

Addition of emulsified beeswax to sodium alginate coatings did not show a significant difference in water vapor permeability of films at various relative humidity gradients (p>0.05). However, when the films incorporated aroma compounds, the addition of beeswax to the coating decreased water vapor permeability at relative humidity gradients of 30-100% and 30-84% (Hambleton and others 2009).

Addition of sunflower oil to calcium alginate films did not have a significant effect on water vapor permeability compared to films without sunflower oil, permeability values ranged from 0.29-0.32x10⁹ g m/Pa s m² (Tapia and others 2007).
3.4. Application of alginate based edible coatings on fresh produce

Fruits and vegetables are often minimally processed by peeling, cutting or slicing, these process steps cause wounding of tissue, increase surface area and make fresh produce more prompt to enzymatic browning, texture breakdown and microbiological spoilage (Rojas-Graü and others 2007; Rojas-Graü and others 2009). Edible coatings have been shown to preserve quality attributes of fresh fruits and vegetables by the regulation of moisture and aroma transfer, oxygen and carbon dioxide equilibrium and oxidative reaction rates (Nisperos-Carriedo and others 1991; Lin and Zhao 2007; Rojas-Graü and others 2009). The capability of edible coatings to carry functional ingredients, such as antimicrobials and antioxidants, help to achieve the microbial safety and color preservation of fresh produce (Cuppett 1994).

3.4.1. Fruits

During storage whole apples can suffer flavor deterioration and loss of texture by softening. Alginate plasticized with glycerol was used to coat “Bravo de Esmolfe” whole apples, coated apples resulted in less texture, color and weight change after 97 days of storage at 20°C (Moldão-Martins and others 2003).

Fresh cut apples undergo enzymatic browning, and faster weight loss given that the surface area is increased and they are no longer protected by the peel which functions as a barrier to the environment (Olivas and others 2007a). Calcium alginate based coatings with 0.25% potassium sorbate have been shown to preserve texture, color and reduce weight loss of fresh cut Gala apples for a period of 10 days stored at 3°C, in this study a calcium alginate coating containing acetylated monoglyceride significantly reduced weight loss (p<0.05) when compared to a calcium alginate coating and a calcium alginate coating containing butter and linoleic acid. The three coatings significantly reduced color change and maintained hardness (p<0.05) compared to control, and no
difference was found between the three coatings ability to improve color and texture (Olivas and others 2007b).

Calcium alginate coating containing malic acid and several essential oils or their active compounds were shown to reduce counts of *E. Coli* O157:H7 in fresh cut apples. The most effective effect was found in coatings containing 0.7% lemongrass oil or 0.5% citral oil resulting in a 4 log CFU/g reduction compared to uncoated control on day 0. Moreover, these two coatings were able to maintain populations of *E. Coli* O157:H7 below 2 log CFU/g throughout 30 days of storage at 5°C. Coated samples also showed significant reduction in native microflora (p<0.05), in this case the most effective coatings were the ones containing 0.3-0.7% cinnamon or 0.3-0.7% clove essential oils. Coated samples were shown to have lower oxygen consumption and lower carbon dioxide and ethanol production compared to uncoated controls. Coatings containing cinnamon, clove or lemongrass essential oil at 0.3% and the coating without essential oil were shown to maintain desired firmness over 30 days of storage, increasing concentrations of these essential oils or their active compounds were shown to significantly decrease firmness (p<0.05) in the coated apple samples (Raybaudi-Massilia and others 2008b).

In a study were fresh cut Fuji apples were coated with alginate and anti-browning agents, an increase in N-acetylcysteine in the coating significantly increase resistance to water diffusion which was calculated from the rate of moisture loss indicated by weight loss. In this study the resistance to water diffusion was increased when sunflower oil was added to the coating. However, increasing the oil in the alginate coating from 0.025% to 0.125% did not have an effect on the water vapor resistance (Rojas Graü and others 2007).

Comparable results were obtained when fresh cut papaya was coated with an alginate containing 2% glycerol, 1% ascorbic acid and sunflower oil was added to the coating. In this study increasing concentrations of glycerol and ascorbic acid were found
to significantly increase water vapor resistance of the coating (p<0.05 and p<0.001 respectively), and no significant differences were obtained in respiration rate and ethylene production of coated and uncoated samples. The alginate coating also increased firmness compared to uncoated samples. (Tapia and others 2008).

Probiotic *Bifidobacterium lactis* Bb-12 was incorporated in calcium alginate coatings plasticized with glycerol containing sunflower oil, ascorbic acid, citric acid or N-acetylcysteine, and used to coat papaya and apple cylinders. Viable cells of the probiotic were shown to be maintained between 6.24-6.89 Log CFU/g in papaya cylinders and between 7.21-7.94 Log CFU/g in apple cylinders for 10 days of storage at 2°C (Tapia and others 2007).

Apple slices treated with commercial browning inhibitor Natureseal® and coated with sodium alginate containing prebiotics inulin, oligofructose or a mixture of inulin and oligofructose were shown to maintain fructan content throughout 14 days of storage at 4°C. Samples coated with alginate, and alginate with oligofructose had less than 1% weight loss after 14 days of storage, uncoated control lost around 2.5%. All coated samples were significantly firmer than uncoated control (p<0.001). However, uncoated samples treated with Natureseal® showed significantly higher firmness than alginate coated treatments, this was attributed to the calcium content of Natureseal®, by adding alginate less calcium would be available to strengthen the cell wall, because of the cross-linking reaction between calcium and alginate. Coated samples also had lower titratable acidity than uncoated control (p<0.05) which is desirable given that a rise in acidity can favor growth of *Salmonella* and *Shigella*. Coated samples maintained their sensory acceptability throughout 14 days of storage, acceptability of uncoated control dropped significantly (p<0.05). No significant differences in oxygen and carbon dioxide headspace concentration were obtained throughout storage (p>0.001) (Rößle and others 2011).
Fresh cut pears are susceptible to enzymatic browning and weight loss. Calcium alginate coating containing N-acetylcysteine and glutathione as anti-browning agents and sunflower oil has shown to increase water vapor resistance (calculated from the rate of moisture loss indicated by weight loss) in fresh cut pears stored for 14 days at 4°C. Coated samples also had lower ethylene production which was further decreased when anti-browning agents were added to the coating; lower ethanol production was also observed in coated samples containing N-acetylcysteine and glutathione after 11 days of storage. The coating with anti-browning agents was able to aid in the retention of vitamin C for up to 11 days and slowed down color change indicated by hue angle and sensory analysis throughout the 14 days of storage. Total aerobic psychrotrophic growth was reduced by the coating with anti-browning agents by 2 Log CFU/g after 14 days of storage compared to uncoated control. Coated samples containing antibrowning agents scored higher in odor and taste acceptance evaluated by sensory analysis (Oms-Oliu and others 2008).

Sodium alginate in combination with water-soluble chitosan was used to coat fresh Elliott blue berries, coated fruit resulted in significantly higher firmness (p<0.05) and lower decay rate indicated by mold growth. No significant differences were found in weight loss between coated and uncoated fruit which was attributed by the hydrophilic nature of the coating (Duan and others).

Calcium alginate coating containing glycerol (2%), palmitic acid (0.5%), glycerol monostearate (0.5%) and β-cyclodextrine (0.5%) and a 10⁹ CFU/mL culture of Cryptococcus laurentii was used to coat strawberries which were stored at 20°C and 70%RH. The coated strawberries showed less decay incidence (indicated by visual mold growth), less weight loss and more firmness compared to uncoated control. Moreover, the coating significantly reduced mold counts by 2 log CFU/g from day 2-5 of storage (Fan and others).
Calcium alginate has shown to significantly decrease moisture loss and respiration rate in whole peaches stored at 15°C and 40%RH. Coated peaches also had a slower change in titratable acidity pH and firmness. The coating did not have an effect on the total soluble solids of the fruit (Maftoonazad and others 2008).

Sodium alginate containing D-L malic acid several essential oils, or their active compounds, cross linked with calcium lactate was used to coat fresh cut melon. Coatings containing 0.5% eugenol, 0.5% geraniol or lemongrass essential oil were the most effective in suppressing microbial growth for up to 21 days at 5°C. Coatings containing cinnamon (0.7%), palmarosa (0.3-0.7%), lemongrass (0.3-0.7%) essential oils, eugenol (0.5%), geraniol (0.5%) or citral (0.5%) extended microbiological shelf life for more than 21 days (indicated by reaching a maximum limit of $10^7$ CFU/g); uncoated and coated controls without essential oils had a shelf life of around 4 and 10 days respectively. The coatings with various essential oils also reduced oxygen consumption, and carbon dioxide and ethanol production compared to uncoated and coated controls without essential oils. Coatings with low concentration of palmarosa and lemongrass essential oils (0.3%) and all concentrations of cinnamon and eugenol were shown to maintain firmness by more than 21 days. Coatings with cinnamon essential oil, eugenol, geraniol or citral were shown to maintain color indicated by chroma values for more than 21 days in fresh cut melons (Raybaudi-Massilia and others 2008a).

Tomato has a limited postharvest shelf life limited by transpiration, mold infection, acceleration of ripening and senescence. Moreover, tomatoes are sensitive to chilling injury and recommended storage temperatures are above 11°C (Zapata and others 2008). Sodium alginate coating plasticized with glycerol was shown to reduce ethylene production and respiration rate compared to uncoated control (p<0.05). Coated samples were also shown to have around 2% less weight loss (p<0.05), higher firmness for up to day 5 of storage at 20°C and no significant change in color indicated by hue angle (p>0.05) for up to day 6 of storage compared to uncoated control, finally coated samples scored higher than control in a 5 ranked scale sensory analysis performed by a trained
panel evaluating crunchiness, juiciness, sweetness, sourness and overall quality (Zapata and others 2008)

3.4.2. Vegetables

Calcium alginate coated lettuce has shown increased crispness throughout 12 days of storage at 2°C and 6°C, no significant difference was found in color change, Russet spotting, or carbon dioxide levels in the package compared to uncoated control (Tay and Perera 2004).

Calcium alginate was used to coat minimally processed carrots. Coated carrots and coated carrots containing 0.1% and 0.5% citric acid were able to maintain firmness. The whiteness index or whiteness blush did not increase significantly (p<0.05) for coated carrots without citric acid, but the coatings that incorporated citric acid resulted in a significant increase of the whiteness index (p<0.05). The coating alone did not have any effect on the total viable count. However, the coating containing 0.5% citric acid reduced the viable count by 2 log CFU/g. Coated and uncoated samples were subjected to several atmospheres; under all atmospheres the coated samples had lower phenolic content. Coated samples under controlled atmospheres of 80%-30%-0%, 70%-30%-0% and 50%-30%-20% of O₂-CO₂-N₂ had significantly higher contents of D-sucrose. Coated samples stored at 8°C and air had significantly lower production of ethanol and ethylene. (Amanatidou and others 2000).

3.4.3. Mushrooms

Mushrooms coated with 1% and 2% calcium alginate and stored at 4°C, maintained significantly higher L* values (p<0.05) from days 2-6 of storage. Mushrooms coated with 2% alginate resulted in significantly lower weight loss (p<0.05) between days 1-4 of storage at 20°C. Mushrooms coated with 1% alginate and stored at
20°C had significantly higher L* values between days 1-4 of storage. After 48 hours of storage at 4°C and 20°C coated mushrooms had lower strain at fracture and higher stiffness (resistance to mastication) characteristics desired in fresh mushrooms (Nussinovitch and Kampf 1993).

Alginate coatings containing lipids have shown to reduce browning on mushrooms on the later days of storage. Mushrooms coated with 2% alginate; 2% alginate + ergosterol; and 2% alginate + 0.2% ergosterol + polysorbate 80; resulted in significantly higher L* values (less browning) between days 7-19 of storage (2-3°C) compared to uncoated mushrooms. Mushrooms coated with calcium alginate containing ergosterol and polysorbate 80 resulted in significantly higher L* values (p<0.05) compared to mushrooms coated with alginate between days 7-19 of storage. Mushrooms coated with calcium alginate and calcium alginate + ergosterol, during 19 days of storage were found to lose less weight compared to uncoated ones, but no difference was found between the two coatings (Hershko and Nussinovitch 1998).

3.5. Application of alginate based edible coatings on meat products

3.5.1. Beef

Calcium alginate (1%) has shown to be an appropriate delivery system for nisin in beef carcasses. Beef tissue coated with calcium alginate containing 100 ppm of nisin was shown to reduce growth of Brochothrix thermosphacta, responsible for spoilage in vacuum-packaged red meat, for up to 7 days (>2.42logCFU/cm²) compared to samples coated with calcium alginate, samples treated with 100µg/mL of nisin solution and untreated samples (>6logCFU/cm²) (Cutter and Siragusa 1996). This system was also shown to significantly reduce growth of Brochothrix thermosphacta (p≤0.05) for up to seven days when applied to beef prior to grinding; compared to samples treated
with 100µg/mL of nisin, samples coated with 1% calcium alginate, and untreated samples (Cutter and Siragusa 1997).

The growth of E. coli O157:H7 in ground beef treated with nisin and chelators immobilized in calcium alginate gels was studied. Nisin immobilized in 1.5% calcium alginate provided significantly higher (P<0.05) inhibition of E. coli O157:H7, as compared to the non-immobilized control when stored at 10°C. The combination of nisin and EDTA in the immobilized system also provided significantly higher inhibition (P<0.05) effect when compared to the non-immobilized combination system. The highest inhibition was achieved with the combination of nisin and acetic acid immobilized in 1.5% calcium alginate. This immobilized combination also provided significantly higher inhibition (P<0.01) when compared to the non-immobilized system (Fang and Tsai 2003).

3.5.2. Poultry

Calcium alginate edible coatings have been used to overcome the high prevalence of Listeria monocytogenes in poached and deli turkey products. Alginate coatings containing 2.4% sodium lactate and 0.3% potassium sorbate, 600IU/g nisin and 2.4% sodium lactate or 500IU/g nisin and 0.25% sodium diacetate significantly reduced growth of L. monocytogenes by 2-3log CFU/g in poached turkey meat over 8 weeks of storage at 4°C compared to uncoated control (p<0.05). Coatings containing nisin in combination with sodium lactate, sodium diacetate or potassium sorbate were also shown to significantly decrease aerobic and anaerobic counts of poached turkey throughout 8 weeks of storage at 4°C (p<0.05). For deli turkey the alginate coating containing antimicrobials only showed significant differences in inhibition of L. monocytogenes on week 3 of storage, and no significant differences were found between the treatments and control on growth of aerobic and anaerobic bacteria (p>0.05) (Juck and others 2010).
Carcass washing with antimicrobials as a treatment of raw poultry to reduce populations of *Salmonella*, has had limited success in increased line speeds because of reduced contact time with antimicrobial agents. Calcium alginate coatings containing acidified sodium chlorite (1,200ppm) were shown to significantly reduce *Salmonella* on inoculated chicken drumettes (p<0.05) compared to control washed with acidified sodium chlorite (1,200ppm). Drumettes coated with alginate containing acidified sodium chlorite also showed less weight loss compared to samples coated with alginate, uncoated control, and control washed with acidified sodium chlorite. Calcium alginate coating with acidified sodium chlorite was shown to have a low absorption rate (0.006g/min) compared to a pea starch coating (0.05g/min) and compared to water (0.06g/min) (Mehyar and others 2007).

### 3.5.3. Seafood

Coatings with nisin and EDTA, calcium alginate without antimicrobial and a dipping solution of nisin and EDTA significantly inhibited the growth of mesophilic and psychrophilic bacteria (P<0.05) on northern snakehead fillets (*Channa argus*) at 4°C. The calcium-alginate coating containing nisin and EDTA and the calcium-alginate coating without antimicrobial significantly (P<0.05) reduced the water loss in the fillets after 7 days of storage as compared to the fillets treated with the dipping solution and the control. A nine-point hedonic sensory analysis with a trained panel was performed. Overall, the calcium-alginate coating with incorporated nisin and EDTA and the calcium-alginate coating without antimicrobial scored significantly higher (P<0.05) than the control and the fillets treated by the dipping solution (Lu and others 2009).

Alginate coatings containing sodium lactate (2.4%) and sodium diacetate (0.25%) have shown to delay growth of *Listeria monocytogenes* in cold smoked salmon slices and fillets over a storage period of 30 days at 4°C. Coated slices and fillets reached 4.1 and 4.4 CFU/g respectively when uncoated controls reached 7.3 and 6.8log CFU/g respectively (Neetoo and others 2009).
Bream is a highly consumed freshwater fish in China with a high content of unsaturated fatty acids (70% of total fat) which makes it very prompt to lipid oxidation. Calcium alginate coating plasticized with glycerol containing vitamin C or tea polyphenols has been shown to significantly reduce total viable counts, weight loss and total volatile basic nitrogen (related to spoilage bacteria and endogenous enzymes) in coated bream. The coated samples were also shown to have significantly lower thiobarbituric acid value (p<0.05) which is related to the extent of lipid oxidation; coated samples were below the threshold of 2 mgMDA/kg after 21 days of storage, whereas uncoated samples reached that limit after 12 days of storage. The coating containing vitamin C was most effective in reducing change in pH between days 4 and 22 of storage and scored significantly better (p<0.05) in a sensory analysis than other coatings and control throughout 21 days of storage (Song and others 2010).

3.6. Application of alginate based edible coatings on dairy products

Edible coatings have been used on fresh cheese to overcome microbial spoilage and to substitute the brine in the package reducing packing weight and transportation cost. Fior di Latte cheese coated with calcium alginate containing lysozyme and Na₂EDTA was shown to maintain weight change compared to uncoated samples packaged with brine (p>0.05), coated samples with and without modified atmosphere packaging and control with brine had significantly lower weight loss compared to uncoated control packaged in modified atmosphere without brine (p<0.05). Coated samples with and without modified atmosphere packaging and uncoated control packaged in modified atmosphere had lower counts of *Pseudomonas* sp. (p<0.05), and did not reach the limit of 10⁶ CFU/g, where alterations to the product start to appear, after 8 days of storage at 10°C; uncoated control packaged in brine reach that limit on the 3rd day of storage. No differences in overall quality were found in the sensory evaluation between coated and uncoated samples with or without modified atmosphere packaging and control with brine (Conte and others 2009).
Calcium alginate coating was used to coat semi-hard cheese, coated samples resulted in 2% less weight loss compared to uncoated control (p<0.05) throughout 25 days of storage at 4°C. Stress at failure after 18 days of storage significantly increased from 50.3 to 58.2kPa (p<0.05) in uncoated control, samples coated with alginate did not have a significant change in stress at failure, the authors explained this increase as a result of drying in the uncoated control. Sensory analysis indicated that coated samples crosslinked with calcium lactate resulted in no bitter taste compared to samples crosslinked with calcium chloride (Kampf and Nussinovitch 2000).

3.7. Summary

Alginate salts are extracted from brown seaweeds. The property of alginate to form a water insoluble matrix when cross-linked with divalent cations such as Ca$^{+2}$, allows it to be applied as a liquid coating to enrobe foods and become insoluble after cross-linking. Alginate based coatings have been applied on whole and cut fresh produce such as fruits, vegetables and mushrooms; to retard moisture loss, enzymatic browning, off-flavor development and microbial spoilage. Alginate as a carrier of antimicrobial agents has also been used to coat meat products such as beef, poultry and seafood; to reduce lipid oxidation and counts of spoilage and pathogenic bacteria. Alginate coatings applied in fresh cheese have shown to reduce microbial spoilage and moisture loss.
References


Chapter 4

SCREENING STUDY TO LOOK AT THE EFFECT OF ALGINATE BASED EDIBLE COATINGS ON FRESH MUSHROOM (Agaricus bisporus) QUALITY

Abstract

Fresh white button mushrooms are a highly perishable crop with a limited shelf life. Biopolymers based coatings can be used as carriers of antimicrobial and chelating agents to increase fresh produce shelf life. The objective of this study was to evaluate the effect of three coatings: alginate (A), alginate containing beeswax (AB), and alginate containing beeswax, nisin and Na₂EDTA (ABN) on the quality of fresh white button mushrooms (Agaricus bisporus) as measured by color change, weight loss, degree of cap opening and microbial growth. Mushrooms were stored at 12°C and 60% R.H. for 16 days. Mushrooms coated with ABN had significantly (p<0.05) lower ΔL* value as compared to uncoated control (C) after day 8 of storage. Coatings AB and A had significantly (p<0.05) lower ΔL* value compared to C after day 14 and 16 respectively.

No significant (p>0.05) differences were found in mushroom WL between coatings A, B, ABN and C. Moreover, no difference was found in the rate at which coated mushrooms lost weight compared to C. Mushrooms coated with A, AB and ABN had significantly (p<0.05) lower MI as compared to C. Mushrooms coated with ABN had significantly (p<0.05) lower BG during the early days of storage. Coating mushrooms with ABN extended the shelf life from 6 to 12 days when stored at 12°C and 60% relative humidity. These results indicate that edible coatings can be a used to extend shelf life of fresh mushrooms.
4.1. Introduction

The most important quality attributes in fresh produce are color, texture, flavor, nutritional value and microbial safety (Lin and Zhao 2007). The white button mushroom is highly perishable with a limited shelf life when sold as a fresh retail commodity. As mushrooms age they suffer enzymatic browning, caused by polyphenol oxidases, which are kept apart from the substrate by intact membrane boundary layers in the cells. However, after picking the mushroom processing and handling, causes tissue damaging which allows the enzymes and substrate to come in contact and start the browning reaction (Nichols 1985). The color of white button mushrooms can also be altered by bacterial contamination, *Pseudomonas tolaasii*, *P. reactans* and *P. gingeri* have been identified as responsible for post-harvest discoloration (Wells and others 1996). The lack of epidermal structure in mushrooms results in high transpiration rates in mushrooms resulting in increased weight loss (San Antonio and Flegg 1964).

Alginate based edible coatings have been shown to increase fresh produce shelf life (Nisperos-Carriedo and others 1991; Lin and Zhao 2007; Rojas-Graü and others 2009). Calcium alginate coatings containing antimicrobial and antibrowning agents have been shown to achieve microbial safety and color preservation of fresh cut apples and fresh cut papaya. Moreover, the incorporation of lipids in alginate coatings has shown an increase in water vapor resistance (Rojas Graü and others 2007; Tapia and others 2008). Wax emulsions are commonly applied in fresh citrus fruits to prevent water loss and shrinkage, to act as a barrier to gas exchange and to carry fungicides and growth regulators to control decay (Kaplan 1986).

The main objective of this study was to evaluate the effect of three coatings: alginate (A), alginate containing beeswax (AB), and alginate containing beeswax, nisin and Na₂EDTA (ABN) on the quality of fresh white button mushrooms (*Agaricus bisporus*) as measured by color change, weight loss, degree of cap opening and microbial growth.
4.2. Materials and methods

4.2.1. Materials

White button mushrooms (*Agaricus bisporus*), were obtained from the Pennsylvania State University Mushroom Test and Demonstration Facility (MTDF). Freshly picked mushrooms were obtained in the morning, and stored at 4°C until used.

High viscosity sodium alginate from brown algae was obtained from Sigma® Life Science (Norway), bleached beeswax was obtained from Aldrich® Chemistry (U.S.A.); anhydrous calcium chloride (CaCl₂) was obtained from J.T. Baker (Japan); Palsgaard® SMS 2008 (sorbitan monostearate) and Palsgaard® Polysorb 7462 (polysorbate) were provided by Palsgaard® (Denmark); Nisaplin® (nisin 20,000 IU/mL) was provided by Danisco® (Denmark); disodium etilendiamintetraacetic acid dihydrate (Na₂EDTA) was obtained from Thermo Scientific® (IL, U.S.A.).

4.2.2. Coating preparation

The coating was prepared two days ahead of the experiment day. Three coatings were prepared: a sodium alginate coating (A), a sodium alginate coating containing beeswax (AB) and a sodium alginate coating containing beeswax, nisin and Na₂EDTA (ABN), the compositions of the coatings are shown in Table 4.1.

**Table 4.1** Composition of alginate based coatings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium Alginate (%)</th>
<th>Beeswax (%)</th>
<th>Nisin (IU/mL)</th>
<th>EDTA (mg/mL)</th>
<th>Polysorbate (%)</th>
<th>Sorbitan monostearate (% of BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated Control (C)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>1.75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>1.75</td>
<td>0.55</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.17</td>
</tr>
<tr>
<td>ABN</td>
<td>1.75</td>
<td>0.55</td>
<td>4000</td>
<td>5.50</td>
<td>0.17</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Disodium EDTA was added to deionized (DI) water and stirred at 800 rpm until dissolved. Sodium alginate was added to the mixture and stirred at 800 rpm with heat until it was completely dissolved and it had reached 70°C. Once dissolved the speed was reduced to 60 rpm and stirred for 30 minutes to remove air bubbles. In separate flask beeswax, polysorbate and sorbitan monostearate were mixed at 60 rpm and heated to 70°C until melted. Once melted, speed was increased to 1000 rpm and part of the alginate mixture was slowly added to the beeswax mixture and stirred for 15 minutes. The emulsion was then added to the remaining alginate mixture and stirred at 800 rpm for 60 minutes. The heat was turned off, and the emulsion was kept stirred at 800 rpm until it reached 25°C.

On the experiment day, Nisaplin® was dissolved in DI water, added to the coating and mixed for 30 minutes. A flow diagram of the steps for the coating preparation is shown in Figure 4.1.
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>Na₂EDTA dissolved in DI water</td>
</tr>
<tr>
<td>Step 2</td>
<td>Sodium alginate dissolved in DI water 70°C</td>
</tr>
<tr>
<td>Step 3</td>
<td>Beeswax, sorbitan monostearate and polysorbate melted 70°C</td>
</tr>
<tr>
<td>Step 4</td>
<td>Alginate slowly added to BW mixture and stirred for 15 min</td>
</tr>
<tr>
<td>Step 5</td>
<td>Emulsion added to remaining alginate and stirred for 1 hour 70°C</td>
</tr>
<tr>
<td>Step 6</td>
<td>Nisin dissolved in DI water, added to emulsion at 24°C and stirred for 30 min</td>
</tr>
</tbody>
</table>

**Figure 4.1.** Coating preparation flow diagram
4.2.3. Mushroom coating, packaging and storage

Freshly picked mushrooms were obtained in the morning from the MTDF and were sorted by size and appearance. Selected mushrooms had a cap diameter between 3 and 4 cm, and were free of any visible signs of disease or physical damage. Mushrooms were placed in polystyrene trays: six mushrooms per tray for trays used to collect color and maturity index data; 6 mushrooms per tray with a total weight of 75g for weight loss data; and 100g of mushrooms per tray for trays used to collect standard plate count data. Mushrooms used for color data were labeled 1-6 on the stipe with a permanent marker. Each tray of mushrooms was washed by soaking in tap water for 15 seconds to remove peat moss particles. Mushrooms were then placed back in trays with a folded napkin on the bottom and allowed to dry for 30 min at 4°C. A flow diagram for the steps involved in sample preparation is shown in Figure 4.2.
Figure 4.2. Sample preparation flow diagram

Mushrooms sorted by size and appearance

Mushrooms labeled

Mushrooms washed by soaking for 15 sec in tap water

Mushrooms placed in trays and allowed to dry for 30 min at 4°C
Washed mushrooms were placed in a plastic bin with a rubber sink protector on the bottom. Mushrooms were coated by spraying with a piston pump driven spray gun (Wagner Power Painter Plus, China). Eight passes of coating were applied at a distance of 30 cm, on the top and bottom of the mushrooms. The coating was cross-linked with 4% CaCl$_2$ solution, applied with an atomizer bottle.

Coated mushrooms were placed back into trays and allowed to dry for 2 hours at 4°C. Mushroom trays were wrapped with perforated Polyvinylchloride (PVC) film with antifogging agent, using a heat sealer (Heat Sealing Equipment LLC., Cleveland, OH, USA). Each tray was wrapped making sure it had 4 perforations on the film.

Packaged trays were stored at 12°C and 60% relative humidity in an environmental chamber (Lunaire Ltd. model CE0932-4, PA, U.S.A.). A flow diagram of the steps for mushroom coating, packaging and storage is shown in Figure 4.3.
Figure 4.3. Mushroom coating flow diagram
4.2.4. Color measurement

Color of mushrooms was evaluated using the CIE L*a*b* color space (Commission International de l’Éclairage or International Commission on Illumination) with a CR-400 Minolta Chromameter (Minolta Sensing Inc., Japan). An observer of 2° (recommended for small objects i.e., equivalent to a circle of 15 mm diameter observed from a distance of 45 cm) and illuminant C (simulated overcast-sky daylight) were used (Billmeyer and Saltzman 1981) (MacDougall 2002). Data was collected with the Spectra Match Q.C. 3.5.0.1 software. The original projection cone with a 10 mm aperture was replaced with a 25 mm aperture projection cone to be able to cover a larger area of the cap.

Color data was obtained prior to coating and on days 2, 4, 6, 8, 10, 12, 14 and 16 of storage. The ΔL* and ΔE* values were calculated for each mushroom using the following equations:

\[
\Delta L^* = L_{time=0}^* - L_{time=t}^* \quad (Equation\ 4.1)
\]

\[
\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (Equation\ 4.2)
\]

Where L*, a* and b* define the location of any given color in the uniform color space: L* is lightness (0=black, 100=white) and a* and b* are chroma coordinates (-a=green, +a=red, -b=blue, +b=yellow) (MacDougall 2002).

The calculated ΔL* and ΔE* values were averaged for each tray (average of 6 mushrooms), and the average of 6 trays, 3 trays per flush, was used as each data point. Figure 4.4 illustrates the color data collection procedure.
4.2.5. Weight loss determination

Weight of the mushrooms was monitored using a laboratory scale (model B502-S, Mettler-Toledo GmbH, Greifensee, Switzerland). Mushrooms were weighed before washing, before coating, after coating, after packaging and then on days 2, 4, 6, 8, 10, 12, 14 and 16 of shelf life. Three trays per treatment were used to monitor weight throughout storage time. Weight was recorded for each tray and the average of 6 trays, 3 trays per flush, was one data point.

\[
\Delta L^*_{A=1-6} \quad \Delta L^*_{B=1-6} \quad \Delta L^*_{C=1-6} \quad \Delta L^*_{D=1-6} \quad \Delta L^*_{E=1-6} \quad \Delta L^*_{F=1-6} \\
\overline{\Delta L^*_{A-F}}
\]
Weight loss throughout the shelf life was calculated by subtracting the weight of the mushrooms at time t from the initial weight of the mushrooms dividing by the initial mushroom weight; using the following equation:

\[
\% \text{ weight loss} = \frac{\text{weight}_{t} - \text{weight}_{\text{initial}}}{\text{weight}_{\text{initial}}} \cdot 100\% \tag{Equation 4.3}
\]

4.2.6. Maturity index measurement

Using the extent of cap opening, the maturity index was assigned to each mushroom based on the following seven point scale:

<table>
<thead>
<tr>
<th>Stage</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of Sporophore</td>
<td>Veil intact (tight)</td>
<td>Veil intact (stretched)</td>
<td>Veil partially broken (less than half)</td>
<td>Veil partially broken (greater than half)</td>
<td>Veil completely broken</td>
<td>Cap open, gills well exposed</td>
<td>Cap open, gill surface flat</td>
</tr>
<tr>
<td><img src="image" alt="Figure 4.5" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.5.** Appearance of sporophore development
Source: (Hammond and Nichols 1976; Guthrie 1984)

The maturity index was assigned to each mushroom on day 0 and on days 2, 4, 6, 8, 10, 12, 14 and 16 of shelf life. The average of six mushrooms in one tray was taken, and the average of 6 trays, 3 trays per flush, was one data point.
4.2.7. Standard plate count

Standard plate count was used to estimate the total aerobic count of mushrooms over storage time. One tray was evaluated for bacterial count on days 0, 3, 6, 10 and 16. The mushrooms in each package were blended with an equal weight of 2% Difco® buffered peptone water (Becton, Dickinson & Company, MD, U.S.A.) in a sterilized variable speed laboratory blender (Model LB10, Waring Commercial, CT, U.S.A.). The homogenate was serially diluted and spread plated on Difco® Eugon Agar (Becton, Dickinson & Company, MD, U.S.A.) Petri dishes were incubated at 25°C for 48 hours (Guthrie 1984). The mean of triplicate plates was a single measurement per package, bacterial growth was calculated as follows:

\[ BG = \log \left( \frac{N_f}{N_0} \right) \]  
\text{(Equation 4.4)}

Where \( N_f \), is the colony forming units per gram at time \( t \) of shelf life; and \( N_0 \), is the colony forming units per gram on day 0 of shelf life.

4.2.8. Shelf life determination

To evaluate the overall shelf life of mushrooms based on color change, the retailer acceptability value (RAV) of Hunter L = 80 was used (Gormley 1975). This value was converted from Hunter L value to CIE L* value using the following equations (Billmeyer and Saltzman 1981):

\[ Y = \left( \frac{\text{Hunter L}}{10} \right)^2 \]  
\text{(Equation 4.5)}

\[ Y = \left( \frac{80}{10} \right)^2 \]
\[ Y = 64 \]
where $Y_n$ is the Y tristimulus value of the reference white for the selected illuminant and observer. $Y_n = 100$ for 2° observer and C illuminant (Billmeyer and Saltzman 1981).

The RAV $L^*$ of 84 was converted to $\Delta L^*$ by subtracting the RAV $L^*$ from the average $L^*$ value on day 0 for the mushrooms obtained from the MTDF as follows:

$$RAV\ \Delta L^* = L^*_{Day\ 0} - RAV\ L^*$$  \hspace{1cm} (Equation 4.7)

$$RAV\ \Delta L^* = 95 - 84$$
$$RAV\ \Delta L^* = 11$$

Shelf life was determined graphically by finding the time (days) when mushrooms reached the RAV.

4.2.9. Statistical analysis

Measurements are the average of six measurements: three taken on second flush mushrooms and three taken on third flush mushrooms. Data analysis was conducted using Duncan’s Multiple Range Test comparing treatments within each day of storage using Statistica (version 6.0 Statsoft Inc Tulsa OK, USA)
4.3. Results and discussion

4.3.1. Color

The ΔL* values for fresh mushrooms coated with A, AB and ABN and the uncoated control (C) are shown in Figure 4.6. Starting on day 8, coating ABN showed significantly lower ΔL* value (p<0.05) compared to C. Coating A and AB showed significantly lower ΔL* value compared to C between 14 to 16 days of storage (p<0.05).

![Figure 4.6](image)

**Figure 4.6.** Effect of alginate based coatings on absolute ΔL* value during storage. (Data represents average of six measurements; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)

Alginate coatings have been shown in the literature to reduce browning on mushrooms. When mushrooms were coated with 1% and 2% alginate and stored at 4°C, coated mushrooms resulted in significantly higher L* values (p<0.05) from days 2-6 of storage (Nussinovitch and Kampf 1993). Alginate coatings containing lipids have been shown to reduce browning on mushrooms between days 7-19 of storage (Hershko and
Nussinovitch 1998). Mushrooms coated with 2% alginate; 2% alginate + ergosterol; and 2% alginate + 0.2% ergosterol + polysorbate 80; have shown to result in significantly higher L* values (less browning) between days 7-19 of storage (2-3°C) as compared to uncoated mushrooms.

In our study no significant difference in ΔL* value was found between coating A and AB. However, coating AB resulted in less visual glossiness on the mushroom surface and had higher resemblance to uncoated mushrooms (Figure 4.7.). These results are in contrast with an earlier study where mushrooms coated with calcium alginate containing lipids resulted in significantly higher L* values (p<0.05) compared to mushrooms coated with alginate between days 7-19 of storage (Hershko and Nussinovitch 1998).

![Figure 4.7. Glossiness of mushrooms coated with A and AB](image)

Other commodities coated with alginate based coatings have shown to reduce changes in color throughout storage. Fresh cut Gala apples coated with calcium alginate or calcium alginate containing lipids, such as acetylated monoglyceride or linoleic acid with butter, have been found to significantly reduce browning (p<0.05) as compared to uncoated control (Olivas and others 2007). Calcium alginate has shown to prevent increase in whitening index on minimally processed carrots after 8 days of storage at 8°C
(Amanatidou and others 2000). However, when the coating contained 0.1% or 0.5% citric acid the whitening index increased significantly (p<0.05).

During preliminary work, between days 1 to 4 of storage, yellow and pink discolorations were observed in some mushrooms. However, a change in L* value was not registered in the chromameter. Hence, ΔE* values were used. ΔE* values for fresh mushrooms coated with A, AB and ABN and the uncoated control (C) are shown in Figure 4.8. Coating ABN showed significantly lower ΔE* value compared to C starting on day 8. Coating AB had significantly lower ΔE* value compared to C starting on day 12, and coating A was significantly different than control until day 14. No significant difference on ΔE* value was found between the coatings (A, AB and ABN) and control on the early days of storage indicating that the color discolorations observed at the beginning of storage did not have a significant impact on the total color difference.

**Figure 4.8.** Effect of alginate based coatings on ΔE* value during storage. (Data represents average of six measurements; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)
Beeswax and shellac have been added to locust bean gum coatings to obtain different hydrophobic/hydrophilic ratios (Rojas-Argudo and others 2005). These coatings were shown to reduce total color difference in coated cherries. In this study the increase in hydrophobicity of the coating significantly reduced the change in ΔE value (p<0.05) after 6 days of storage at 1°C and 1 day at 20°C. However, when cherries were stored for 11 days at 1°C and 1 day at 20°C no significant difference was found among the coatings or uncoated control. In our study, no significant difference in total color was found between the three coatings. At 0.55% beeswax the increase in hydrophobicity of the coating does not appear to have an effect on ΔE* value when compared with the calcium alginate coating without beeswax (A).

4.3.2. Weight loss

The effect of alginate based coatings on mushroom weight loss is shown in Figure 4.9. No significant difference was found between mushrooms coated with A, AB or ABN and C throughout the 16 days of storage (p>0.05). These results are in contrast with a study where mushrooms were coated with calcium alginate and calcium alginate + ergosterol (Hershko and Nussinovitch 1998). During 19 days of storage, coated mushrooms lost less weight as compared to uncoated ones. However, no difference was found between the two coatings. Alginate coated apples containing lipids such as acetylated monoglyceride have been found to reduce weight loss when compared to alginate coating, or alginate coating with linoleic acid and butter (Olivas and others 2007). Moreover, the three coatings significantly reduced weight loss compared to uncoated control.
Figure 4.9. Effect of alginate based coatings on % weight loss during storage. (Data represents average of six measurements; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)

The rate of change in weight loss for each treatment is shown in Table 4.2. No significant differences were found in the rate at which mushrooms lost weight during the 16 days of storage. Table 4.2 shows that the overall rate of change in weight loss per day is the same for the three treatments and control, this means that the coating was not able to act as a moisture barrier for the mushrooms.

Table 4.2 Rate of change in weight loss during storage of mushrooms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate of WL (% WL/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alg+BW+Ni+Na₂EDTA</td>
<td>1.57ᵃ ± 0.08</td>
</tr>
<tr>
<td>Alg</td>
<td>1.64ᵃ ± 0.02</td>
</tr>
<tr>
<td>Alg+BW</td>
<td>1.69ᵃ ± 0.09</td>
</tr>
<tr>
<td>Uncoated Control</td>
<td>1.65ᵃ ± 0.03</td>
</tr>
</tbody>
</table>

Note: Average of six measurements. Different letters within each column are significantly different by Duncan's Multiple Range Test at 5% level.
Given that the calcium alginate coating is insoluble in water, it was expected to result in lower water vapor permeability, and it was expected to decrease even further when beeswax was added to the coating, resulting in less overall weight loss. The results obtained in this study can be explained on the basis of the coating thickness and the high transpiration rate of mushrooms. Other studies have shown that alginate coatings containing lipids decrease weight loss on fresh cut apples (Olivas and others 2007; Rojas-Graü and others 2007). However, in these studies the coating was made by dipping, and the apples were stored at lower temperatures. Cut apples have lower transpiration rate than mushrooms; mushrooms loose 21% of their weight after 6 days of storage at 4°C (Nussinovitch and Kampf 1993) and the weight loss of fresh cut apple cultivars ranges between 0.53% - 1.29% after 12 days at 2°C (Kim and others 1993).

Coating by dipping results in more uniform thickness and less pinholes than spray coating, because solvents evaporate at a faster rate from sprayed films which causes premature immobilization of polymer chains. Coatings with non-uniform surfaces and pinholes have higher water vapor permeability (Kester and Fennema 1986), and more weight loss can be obtained with these coatings. Alginate coatings can act as a sacrificing agent where they give up moisture within the polymer before the food starts dehydrating (Kester and Fennema 1986). Coatings with a more uniform thickness have more moisture to give up before the food starts drying.

4.3.3. Maturity index

The change of the maturity index of coated and uncoated mushrooms throughout storage is shown in Figure 4.10. Mushrooms coated with A, AB and ABN had significantly lower opening of the cap (p<0.05) from day 6 to day 16 of storage. No significant difference was found between the three coating treatments throughout storage.
Figure 4.10. Effect of alginate based coatings on maturity index during storage. (Data represents average of six measurements; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)

Development stage of the sporophore has been related to carbon dioxide concentrations in controlled atmosphere packaging. After 7 days, mushrooms stored at 10°C under 4 different O₂ concentrations (5-20%) and 15% CO₂ did not have a broken veil whatever the oxygen concentration (Briones and others 1992). In this study higher concentrations of CO₂ resulted in slower opening of the cap. In another study where mushrooms were packaged with various perforated films to achieve modified atmospheres, development of the cap was significantly delayed with increasing concentrations of CO₂ (6 - 20%) and decreasing concentrations of O₂ (15-2%) when stored at 18°C for 4 days (Burton and others 1987). Extent of cap opening has been shown to be increased by controlling oxygen atmosphere concentration between 2-5%, the maximum effect was found at 5% O₂ concentration (Murr and Morris 1975). In this study the extent of cap opening was reduced by increasing CO₂ concentration between 25-50% or by storing mushrooms under 0% O₂.
The effect of the coating on maturity index could be attributed to the selective permeability of alginate to oxygen (Conca and Yang 1993). Low oxygen permeability of alginate films has been reported (Hambleton and others 2009), and as mentioned before low oxygen concentration has been related to slower opening of the cap.

In a different study, chitosan coated mushrooms were packaged in various films to achieve modified atmosphere; whole and sliced coated mushrooms were packaged with polyolefin PD-941 film, and sliced coated mushrooms were packaged with PVC film (Kim and others 2006). All treatments exhibited lower maturity index compared to uncoated control. The authors related this effect to the limited oxygen concentration.

**4.3.4. Standard plate count**

Microbial quality of mushrooms was recorded by standard plate count. Figure 4.11 shows the bacterial growth throughout storage. Mushrooms coated with ABN showed a reduction on total bacterial growth on day 3 of storage (p<0.05). No difference was found between the three coatings or control on days 6 and 10 of storage. This is in agreement with a study where alginate based coatings containing potassium sorbate were used to coat apples (Olivas and others 2007). In this study no significant difference was found in mesophilic, psychrotrophic and yeast and mold counts among treatments or control. Calcium alginate was used to coat minimally processed carrots, the coating alone did not have any effect on the total viable count (Amanatidou and others 2000). However, the coating containing 0.5% citric acid reduced the viable count by 2 log CFU/g.

On day 16, mushrooms coated with ABN had significantly higher bacterial counts (p<0.05) than the uncoated control and the mushrooms coated with alginate.
Figure 4.11. Effect of alginate based coatings on bacterial growth indicated by standard plate count during storage. (Data represents average of six measurements; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)

4.3.5. Shelf life determination

Shelf life was determined as shown in Figure 4.12. The uncoated control reached RAV ΔL* of 11 between days 6 and 8 of storage, and the mushrooms coated with ABN reached a RAV ΔL* of 11 between days 12 and 14 of storage. ABN coating was thus able to extend the shelf life of mushrooms by 6 days when stored at 12°C and 60% relative humidity. Shelf life of mushrooms coated with ABN was nearly doubled as compared of that of the uncoated control. Further shelf life extension might be achieved in mushrooms stored at 4°C. Visual appearance of uncoated mushrooms and mushrooms coated with ABN at the beginning and end of shelf life is shown in Figure 4.13.
Figure 4.12. Shelf life determination of mushrooms coated with ABN and uncoated control (C) (Data represents average of six measurements; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ABN</td>
<td><img src="image" alt="ABN Day 0" /></td>
</tr>
<tr>
<td>C</td>
<td><img src="image" alt="C Day 0" /></td>
</tr>
</tbody>
</table>

**Figure 4.13.** Mushrooms at the beginning and end of shelf life: day 6 for C and day 12 for ABN
4.4. Conclusions

Mushrooms treated with coating ABN had significantly (p<0.05) lower browning during storage as compared to uncoated control. This composite coating extended the shelf life of fresh mushrooms from 6 to 12 days when stored at 12°C and 60% R.H.. Mushrooms coated with AB and A had significantly (p<0.05) lower browning after days 14 and 16 of storage respectively.

No significant (p>0.05) differences were found in mushroom weight loss between coatings A, AB, ABN and uncoated control throughout storage. Moreover, no difference was found in the rate at which coated mushrooms lost weight compared to uncoated control.

Mushrooms coated with A, AB and ABN had significantly (p<0.05) lower extent of cap opening compared to uncoated ones, but no difference was found among the three coatings (p>0.05).

Mushrooms coated with ABN had significantly (p<0.05) lower total bacterial count during the early days of storage.
References


Chapter 5

OPTIMIZATION OF EDIBLE COATING WITH ALGINATE, BEESWAX, NISIN AND Na₂EDTA TO MAXIMIZE SHELF LIFE OF FRESH WHITE BUTTON MUSHROOM (Agaricus bisporus)

Abstract

Enzymatic browning, bacterial spoilage and high respiration rate are the main causes for the short shelf life of fresh mushrooms (Agaricus Bisporus). Alginate coatings with lipids, antimicrobials and chelating agents can delay quality deterioration and extend shelf life. The main objectives of this study were to determine the effect of the concentration of alginate, beeswax, nisin and Na₂EDTA on the quality of fresh white button mushrooms as measured by lightness (ΔL*), weight loss (WL), maturity index (MI) and bacterial growth (BG); and to find the optimal coating composition that maximizes shelf life using response surface methodology (RSM). Coatings with various concentrations of alginate, beeswax, nisin and Na₂EDTA, as determined by a central composite experimental design, were used to coat mushrooms. Mushrooms were then stored at 12°C/60%RH for 16 days and measurements were taken throughout storage. Results showed that alginate significantly (p<0.01) reduced the rate of BG throughout storage. Beeswax significantly decreased ΔL* (p<0.01) and BG (p<0.05) after 6 days in storage, but increased WL (p<0.10). Na₂EDTA significantly reduced ΔL* (p<0.05) and BG (p<0.01), while nisin significantly decreased ΔL*(p<0.10) and MI (p<0.01) during days 0-6. Nisin also increased WL significantly (p<0.05) throughout storage, but significant (p<0.01) interactions indicated that at high concentrations of Na₂EDTA and alginate, this effect was minimal. RSM was used to find the optimal concentration of each compound to maximize shelf life by reducing ΔL*, MI and BG. The optimal coating (OC) contained 2.49% alginate, 0.82% beeswax, 8.18mg/mL Na₂EDTA and 4000IU/mL nisin. In a validation study, ΔL* and MI of mushrooms with OC were compared to those
of the uncoated control (C). The OC significantly (p<0.05) reduced ΔL* and MI, extending the shelf life by 7 past that of the control.
5.1. Introduction

The use of edible coatings has been considered as a potential approach to extend shelf life, in response to the increasing consumer demand for fresh and minimally processed foods (Lin and Zhao 2007). The crucial quality attributes in fresh produce are appearance, color, texture, flavor, nutritional value, and microbial safety; edible coatings have been proven to preserve these quality attributes by regulating moisture and aroma transfer, and oxygen and carbon dioxide equilibrium (Lin and Zhao 2007). The capability of edible coatings to carry functional ingredients, such as antimicrobials and antioxidants, can improve the microbial safety and color preservation of fresh produce (Cuppett 1994). Edible coatings applied on minimally processed fresh produce have been shown to work better when a combination of bioactive compounds is incorporated into the coating rather than a single individual compound (Rojas Graü and others 2007; Tapia and others 2008).

Enzymatic browning caused by polyphenol oxidases, bacterial spoilage by Pseudomonas spp. and high respiration rate are the main causes contributing to the short shelf life of fresh white button mushrooms Agaricus Bisporus (Nichols 1985; Wells and others 1996; Saltveit 2004). Technologies such as modified atmosphere packaging, humidity controlled packaging, irradiation and antimicrobial wash have been proposed to extend the shelf life of mushrooms (Burton and others 1987b; Sapers and others 1994; Roy and others 1995a; b; 1996; Gautam and others 1998; Barron and others 2002; Beelman and Demirci 2007). However, biopolymer coatings with antimicrobials and chelating agents have not been explored extensively to increase the shelf life of fresh mushrooms.

Unlike fruits and vegetables, mushrooms lack epidermal structure. This causes high transpiration rate from the fruiting body, and water loss comparable to that of a free
water surface (San Antonio and Flegg 1964). Edible coatings can act as a skin replacement or fortifier of natural epidermal layers that commonly protect fruits and vegetables.

Analysis and modeling of problems that require the optimization of responses affected by several variables can be achieved by response surface methodology (Montgomery 2009). This approach has been used to optimize the concentration of bioactive compounds added to edible coatings, and using quality parameters as responses (Matsos 2006; Rojas-Graü and others 2007; Tapia and others 2008).

In many cases, several quality parameters or responses are important, such as color change, bacterial growth and maturity index, because all of them are involved in quality deterioration and shelf-life. When multiple responses are involved to obtain an optimum coating composition, an adequate approach is to overlay the contour plots for each response and find the region where all contours overlap closest to the desired response range (Montgomery 2009).

The main objectives of this study were to determine the effect of alginate, beeswax, nisin and Na₂EDTA on the quality of fresh white button mushroom (*Agaricus bisporus*) as measured by color change, weight loss, degree of cap opening and bacterial growth and to find an optimal coating composition that maximizes shelf life using response surface methodology.
5.2. Materials and methods

5.2.1. Materials

The materials used for this experiment are the same as the ones described in Chapter 4 (see Section 4.2.1).

5.2.2. Coating preparation

The procedure used to prepare the coating was the same as the one described in Chapter 4 (see Section 4.2.2).

5.2.3. Mushroom coating, packaging and storage

Mushrooms were coated packaged and stored as described in Chapter 4 (see Section 4.2.3).

5.2.4. Color measurement

Color of mushrooms was evaluated using the CIE L*a*b* color space (Commission International de l’Éclairage or International Commission on Illumination) with a CR-400 Minolta Chromameter (Minolta Sensing Inc., Japan). An observer of 2° (recommended for small objects i.e., equivalent to a circle of 15 mm diameter observed from a distance of 45 cm) and illuminant C (simulated overcast-sky daylight) were used (Billmeyer and Saltzman 1981) (MacDougall 2002). Data was collected with the Spectra Match Q.C. 3.5.0.1 software. The original projection cone with a 10 mm aperture was replaced with a 25 mm aperture projection cone to be able to cover a larger area of the cap.
Color data was obtained prior to coating and on days 2, 4, 6, 8, 10, 12, 14 and 16 of storage. ΔL* and ΔE* values were calculated for each mushroom using the following equations:

\[ \Delta L^* = L_{time = 0}^* - L_{time = t}^* \quad (\text{Equation 5.1}) \]

\[ \Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (\text{Equation 5.2}) \]

Where L*, a* and b* define the location of any given color in the uniform color space: L* is lightness (0=black, 100=white) and a* and b* are chroma coordinates (-a=green, +a=red, -b=blue, +b=yellow) (MacDougall 2002).

The calculated ΔL* and ΔE* values were averaged for each tray (average of 6 mushrooms), and the average of 3 trays was used as each data point. ΔL* and ΔE* values were plotted against storage time for each treatment (coating). The data set was broken into two time periods: days 0-6 and days 6-16 of storage. A linear regression model was fitted to each time period. The slope of the regression model represents the rate of change in ΔL* and ΔE* value over time. The absolute value of these slopes were designated as dΔL* and dΔE* respectively. Figure 5.1 illustrates the color data collection procedure and the calculations made to obtain dΔL* for days 0-6 and 6-16 of storage.
Figure 5.1. Graphical illustration of color measurement and related calculations
5.2.5. Weight gain and weight loss determination

The weight of the mushrooms was monitored using a laboratory scale (model B502-S, Mettler-Toledo GmbH, Greifensee, Switzerland). Mushrooms were weighed before washing, before coating, after coating, after packaging and then on days 2, 4, 6, 8, 10, 12, 14 and 16 of storage. Three trays per treatment were used to monitor the weight of mushrooms throughout the entire storage time.

To estimate the amount of coating retained on the mushroom surface, the percent weight gain was calculated from the weight of the mushrooms after 2 hours of drying and the initial mushroom weight using the following equation:

\[
\%WG = \frac{\text{weight}_\text{dried} - \text{weight}_\text{initial}}{\text{weight}_\text{initial}} \times 100\% \quad (\text{Equation 5.3})
\]

The weight loss throughout storage was also calculated by monitoring the weight of the mushrooms. A linear regression model was fitted to the data, and the rate of weight change over time (dw) was obtained from the slope.
5.2.6. Maturity index measurement

Using the extent of cap opening, the maturity index was assigned to each mushroom based on the following seven point scale:

<table>
<thead>
<tr>
<th>Stage</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>Description of Sporophore</td>
<td>Veil intact (tight)</td>
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<td>Veil partially broken (less than half)</td>
<td>Veil partially broken (greater than half)</td>
<td>Veil completely broken</td>
<td>Cap open, gills well exposed</td>
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<th>5</th>
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<th>7</th>
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<tr>
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<td>Veil partially broken (greater than half)</td>
<td>Veil completely broken</td>
<td>Cap open, gills well exposed</td>
<td>Cap open, gill surface flat</td>
</tr>
</tbody>
</table>

Figure 5.2. Appearance of sporophore development
(Hammond and Nichols 1976; Guthrie 1984)

The maturity index was assigned to each mushroom on day 0 and on days 2, 4, 6, 8, 10, 12, 14 and 16 of storage. The data points were plotted against time for each treatment (coating). To fit a linear regression model, the data set was broken into two time periods: days 0-6 and days 6-16 of storage. The rate of change in maturity index over time was obtained from the slope (dMI).
5.2.7. Standard plate count

Standard plate count was used to estimate the total aerobic count of mushrooms over storage time. One tray was evaluated for bacterial count on days 0, 3, 6, 10 and 16. Mushrooms in each package were blended with an equal weight of 2% Difco® buffered peptone water (Becton, Dickinson & Company, MD, U.S.A.) in a sterilized variable speed laboratory blender (Model LB10, Waring Commercial, CT, U.S.A.). The homogenate was serially diluted and spread plated on Difco® Eugon Agar (Becton, Dickinson & Company, MD, U.S.A.). Petri dishes were incubated at 25°C for 48 hours (Guthrie 1984). The mean of triplicate plates was a single measurement per package, bacterial growth was calculated as follows:

\[ \text{BG} = \log \left( \frac{N_t}{N_0} \right) \]  (Equation 5.4)

Where \( N_t \) is the colony forming units per gram at time \( t \) of storage; and \( N_0 \) is the colony forming units per gram on day 0 of storage.

Each measurement was plotted against time for each treatment (coating). To fit a linear regression model, the data set was broken into two time periods: days 0-6 and days 6-16 of storage. The rate of change in bacterial growth (dBG) was obtained from the slope of each regression line.
5.2.8. Experimental design

A central composite rotatable design with 2 blocks, 4 factors at 5 levels each, and a total of 24 runs including 4 center points per block was used for this experiment. The star distance (α) was 1.68. The experimental order was randomized. The experimental design was constructed using Statistica (version 6.0 Statsoft Inc Tulsa OK, USA). Table 5.1, shows the experimental design with coded values sorted by experiment number.

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<th>Nisin</th>
<th>Na&lt;sub&gt;2&lt;/sub&gt;EDTA</th>
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For each experimental run, 11 trays with 6 mushrooms each were prepared and coated (3 for color and maturity index, 3 for weight and 5 for standard plate count). The experimental design was blocked to differentiate two flushes of a single mushroom spawn (1\textsuperscript{st} flush for block 1, and 2\textsuperscript{nd} flush for block 2). To be able to coat the 132 mushroom trays, each block was divided in two consecutive days that corresponded to the highest picking days of each flush, so that each day 66 trays were coated. Table 5.2 shows the experimental design with the coded values sorted by run order, and Table 5.3 shows the actual concentrations that correspond to the coded values for each component of the coating.

### Table 5.2. Experimental design sorted by run order (coded values)

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Table 5.3. Coded values and actual concentrations

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<th>Coded Value</th>
<th>Sodium Alginate %</th>
<th>Beeswax %</th>
<th>Nisin IU/mL</th>
<th>Na₂EDTA mg/mL</th>
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<td>1621.59</td>
<td>2.82</td>
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<td>0</td>
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<td>0.55</td>
<td>4000.00</td>
<td>5.50</td>
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<tr>
<td>1</td>
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<td>0.82</td>
<td>6378.41</td>
<td>8.18</td>
</tr>
<tr>
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<td>3.00</td>
<td>1.00</td>
<td>8000.00</td>
<td>10.00</td>
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</table>

Data was analyzed using multiple linear regression procedures with MODDE statistical software for design of experiments and optimization (version 7.0.0.1, Umetrics, Umeå, Sweden). A second order polynomial was fitted to each response data set:

\[ R_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + \varepsilon_i \]

(Equation 5.5)

5.2.9. Graphic optimization

The optimization procedure was performed with the data obtained from the experimental design described in Section 5.2.8.

Response surface methodology was utilized to find the concentration combination for an optimal and a sub-optimal coating. Identification of the two coatings was done by overlaying the contour plots of each response and finding the region where all contours overlap closest to the desired value of quality.
5.2.10. Validation study

The models and optimization process were validated by coating mushrooms with the identified optimal coating, a coating that belonged to the center point of the experimental design and a combination identified as a sub-optimal coating. The coating performance predicted by the models was then compared to that found by the experimental validation study.

The three different coating treatments were supplemented by two uncoated controls: washed and unwashed mushrooms. The validation study was performed in triplicate within the same flush of mushrooms to eliminate the variability of each flush. For each coating 9 trays of mushrooms were coated (3 per replicate).

Data analysis was conducted using Duncan’s Multiple Range Test to compare treatments within each day of storage. A two sample T-test was used to compare slopes of quality parameters predicted by the models and the actual slopes obtained from the validation experiments data. All analysis were done using Statistica (version 6.0 Statsoft Inc Tulsa OK, USA)

5.2.11. Shelf life determination

The procedure used to determine shelf life of fresh mushrooms was the same as the one described in Chapter 4 (see Section 4.2.8).
5.3. Results and discussion

To analyze the data the slope of each quality parameter vs. time was obtained for each treatment. Table 5.4 shows the rate of change in each quality parameter throughout storage.

5.3.1. Color

5.3.1.1. ΔL* Value

Browning of mushrooms increased over storage time, this is expressed by decrease in lightness (L*value). To standardize the data, ΔL* values where calculated by subtracting the L* value at any given time during storage from the initial L* value:

\[ \Delta L^* = L^*_{t=0} - L^*_t \]

Delta L* values during storage time for mushrooms coated with several combinations of sodium alginate, beeswax, nisin and Na₂EDTA are shown in Error! Reference source not found. To analyze the data the slope of each quality parameter vs. time was obtained for each treatment. Table 5.4 shows the rate of browning expressed by rate change in ΔL* value (dΔL*), higher values of dΔL* indicate faster change in lightness or browning.

To evaluate the effect of coating composition on mushroom lightness the data set was broken into two parts: days 0-6, and days 6-16 of storage. For days 0-6 the regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination (R²) of 0.74 (Table 5.5)
Figure 5.3. Examples of $\Delta L^*$ value changes during storage time of coated mushrooms with various concentrations of sodium alginate, beeswax, nisin and Na$_2$EDTA
Table 5.4. Rate of change in quality parameters during storage of mushrooms at 12°C

<table>
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<tr>
<th>Exp. No.</th>
<th>Alg %</th>
<th>BW IU/mL</th>
<th>Nisin Na₂EDTA mg/mL</th>
<th>dΔL* 0-6</th>
<th>dΔL* 6-16</th>
<th>dΔE* 0-6</th>
<th>dΔE* 6-16</th>
<th>%WG</th>
<th>dw</th>
<th>dMI 0-6</th>
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<td>1.19</td>
<td>0.98</td>
<td>5.47</td>
<td>1.39</td>
<td>0.31</td>
<td>0.25</td>
<td>0.36</td>
</tr>
<tr>
<td>14</td>
<td>3.00</td>
<td>0.55</td>
<td>4000.00</td>
<td>5.50</td>
<td>0.83</td>
<td>0.87</td>
<td>1.17</td>
<td>0.91</td>
<td>5.98</td>
<td>1.56</td>
<td>0.40</td>
<td>0.25</td>
<td>0.19</td>
</tr>
<tr>
<td>15</td>
<td>1.75</td>
<td>0.10</td>
<td>4000.00</td>
<td>5.50</td>
<td>1.32</td>
<td>1.82</td>
<td>1.27</td>
<td>5.69</td>
<td>6.14</td>
<td>0.41</td>
<td>0.39</td>
<td>0.28</td>
<td>0.27</td>
</tr>
<tr>
<td>16</td>
<td>1.75</td>
<td>1.00</td>
<td>4000.00</td>
<td>5.50</td>
<td>0.87</td>
<td>0.73</td>
<td>1.24</td>
<td>0.80</td>
<td>5.13</td>
<td>1.49</td>
<td>0.36</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>17</td>
<td>1.75</td>
<td>0.55</td>
<td>0.00</td>
<td>5.50</td>
<td>0.96</td>
<td>1.04</td>
<td>1.49</td>
<td>0.94</td>
<td>6.84</td>
<td>1.35</td>
<td>0.49</td>
<td>0.27</td>
<td>0.30</td>
</tr>
<tr>
<td>18</td>
<td>1.75</td>
<td>0.55</td>
<td>8000.00</td>
<td>5.50</td>
<td>1.05</td>
<td>1.01</td>
<td>1.76*</td>
<td>1.03</td>
<td>7.52</td>
<td>1.41</td>
<td>0.36</td>
<td>0.29</td>
<td>0.30</td>
</tr>
<tr>
<td>19</td>
<td>1.75</td>
<td>0.55</td>
<td>4000.00</td>
<td>1.00</td>
<td>1.36</td>
<td>0.99</td>
<td>1.87</td>
<td>0.96</td>
<td>5.92</td>
<td>1.31</td>
<td>0.34</td>
<td>0.23</td>
<td>0.37</td>
</tr>
<tr>
<td>20</td>
<td>1.75</td>
<td>0.55</td>
<td>4000.00</td>
<td>10.00</td>
<td>0.77</td>
<td>1.04</td>
<td>1.31</td>
<td>1.18</td>
<td>6.77</td>
<td>1.33</td>
<td>0.36</td>
<td>0.29</td>
<td>0.21</td>
</tr>
<tr>
<td>21</td>
<td>1.75</td>
<td>0.55</td>
<td>4000.00</td>
<td>5.50</td>
<td>0.74</td>
<td>0.95</td>
<td>1.01*</td>
<td>1.07</td>
<td>5.94</td>
<td>1.30</td>
<td>0.31</td>
<td>0.23</td>
<td>0.31</td>
</tr>
<tr>
<td>22</td>
<td>1.75</td>
<td>0.55</td>
<td>4000.00</td>
<td>5.50</td>
<td>0.59</td>
<td>0.82</td>
<td>1.11</td>
<td>0.84</td>
<td>6.64</td>
<td>1.39</td>
<td>0.42</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td>23</td>
<td>1.75</td>
<td>0.55</td>
<td>4000.00</td>
<td>5.50</td>
<td>0.63</td>
<td>1.12</td>
<td>1.05</td>
<td>1.09</td>
<td>6.20</td>
<td>-0.63*</td>
<td>0.43</td>
<td>0.26</td>
<td>0.23</td>
</tr>
<tr>
<td>24</td>
<td>1.75</td>
<td>0.55</td>
<td>4000.00</td>
<td>5.50</td>
<td>0.92</td>
<td>1.18</td>
<td>1.22</td>
<td>1.17</td>
<td>5.35</td>
<td>-0.92</td>
<td>0.41</td>
<td>0.23</td>
<td>0.37*</td>
</tr>
</tbody>
</table>

*Data point not included in statistical analysis
The concentration of Na\(_2\)EDTA in the coating significantly decreased d\(\Delta L^*\) at the 5\% level (Table 5.6). Thus, Na\(_2\)EDTA can significantly slow down the rate of mushroom browning during early days of storage. The main effect of Na\(_2\)EDTA is shown graphically in Figure 5.4, the parabolic shape of this graph explains why the quadratic effect of Na\(_2\)EDTA is significant in the model (p<0.10). The decrease in d\(\Delta L^*\) reached a plateau when the concentration of Na\(_2\)EDTA was 8.18mg/mL (coded value 1) and no further decrease in d\(\Delta L^*\) was achieved when the concentration was increased to 10mg/mL (coded value 1.68).

The concentration of nisin in the coating significantly decreased d\(\Delta L^*\) at the 10\% level (Table 5.6). Thus, nisin can significantly slow down the rate of mushroom browning during the beginning of storage time. The main effect of nisin is shown graphically in Figure 5.5.
Table 5.6: Regression coefficients of alginate, beeswax, nisin and Na2EDTA concentrations on rate of change in lightness (dΔL*) during days 0-6 of storage at 12°C

<table>
<thead>
<tr>
<th>dΔL* 0-6</th>
<th>Coeff. N</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.56091</td>
<td>0.150999</td>
<td>1.19589e-011</td>
<td>0.263627</td>
</tr>
<tr>
<td>Alg</td>
<td>0.130799</td>
<td>0.164225</td>
<td>0.437423</td>
<td>0.286717</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.121827</td>
<td>0.164225</td>
<td>0.468941</td>
<td>0.286717</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.294018</td>
<td>0.164225</td>
<td>0.092339</td>
<td>0.286717</td>
</tr>
<tr>
<td>EDT</td>
<td>-0.681856</td>
<td>0.255169</td>
<td>0.0166958</td>
<td>0.445494</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Block</th>
<th>DF = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (B1)</td>
<td>-0.70393</td>
</tr>
<tr>
<td>Block (B2)</td>
<td>0.70393</td>
</tr>
</tbody>
</table>

| EDT*EDT   | 0.288125 | 0.151724 | 0.0757486 | 0.264893    |
| Alg*Bee   | -0.948948| 0.333394 | 0.0116693 | 0.582066    |

| N = 24 | Q2 = 0.384 | Cond. no. = 4.0195 |
| DF = 16 | R2 = 0.744 | Y-miss = 0 |
| R2 Adj. = 0.632 | RSD = 0.1559 | Conf. lev. = 0.90 |

![Graph](image.png)

Figure 5.4. Main effect of Na2EDTA on dΔL* during days 0-6 of storage at 12°C
Figure 5.5. Main effect of nisin on $d\Delta L^*$ during days 0-6 of storage at 12°C

The concentrations of sodium alginate and beeswax did not show a significant effect on the rate of browning (Table 5.6). The interaction between alginate and beeswax was significant at the 5% level and is shown in Figure 5.6, according to the graph when alginate is held at the high level an increase in beeswax decreases browning, but when alginate is held at low level increasing beeswax concentration increases browning. The interaction effect of alginate and beeswax is highly confounded with the linear effect of Na$_2$EDTA (correlation = 0.77). Given that the main effect of Na$_2$EDTA is significant and the main effect of alginate and beeswax are not significant, it is very likely that this interaction turned out to be significant because it was confounded.
For days 6-16 of storage the regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination ($R^2$) of 0.72 (Table 5.7).

The concentration of beeswax in the coating significantly decreased $d\Delta L^*$ at the 1% level (Table 5.8). Thus, in the later days of storage beeswax can significantly slow down the rate of mushroom browning. The main effect of beeswax is shown graphically in Figure 5.7. The concentrations of sodium alginate, nisin and beeswax did not show a significant effect on the rate of browning between days 6-16 of storage (Table 5.8).
Table 5.7. ANOVA table for rate of change in lightness (dΔL*) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>dΔL* 6-16</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>23</td>
<td>19.5934</td>
<td>0.851885</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>18.075</td>
<td>18.075</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>22</td>
<td>1.51838</td>
<td>0.0690171</td>
<td>0.262711</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>1.08617</td>
<td>0.135772</td>
<td>0.008</td>
<td>0.368472</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.432204</td>
<td>0.0308717</td>
<td>0.175704</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>9</td>
<td>0.151444</td>
<td>0.0168271</td>
<td>0.299669</td>
<td>0.944</td>
<td>0.129719</td>
</tr>
<tr>
<td>(Model Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>5</td>
<td>0.280761</td>
<td>0.0561522</td>
<td>0.236964</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Replicate Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 23  Q2 = 0.319  Cond. no. = 3.2004  DF = 14  R2 = 0.715  Y-miss = 0  R2 Adj. = 0.553  RSD = 0.1757

Table 5.8. Regression coefficients of alginate, beeswax, nisin and Na₂EDTA concentrations on rate of change in lightness (dΔL*) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>dΔL* 6-16</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.881483</td>
<td>0.0366714</td>
<td>8.77761e-013</td>
<td>0.078653</td>
</tr>
<tr>
<td>Alg</td>
<td>-0.00724883</td>
<td>0.0738744</td>
<td>0.923226</td>
<td>0.158446</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.176928</td>
<td>0.0475451</td>
<td>0.00227932</td>
<td>0.101975</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.045651</td>
<td>0.0475451</td>
<td>0.353264</td>
<td>0.101975</td>
</tr>
<tr>
<td>EDT</td>
<td>0.0207842</td>
<td>0.0475451</td>
<td>0.668672</td>
<td>0.101975</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>0.115228</td>
<td>0.0366714</td>
<td>0.00720294</td>
<td>0.078653</td>
</tr>
<tr>
<td>Alg*Nis</td>
<td>0.0855936</td>
<td>0.0621206</td>
<td>0.189872</td>
<td>0.133237</td>
</tr>
<tr>
<td>Bee*EDT</td>
<td>-0.131231</td>
<td>0.0965214</td>
<td>0.195452</td>
<td>0.207020</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>0.131159</td>
<td>0.0621206</td>
<td>0.0532016</td>
<td>0.133237</td>
</tr>
</tbody>
</table>

N = 23  Q2 = 0.319  Cond. no. = 3.2004  DF = 14  R2 = 0.715  Y-miss = 0  R2 Adj. = 0.553  RSD = 0.1757  Conf. lev. = 0.90
The interaction between nisin and Na$_2$EDTA had a significant effect on decreasing dΔL* (p<0.10). However, none of the main effects of this interaction are significant so it is difficult to speculate about the meaning of this interaction. According to the graph (see Figure 5.8), when the concentration of Na$_2$EDTA is low, an increase in nisin concentration decreases the change in ΔL* value; but when the concentration of Na$_2$EDTA is high, nisin slightly increases change in ΔL* value.

**Figure 5.7.** Main effect of beeswax on dΔL* during days 6-16 of storage at 12°C
Figure 5.8. Interaction between nisin and Na$_2$EDTA on dΔL* during days 6-16 of storage at 12°C
5.3.1.2. ΔE* Value

To evaluate the effect of coating composition on mushroom overall color change the data set was broken in two parts: days 0-6, and days 6-16 of storage. Table 5.4 shows the rate of browning expressed by rate of change in ΔE* value (dΔE*). Larger values of dΔE* indicate a faster change in color. For days 0-6 of storage the regression model fitted to the data was significant (p<0.05) with no significant lack of fit and a coefficient of determination (R²) of 0.74 (Table 5.9).

Table 5.9. ANOVA table for rate of change in color (dΔE*) during days 0-6 of storage at 12°C

<table>
<thead>
<tr>
<th>Slope Delta E 0-6</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>22</td>
<td>34.8286</td>
<td>1.58312</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>32.975</td>
<td>32.975</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>21</td>
<td>1.85357</td>
<td>0.088265</td>
<td>3.77738</td>
<td>0.018</td>
<td>0.390155</td>
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<tr>
<td>Regression</td>
<td>9</td>
<td>1.36999</td>
<td>0.152221</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
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<td>0.483577</td>
<td>0.040298</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit (Model Error)</td>
<td>7</td>
<td>0.323215</td>
<td>0.0461736</td>
<td>1.43967</td>
<td>0.356</td>
<td>0.21488</td>
</tr>
<tr>
<td>Pure Error (Replicate Error)</td>
<td>5</td>
<td>0.160361</td>
<td>0.0320723</td>
<td>0.179087</td>
<td></td>
<td></td>
</tr>
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</table>

N = 22  Q2 = -0.676  Cond. no. = 3.9918
DF = 12  R2 = 0.739  Y-miss = 0
R2 Adj. = 0.543  RSD = 0.2007

The concentration of beeswax and nisin in the coating significantly decreased dΔE* at the 5% level (Table 5.10). Thus, beeswax and nisin can significantly slow down the rate of mushroom color change during the early days of storage. The main effect of beeswax and nisin are shown graphically in Figure 5.9 and Figure 5.10 respectively.
Table 5.10. Regression coefficients of alginate, beeswax, nisin and Na$_2$EDTA concentrations on rate of change in color (dΔE*) during days 0-6 of storage at 12°C

<table>
<thead>
<tr>
<th>Slope Delta E 0-6</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.16575</td>
<td>0.0537904</td>
<td>5.45166e-011</td>
<td>0.0958684</td>
</tr>
<tr>
<td>Alg</td>
<td>0.0496894</td>
<td>0.0543209</td>
<td>0.378343</td>
<td>0.0968138</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.129541</td>
<td>0.0543209</td>
<td>0.0344647</td>
<td>0.0968138</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.161677</td>
<td>0.0619891</td>
<td>0.0228795</td>
<td>0.11048</td>
</tr>
<tr>
<td>EDT</td>
<td>-0.165312</td>
<td>0.0844025</td>
<td>0.0738107</td>
<td>0.150427</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>0.104051</td>
<td>0.0433456</td>
<td>0.0334887</td>
<td>0.0772529</td>
</tr>
<tr>
<td>EDT*EDT</td>
<td>0.0896111</td>
<td>0.0511862</td>
<td>0.105497</td>
<td>0.091227</td>
</tr>
<tr>
<td>Alg*Bee</td>
<td>-0.284687</td>
<td>0.110277</td>
<td>0.0240287</td>
<td>0.196542</td>
</tr>
<tr>
<td>Alg*Nis</td>
<td>-0.112528</td>
<td>0.0709736</td>
<td>0.138841</td>
<td>0.126493</td>
</tr>
<tr>
<td>Bee*Nis</td>
<td>0.103512</td>
<td>0.0709736</td>
<td>0.170389</td>
<td>0.126493</td>
</tr>
</tbody>
</table>

N = 22  Q2 = -0.676  Cond. no. = 3.9918  DF = 12  R2 = 0.739  Y-miss = 0  R2 Adj. = 0.543  RSD = 0.2007  Conf. lev. = 0.90

Figure 5.9. Main effect of beeswax on dΔE* during days 0-6 of storage at 12°C
The concentration of Na₂EDTA in the coating significantly decreased $d\Delta E^*$ at the 10% level (Table 5.10). Thus, Na₂EDTA can slow down the rate of mushroom color change during the early days of storage. The main effect of Na₂EDTA is shown graphically in Figure 5.11, the decrease in $d\Delta E^*$ reached a plateau when the concentration of Na₂EDTA was 8.18mg/mL (coded value 1) and no further decrease in $d\Delta E^*$ was achieved when the concentration was further increased to 10mg/mL (coded value 1.68).

**Figure 5.10.** Main effect of nisin on $d\Delta E^*$ during days 0-6 of storage at 12°C
The concentration of sodium alginate did not show a significant effect on $d\Delta E^*$ (Table 5.10). The interaction between alginate and beeswax was significant at the 5% level and is shown in Figure 5.12, according to the graph when beeswax is held at the high level an increase in alginate decreases change in color, but when beeswax is held at low level increasing alginate concentration increases color change. The interaction effect of alginate and beeswax is highly confounded with the linear effect of Na$_2$EDTA (correlation = 0.77). Given that the main effect of Na$_2$EDTA is significant and the main effect of alginate is not significant, it is likely that this interaction turned out to be significant because it was confounded.
For days 6-16 of storage the regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination ($R^2$) of 0.72 (Table 5.11).

The concentration of beeswax in the coating significantly decreased $d\Delta E^*$ at the 1% level (Table 5.12). Thus, in the later days of the storage beeswax can significantly slow down the rate of color change in mushrooms. The main effect of beeswax is shown graphically in Figure 5.13.
Table 5.11. ANOVA table for rate of change in color (dΔE*) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>Slope Delta E 6-16</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>23</td>
<td>22.4578</td>
<td>0.976426</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>21.0139</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>22</td>
<td>1.44392</td>
<td>0.0656328</td>
<td></td>
<td>0.256189</td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>1.03008</td>
<td>0.12876</td>
<td>4.35587</td>
<td>0.008</td>
<td>0.358831</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.413842</td>
<td>0.0295601</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>9</td>
<td>0.114367</td>
<td>0.0127075</td>
<td>0.212163</td>
<td>0.978</td>
<td>0.112728</td>
</tr>
<tr>
<td>(Model Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>5</td>
<td>0.299475</td>
<td>0.0598949</td>
<td></td>
<td></td>
<td>0.244734</td>
</tr>
<tr>
<td>(Replicate Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 23  Q2 = 0.171  Cond. no. = 3.2004  DF = 14  R2 = 0.713  Y-miss = 0  Comp. = 2  R2 Adj. = 0.550  RSD = 0.1719

Table 5.12. Regression coefficients of alginate, beeswax, nisin and Na2EDTA concentrations on rate of change in color (dΔE*) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>Slope Delta E 6-16</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.952916</td>
<td>0.0358839</td>
<td>2.23785e-013</td>
<td>0.0632032</td>
</tr>
<tr>
<td>Alg</td>
<td>-0.0206103</td>
<td>0.0722881</td>
<td>0.779732</td>
<td>0.127323</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.170863</td>
<td>0.0465241</td>
<td>0.00251002</td>
<td>0.081944</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.0265524</td>
<td>0.0465241</td>
<td>0.577234</td>
<td>0.081944</td>
</tr>
<tr>
<td>EDT</td>
<td>0.0647367</td>
<td>0.0465241</td>
<td>0.185801</td>
<td>0.081944</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>0.0674567</td>
<td>0.0358839</td>
<td>0.0811001</td>
<td>0.0632032</td>
</tr>
<tr>
<td>Bee*Nis</td>
<td>-0.0951256</td>
<td>0.0607867</td>
<td>0.139922</td>
<td>0.107065</td>
</tr>
<tr>
<td>Bee*EDT</td>
<td>-0.171493</td>
<td>0.0944488</td>
<td>0.0908863</td>
<td>0.166355</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>0.159347</td>
<td>0.0607867</td>
<td>0.0201204</td>
<td>0.107065</td>
</tr>
</tbody>
</table>

N = 23  Q2 = 0.171  Cond. no. = 3.2004  DF = 14  R2 = 0.713  Y-miss = 0  R2 Adj. = 0.550  RSD = 0.1719  Conf. lev. = 0.90
Figure 5.13. Main effect of beeswax on $d\Delta E^*$ during days 6-16 of storage at 12°C

The concentrations of sodium alginate, nisin and Na$_2$EDTA did not show a significant effect on the rate of color change between days 6-16 of storage (Table 5.12). The interaction between nisin and Na$_2$EDTA was significant ($p<0.05$), but the main effects of this interaction were not significant so it is difficult to speculate about the meaning of this interaction. According to Figure 5.14, when the concentration of Na$_2$EDTA is low, an increase in nisin concentration decreases the change in $\Delta E^*$ value; but when the concentration of Na$_2$EDTA is high, nisin increases change in $\Delta E^*$ value.

The interaction between beeswax and Na$_2$EDTA was significant ($p<0.10$). According to Figure 5.15 when the concentration of Na$_2$EDTA was low, beeswax had no effect on decreasing color change. However, when the concentration of Na$_2$EDTA was high beeswax was able to decrease the change in color. This interaction is highly confounded with the main effect of alginate (correlation = 0.77), but in this case the main effect of alginate is not significant and the main effect for beeswax is highly significant ($p<0.01$).
Figure 5.14. Interaction between nisin and Na$_2$EDTA on $d\Delta E^*$ during days 6-16 of storage at 12°C

Figure 5.15. Interaction between beeswax and Na$_2$EDTA on $d\Delta E^*$ during days 6-16 of storage at 12°C
Na₂EDTA had a significant effect on decreasing overall color change rate (dΔE*) in coated mushrooms and on reducing the rate of mushroom browning (dΔL*) on the early days of storage. This is in agreement with a study where cellulose based coating containing lipids, CaCl₂, calcium disodium EDTA and ascorbic acid was used to coat mushrooms. Several combinations of the active ingredients were used. The coating by itself, the coating with EDTA and ascorbic acid, and a treatment of EDTA and ascorbic acid alone resulted in higher L* values compared to mushrooms washed with water, coated mushrooms containing ascorbic acid and CaCl₂, and a treatment of ascorbic acid and CaCl₂ alone after 8 hours of storage at 70°F (Nisperos-Carriedo and others 1991). In another study, Na₂EDTA in combination with sodium erythorbate and cysteine in a mushroom wash has shown to reduce browning compared to unwashed control on whole and sliced mushrooms after 6 and 5 days of storage respectively at 4°C (Sapers and others 1994). Mushrooms washed with solutions containing 1000ppm of EDTA have shown to preserve whiteness more effectively than wash treatments containing combinations of sulfite, fatty acids and ascorbic acid esters (McConnell 1991). Tetrasodium EDTA was shown to improve color in canned mushrooms when added in the brine after vacuum hydration in citric acid (Kilara and others 1984).

Nisin had a significant effect on decreasing overall color change and reducing browning on the early days of storage, this effect is attributed to the inhibition effect of nisin in combination with Na₂EDTA against Pseudomonas sp. (Ukuku and Fett 2002; Economou and others 2009; Lu and others 2010). Microbial load has been related to the overall color quality of fresh mushrooms (Beelman and others 1989), if nisin was able to inhibit growth of Pseudomonas sp. in the early days of storage, this would reduce overall color change and browning.

Beeswax had a significant effect on reducing overall color change throughout the 16 days of storage and reducing change in dΔL* value in the later days of storage (6-16). In sections 5.3.2 and 5.3.3 it will be shown that beeswax had a significant effect in reducing weight gain and increasing weight loss of coated mushrooms. Other studies
have also shown that when a treatment results in higher weight loss, color is improved. Mushrooms treated with vacuum cooling and stored at 18°C after various periods of storage at 5°C have shown less degree of mushrooms browning compared to conventionally cooled mushrooms. The rate of weight loss of the vacuum cooled mushrooms was significantly greater than that of the conventionally cooled mushrooms (p<0.02), after 102 hours of storage at 5°C vacuum cooled mushrooms lost 1.7% more water; the authors speculated that the evaporated water was extracellular and by removing it, bacterial growth was suppressed resulting in less browning (Burton and others 1987a). Fresh mushrooms packaged with 15 g pouches of sorbitol to control humidity of the package have shown less surface moisture, higher weight loss and higher L values (p<0.05) (Roy and others 1995b).

Sodium caseinate coating containing beeswax has shown to preserve green color in fresh cut asparagus spears after 14 days of storage at 4°C and 66% R.H. expressed by significantly lower a* values (more negative = more green) (p<0.05), in this study coated samples with chitosan containing beeswax had significantly higher a* values (less negative = less green). However, the authors attributed this effect to the low pH of the chitosan coating degrading chlorophyll to pheophytin and reducing green color (Fuchs and others 2008). Whey protein concentrate, whey protein isolate and hydroxypropyl methyl cellulose coatings, all of them containing beeswax have shown to significantly reduce browning in fresh cut apple pieces (p<0.05) compared to the same polymers containing carnauba wax. Moreover, both waxes were able to significantly reduce browning index compared to uncoated control stored for 7 days at 5°C or 1 day at 20°C (Perez-Gago and others 2005).

The interaction between nisin and Na₂EDTA was significant during the later days of storage. When Na₂EDTA was held at a low level and nisin was increased dΔL* and dΔE* were reduced. In sections 5.3.2 and 5.3.3 it will be shown that the interaction between nisin and Na₂EDTA had a significant effect in reducing weight gain and increasing weight loss of coated mushrooms. As mentioned before for the main effect of
beeswax, other studies have shown that when a treatment results in higher weight loss, color is improved (Burton and others 1987a; Roy and others 1995b).

5.3.2. Weight gain

Table 5.4 shows % weight gain (%WG) of mushrooms after one hour of drying at 4°C. The regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination (R^2) of 0.74 (Table 5.13).

<table>
<thead>
<tr>
<th>% Weight Gain</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>23</td>
<td>745.783</td>
<td>32.4253</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>705.331</td>
<td>705.331</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>22</td>
<td>40.4512</td>
<td>1.83869</td>
<td></td>
<td>1.35598</td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>7</td>
<td>29.9752</td>
<td>4.28217</td>
<td>6.13139</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>10.476</td>
<td>0.698402</td>
<td>0.835704</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit (Model Error)</td>
<td>10</td>
<td>7.72059</td>
<td>0.772059</td>
<td>1.40097</td>
<td>0.373</td>
<td></td>
</tr>
<tr>
<td>Pure Error (Replicate Error)</td>
<td>5</td>
<td>2.75544</td>
<td>0.551088</td>
<td>0.742353</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 23</td>
<td>Q2 = 0.134</td>
<td>Cond. no. = 2.1574</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>DF = 15</td>
<td>R2 = 0.741</td>
<td>Y-miss = 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Comp. = 2</td>
<td>R2 Adj. = 0.620</td>
<td>RSD = 0.8357</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The concentration of alginate in the coating significantly increased %WG after drying at the 5% level (Table 5.14). The main effect of alginate is shown graphically in Figure 5.16. This effect can be related to the viscosity of the coating, the concentration of alginate significantly increased the viscosity of the coating at the 0.001% level (data not shown), increasing viscosity of the coating results in thicker films (Cisneros Zevallos and Krochta 2003); thus %WG is increased.

The concentration of beeswax in the coating significantly decreased %WG at the 5% level after drying (Table 5.14). The main effect of beeswax is shown graphically in
Figure 5.17. Increasing the amount of beeswax increases hydrophobicity of the coating, and the capacity of the film to bind water might be reduced. After the drying step at 4°C more water would evaporate from the film resulting in lower coating weight.

Table 5.14. Regression coefficients of alginate, beeswax, nisin and Na₂EDTA concentrations on % weight gain after coating

<table>
<thead>
<tr>
<th>% Weight Gain</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>5.21053</td>
<td>0.215852</td>
<td>2.03492e-013</td>
<td>0.378394</td>
</tr>
<tr>
<td>Alg</td>
<td>0.496794</td>
<td>0.22614</td>
<td>0.044161</td>
<td>0.396428</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.58502</td>
<td>0.22614</td>
<td>0.0206265</td>
<td>0.396428</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.288844</td>
<td>0.22614</td>
<td>0.220921</td>
<td>0.396428</td>
</tr>
<tr>
<td>EDT</td>
<td>0.371417</td>
<td>0.22614</td>
<td>0.121296</td>
<td>0.396428</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>0.672368</td>
<td>0.176521</td>
<td>0.00171144</td>
<td>0.309445</td>
</tr>
<tr>
<td>Nis*Nis</td>
<td>0.501835</td>
<td>0.212166</td>
<td>0.0319144</td>
<td>0.371932</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>0.902026</td>
<td>0.295466</td>
<td>0.00805579</td>
<td>0.517958</td>
</tr>
</tbody>
</table>

N = 23    Q² = 0.134    Cond. no. = 2.1574
DF = 15    R² = 0.741    Y-miss = 0
R² Adj. = 0.620    RSD = 0.8357
Conf. lev. = 0.90

Figure 5.16. Main effect of alginate on weight gain after coating
The concentrations of nisin and Na$_2$EDTA did not show a significant effect on coating weight (Table 5.14). The interaction between Na$_2$EDTA and nisin had a significant effect on increasing coating weight (p<0.01) when the concentration of Na$_2$EDTA was high and nisin was increased (Figure 5.18). Since the main effect of nisin and Na$_2$EDTA are not significant it is difficult to speculate about the meaning of this interaction. Chelating agents such as Na$_2$EDTA can be used to control the cross-linking reaction between sodium alginate and calcium chloride (King 1983; Draget 2009). Higher concentration of Na$_2$EDTA would result in less cross-linking, and less cross-linking results in less rigidity and increased moisture content of film (Rhim 2004). This would allow for more space within the matrix to hold bulky molecules like nisin preventing nisin from disrupting the alginate matrix and improving weight gain.

**Figure 5.17.** Main effect of beeswax on weight gain after coating
Figure 5.18. Interaction between Na₂EDTA and nisin on % weight gain after coating
5.3.3. Weight loss

When the data set for mushroom weight loss was evaluated the plots were found to be linear, so for this analysis the data set was not split into two time periods. Table 5.4 shows the rate of mushroom weight loss expressed by the absolute slope of weight (dw). Larger absolute values of dw indicate a faster change in weight. The regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination (R^2) of 0.78 (see Table 5.15)

Table 5.15. ANOVA table for absolute rate of change in weight (dw) during storage of mushrooms at 12°C

<table>
<thead>
<tr>
<th>dw</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>23</td>
<td>42.6833</td>
<td>1.8558</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>42.2091</td>
<td>42.2091</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>22</td>
<td>0.474205</td>
<td>0.0215548</td>
<td>0.146815</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>9</td>
<td>0.369027</td>
<td>0.0410031</td>
<td>5.068</td>
<td>0.004</td>
<td>0.202492</td>
</tr>
<tr>
<td>Residual</td>
<td>13</td>
<td>0.105178</td>
<td>0.00809058</td>
<td>0.0899476</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit (Model Error)</td>
<td>8</td>
<td>0.0582893</td>
<td>0.00728616</td>
<td>0.776971</td>
<td>0.643</td>
<td>0.085359</td>
</tr>
<tr>
<td>Pure Error (Replicate Error)</td>
<td>5</td>
<td>0.0468883</td>
<td>0.00937765</td>
<td>0.0968383</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The concentration of beeswax in the coating significantly increased dw at the 10% level (Table 5.16). Thus, beeswax can significantly increase the rate of mushroom weight loss. The main effect of beeswax is shown graphically in Figure 5.19. In a study where fresh cut Fuji apples were coated with alginate and antibrowning agents, the resistance to water diffusion (calculated from the rate of moisture loss indicated by weight loss) was increased when sunflower oil was added to the coating. However, increasing the oil in the alginate coating from 0.025% to 0.125% did not have an effect on the water vapor resistance (Rojas-Graü and others 2009). Similar results were obtained when fresh cut
papaya was coated with an alginate containing 2% glycerol, 1% ascorbic acid and sunflower oil was added to the coating in the same proportions (Tapia and others 2008).

**Table 5.16.** Regression coefficients of alginate, beeswax, nisin and Na₂EDTA concentrations on absolute rate of change in weight (dw) during storage of mushrooms at 12°C

<table>
<thead>
<tr>
<th></th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.30771</td>
<td>0.026759</td>
<td>4.031e-016</td>
<td>0.0473876</td>
</tr>
<tr>
<td>Alg</td>
<td>0.0278221</td>
<td>0.0243397</td>
<td>0.273622</td>
<td>0.0431032</td>
</tr>
<tr>
<td>Bee</td>
<td>0.0488196</td>
<td>0.0243397</td>
<td><strong>0.0661538</strong></td>
<td>0.0431032</td>
</tr>
<tr>
<td>Nis</td>
<td>0.0563294</td>
<td>0.0243397</td>
<td><strong>0.0376457</strong></td>
<td>0.0431032</td>
</tr>
<tr>
<td>EDT</td>
<td>-0.0368855</td>
<td>0.0243397</td>
<td>0.15359</td>
<td>0.0431032</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>0.0326896</td>
<td>0.0189337</td>
<td>0.107923</td>
<td>0.0335299</td>
</tr>
<tr>
<td>Alg*Alg</td>
<td>0.0446833</td>
<td>0.022662</td>
<td><strong>0.0703039</strong></td>
<td>0.0401322</td>
</tr>
<tr>
<td>Bee*Bee</td>
<td>0.0368273</td>
<td>0.022662</td>
<td>0.128134</td>
<td>0.0401322</td>
</tr>
<tr>
<td>Alg*Nis</td>
<td>-0.126056</td>
<td>0.0318013</td>
<td><strong>0.00161861</strong></td>
<td>0.0563171</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>-0.0926937</td>
<td>0.0318013</td>
<td><strong>0.0120609</strong></td>
<td>0.0563171</td>
</tr>
<tr>
<td>N = 23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2 =</td>
<td>0.181</td>
<td></td>
<td></td>
<td>2.5129</td>
</tr>
<tr>
<td>Cond. no. =</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF = 13</td>
<td>0.778</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>R2 Adj. =</td>
<td>0.625</td>
<td></td>
<td></td>
<td>0.0899</td>
</tr>
<tr>
<td>R2 Adj. =</td>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cond. lev. =</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5.19.** Main effect of beeswax on absolute slope of weight during storage at 12°C
In our work increasing the concentration of beeswax from 0.1 to 0.5% (coded values -1.68 to 0) did not have any effect on the weight loss. However, when beeswax content was increased from 0.5% to 1% (coded values 0 to 1.68) it significantly increased weight loss (p<0.1). This is in agreement with a study where calcium alginate films were incorporated with garlic oil, increasing concentrations of garlic oil from 0 to 0.3% (v/v) did not show significant differences (p>0.05) in water vapor permeability in the films (range 18.73 - 23.42 g mm/m² day kPa). However, when the concentration was increased to 0.4% water vapor permeability significantly increased to 30.89 g mm/m² day kPa (p<0.05). The authors explained this effect with the hydrophobicity of garlic oil which would extend intermolecular interactions of the alginate matrix, allowing moisture to pass through the film (Pranoto and others 2005).

Cohesive strength in films provides a reduction in porosity and permeability to gases and vapors, cohesive strength is enhanced by polarity of the polymer (Kester and Fennema 1986). Increasing concentration of beeswax would reduce the polarity of the alginate matrix decreasing cohesive strength and providing a more permeable film which would result in more weight loss.

In section 5.3.2 it was observed that beeswax significantly reduced the weight gain after coating (p<0.05), increasing amount of beeswax means less alginate available to form the hydrophilic film so the capacity of the film to bind water is reduced. Alginate coatings can act as a sacrificing agent which gives up moisture within the polymer before the food starts dehydrating (Kester and Fennema 1986). A coating with less capacity to bind water would have a reduced capacity to act as a sacrificing agent and weight loss would be increased.

The concentration of nisin in the coating significantly increased dw at the 5% level (Table 5.16). Thus, nisin can significantly increase the rate of mushroom weight loss. The main effect of nisin is shown graphically in Figure 5.20. The negative effect of nisin seemed to be reduced by increasing the concentration of alginate or Na₂EDTA, as
indicated by the significant interactions of nisin and Na₂EDTA and nisin and alginate (Figure 5.21 and Figure 5.22). The concentrations of sodium alginate and Na₂EDTA did not show a significant effect on the rate of weight loss (Table 5.16).

![Graph](image_url)

**Figure 5.20.** Main effect of nisin on absolute slope of weight during storage at 12°C

The interaction between alginate and nisin was significant at the 1% level, when alginate was kept constant at a high level, an increase in nisin concentration had no effect, but when the concentration of alginate was low an increase in nisin concentration significantly increased the rate of weight loss (Figure 5.21). A lower concentration of alginate results in a thinner film which might be disrupted when nisin concentration is increased. If the film is disrupted the barrier properties of the film are reduced resulting in increased weight loss. If the film is thick enough (higher concentration of alginate) increasing the amount of nisin would not disrupt the integrity of the film. In a study where the properties of biodegradable Poly butylene adipate-co-terephthalate (PBAT) films containing nisin were studied, it was found that increasing concentrations of nisin resulted in small pores and holes in the PBAT matrix as observed by scanning electron
microscopy, the authors concluded that the interaction between the polymer and nisin reduced the ability of the polymer to build bonds within its matrix, this would cause a reduction in barrier properties of the film (Bastarrachea and others 2010).

![Graph](image)

**Figure 5.21.** Interaction between alginate and nisin on absolute slope of weight during storage at 12°C. The remaining two factors kept constant at center level.

The interaction between nisin and Na₂EDTA was significant at the 5% level, when Na₂EDTA was kept constant at a high level, an increase in nisin concentration had no effect, but when the concentration of Na₂EDTA was low an increase in nisin concentration significantly increase the rate of weight loss (Figure 5.22). As mentioned in the previous section, Na₂EDTA is used to control cross-linking (King 1983; Draget 2009). Higher concentration of Na₂EDTA would result in less cross-linking, reducing rigidity and increasing moisture content of the film (Rhim 2004). This would allow for more space within the matrix to hold bulky molecules, like nisin, preventing nisin from disrupting the alginate matrix and reducing weight loss.
Figure 5.22. Interaction between Na$_2$EDTA and nisin on absolute slope of weight during storage at 12°C. The remaining two factors kept constant at center level.
5.3.4. Maturity index

To evaluate the effect of coating composition on maturity index the data set was broken in two parts: days 0-6, and days 6-16 of storage. Table 5.4 shows the maturity index expressed by rate change in maturity index (dMI). Higher values of dMI indicate a faster opening of the cap.

For days 0-6 of storage the regression model fitted to the data was not significant (p>0.05), so the response variable (slope of maturity index) was transformed using the power transformation (dMI²) as indicated by the Box-Cox plot. The regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination (R²) of 0.72 (Table 5.17).

<table>
<thead>
<tr>
<th>dMI² 0-6</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>23</td>
<td>0.74598</td>
<td>0.0324339</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>0.694752</td>
<td>0.694752</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>22</td>
<td>0.0512277</td>
<td>0.00232853</td>
<td>0.0482549</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>0.0367496</td>
<td>0.0045937</td>
<td>4.44197</td>
<td>0.007</td>
<td>0.067768</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.0144782</td>
<td>0.00103416</td>
<td>0.0321583</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>9</td>
<td>0.00786381</td>
<td>0.000873756</td>
<td>0.660497</td>
<td>0.723</td>
<td>0.0295594</td>
</tr>
</tbody>
</table>
<pre><code>   | (Model Error) |           |               |         |      |    |
</code></pre>
<p>| Pure Error | 5  | 0.00661438 | 0.00132288    | 0.0363714 |      |    |
| (Replicate Error) |         |               |         |      |    |</p>

N = 23  Q2 = 0.200  Cond. no. = 2.1574
DF = 14  R² = 0.717  Y-miss = 0
R² Adj. = 0.556  RSD = 0.0322
The concentration of nisin in the coating significantly decreased the slope of maturity index at the 1% level (Table 5.18). Thus, nisin can significantly slow down the rate of cap opening during the early days of storage. The main effect of nisin is shown graphically in Figure 5.23, the parabolic shape of this graph explains why the quadratic effect of nisin is significant in the model (p<0.05). The decrease in the slope of maturity index reached a plateau when the concentration of nisin was 6378 mg/mL (coded value 1) and no further decrease in the slope of maturity index was achieved when the concentration was further increased to 8000mg/mL (coded value 1.68). The concentration of sodium alginate, beeswax and Na₂EDTA did not show a significant effect on the slope of maturity index.

**Table 5.18.** Regression coefficients of alginate, nisin and Na₂EDTA concentrations on rate of change in maturity index during days 0-6 of storage at 12°C

<table>
<thead>
<tr>
<th>dMI' 0-6</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.163157</td>
<td>0.0083061</td>
<td>1.37248e-011</td>
<td>0.0146297</td>
</tr>
<tr>
<td>Alg</td>
<td>0.00577591</td>
<td>0.00870198</td>
<td>0.517634</td>
<td>0.015327</td>
</tr>
<tr>
<td>Bee</td>
<td>0.0010772</td>
<td>0.00870198</td>
<td>0.903243</td>
<td>0.015327</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.0257129</td>
<td>0.00870198</td>
<td>0.0104456</td>
<td>0.015327</td>
</tr>
<tr>
<td>EDT</td>
<td>0.00212593</td>
<td>0.00870198</td>
<td>0.810539</td>
<td>0.015327</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>-0.0243066</td>
<td>0.0067926</td>
<td>0.00302546</td>
<td>0.011964</td>
</tr>
<tr>
<td>Nis*Nis</td>
<td>0.0197045</td>
<td>0.00816425</td>
<td>0.0300798</td>
<td>0.0143799</td>
</tr>
<tr>
<td>Alg*Nis</td>
<td>-0.0169148</td>
<td>0.0113697</td>
<td>0.159001</td>
<td>0.0200257</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>-0.0171467</td>
<td>0.0113697</td>
<td>0.15376</td>
<td>0.0200257</td>
</tr>
</tbody>
</table>

N = 23  Q² = 0.200  Cond. no. = 2.1574  DF = 14  R² = 0.717  Y-miss = 0  R² Adj. = 0.556  RSD = 0.0322  Conf. lev. = 0.90
Figure 5.23. Main effect of nisin concentration on rate of change in maturity index during days 0-6 of storage at 12°C

The rate of cap opening has been related to microbial growth throughout storage (Doores and others 1986). Decrease in extent of cap opening together with lower stipe elongation and less microbial counts have been observed when oxine (stabilized chlorine dioxide) was added to watering treatments on mushrooms. The effect was intensified when a combination treatment of oxine and CaCl$_2$ was added to irrigation water reducing not only bacterial counts and both senescence symptoms but also browning (Solomon and others 1991). Both oxine and nisin are antimicrobials, high microbial counts might cause stress to the mushrooms resulting in faster senescence symptoms. If the microbial counts are reduced by an antimicrobial this would slow down the extent of cap opening resulting in lower maturity index. This is in agreement with the main effect of nisin also being significant in decreasing overall color change and reducing browning on the early days of storage (See section 5.3.1.)
For days 6-16 of storage the regression model fitted to the data was not significant (p>0.05), so the response variable (slope of maturity index) was transformed using the power transformation (dMI²) as indicated by the Box-Cox plot. The regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination (R²) of 0.82 (Table 5.19).

Table 5.19. ANOVA table for rate of change in maturity index (dMI)² during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>dMI² 6-16</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24</td>
<td>0.0263134</td>
<td>0.00109639</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>0.0236789</td>
<td>0.0236789</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>23</td>
<td>0.000263444</td>
<td>0.000114541</td>
<td>0.0107024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>11</td>
<td>0.000215653</td>
<td>0.000196049</td>
<td>4.92267</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>12</td>
<td>0.000477908</td>
<td>3.98257e-005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>6</td>
<td>0.00018092</td>
<td>3.01533e-005</td>
<td>0.60918</td>
<td>0.719</td>
<td></td>
</tr>
<tr>
<td>(Model Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>6</td>
<td>0.000296989</td>
<td>4.94981e-005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Replicate Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 24</td>
<td></td>
<td>Q2 = 0.266</td>
<td></td>
<td>Cond. no. = 4.9451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF = 12</td>
<td></td>
<td>R2 = 0.819</td>
<td></td>
<td>Y-miss = 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2 Adj. =</td>
<td></td>
<td>0.652</td>
<td></td>
<td>RSD = 0.0063</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The concentrations of sodium alginate, beeswax, nisin and Na₂EDTA did not show a significant effect on the rate of change in maturity index between days 6-16 of storage time (Table 5.20). The interaction between nisin and Na₂EDTA had a significant effect on decreasing the slope of maturity index (p<0.05) when the concentration of Na₂EDTA was low and nisin was increased (Figure 5.24). However, the main effects of nisin and Na₂EDTA were not significant so it is difficult to speculate about the meaning of this interaction. Nonetheless, this interaction was also significant for dΔL* and dΔE* in the later days of storage and for weight loss throughout storage. As mentioned before for the main effect of nisin, some studies have shown that antimicrobial treatments can
reduce changes in both color and maturity index (Doores and others 1986; Solomon and others 1991). Other studies have shown that when a treatment results in higher weight loss it reduces color change (Burton and others 1987a; Roy and others 1995b). In our work, increasing nisin concentration in the early days of storage increased the rate of weight loss, decreased the rate of color change and maturity index; and in the later days of storage increasing nisin concentration at low levels of Na₂EDTA increased the rate of weight loss, reduced color change and maturity index. It seems that there might be a relationship between the rate of weight loss and the rate of cap opening similar to the one found for the rate of change in color and the rate of cap opening.

**Table 5.20:** Regression coefficients of alginate, beeswax, nisin and Na₂EDTA concentrations on rate of change in maturity index (dMI) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>dMI^6-16</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.0267105</td>
<td>0.0020192</td>
<td>1.62152e-008</td>
<td>0.00359881</td>
</tr>
<tr>
<td>Alg</td>
<td>-0.00462887</td>
<td>0.00265335</td>
<td>0.106598</td>
<td>0.00472895</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.000958176</td>
<td>0.00170768</td>
<td>0.585057</td>
<td>0.00304353</td>
</tr>
<tr>
<td>Nis</td>
<td>0.00174622</td>
<td>0.00170768</td>
<td>0.326684</td>
<td>0.00304353</td>
</tr>
<tr>
<td>EDT</td>
<td>0.00171368</td>
<td>0.00170768</td>
<td>0.335421</td>
<td>0.00304353</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>-0.00561574</td>
<td>0.00131552</td>
<td>0.00109031</td>
<td>0.00234459</td>
</tr>
<tr>
<td>Bee*Bee</td>
<td>0.0027519</td>
<td>0.00157769</td>
<td>0.106649</td>
<td>0.00281186</td>
</tr>
<tr>
<td>Nis*Nis</td>
<td>0.00267212</td>
<td>0.00157769</td>
<td>0.116099</td>
<td>0.00281186</td>
</tr>
<tr>
<td>EDT*EDT</td>
<td>0.00283555</td>
<td>0.00157769</td>
<td>0.0974836</td>
<td>0.00281186</td>
</tr>
<tr>
<td>Alg*Nis</td>
<td>0.00267668</td>
<td>0.00223119</td>
<td>0.25342</td>
<td>0.00397656</td>
</tr>
<tr>
<td>Bee*EDT</td>
<td>-0.00958023</td>
<td>0.00346677</td>
<td>0.0171692</td>
<td>0.00617867</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>0.00645843</td>
<td>0.00223119</td>
<td>0.0134604</td>
<td>0.00397656</td>
</tr>
</tbody>
</table>

| N = 24 | Q2 = 0.266 | Cond. no. = 4.9451 |
| DF = 12 | R2 = 0.819 | Y-miss = 0 |
| R2 Adj. = 0.652 | RSD = 0.0063 |
| Conf. lev. = 0.90 |
Figure 5.24. Interaction between Nisin and Na$_2$EDTA on rate of change in maturity index during days 6-16 of storage at 12°C

The interaction between beeswax and Na$_2$EDTA had a significant effect on decreasing the slope of maturity index (p<0.05) when Na$_2$EDTA was at a high level and beeswax concentration was increased (Figure 5.25). However, this interaction is highly confounded with the main effect of alginate (correlation =0.77), which in this case is almost significant (P=0.106), the contour plots (see Figure 5.41 and Figure 5.42) show that alginate only decreases dMI when beeswax concentration is high and alginate concentration is increased, at medium and lower levels of beeswax, alginate does not affect the slope of maturity index. A higher concentration of alginate results in a thicker coating which will have better barrier properties and lower permeability to gases.
Figure 5.25. Interaction between beeswax and Na$_2$EDTA on rate of change in maturity index during days 6-16 of storage at 12°C

5.3.5. Standard plate count

To evaluate the effect of coating composition on the bacterial growth indicated by standard plate count the data set was broken in two parts: days 0-6, and days 6-16 of storage. Table 5.4 shows the rate of bacterial growth expressed by rate change in Log(Nf/No), (dBG). Larger values of dBG indicate a faster bacterial growth. For days 0-6 of storage the regression model fitted to the data was significant (p<0.05) with no significant lack of fit and a coefficient of determination ($R^2$) of 0.79 (Table 5.21).
Table 5.21. ANOVA table for rate of bacterial growth (dBG) during days 0-6 of storage at 12°C

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>21</td>
<td>1.72668</td>
<td>0.0822228</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>1.6713</td>
<td>1.6713</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>20</td>
<td>0.05538</td>
<td>0.002769</td>
<td></td>
<td></td>
<td>0.0526213</td>
</tr>
<tr>
<td>Regression</td>
<td>10</td>
<td>0.0439044</td>
<td>0.00439044</td>
<td>3.82589</td>
<td>0.023</td>
<td>0.0662604</td>
</tr>
<tr>
<td>Residual</td>
<td>10</td>
<td>0.0114756</td>
<td>0.00114756</td>
<td></td>
<td></td>
<td>0.0338757</td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>7</td>
<td>0.00134329</td>
<td>0.000191898</td>
<td>0.0568177</td>
<td>0.999</td>
<td>0.0138527</td>
</tr>
<tr>
<td>(Model Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>3</td>
<td>0.0101323</td>
<td>0.00337744</td>
<td></td>
<td></td>
<td>0.0581158</td>
</tr>
<tr>
<td>(Replicate Error)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 21  Q2 = 0.637  Cond. no. = 3.9327
DF = 10  R2 = 0.793  Y-miss = 0
Comp. = 3  R2 Adj. = 0.586  RSD = 0.0339

The concentration of alginate in the coating significantly decreased the rate of bacterial growth at the 1% level (Table 5.22). Thus, alginate can significantly slow down the rate of bacterial growth on mushrooms during the early days of storage. The main effect of alginate is shown graphically in Figure 5.26.
Table 5.22. Regression coefficients of alginate, beeswax, nisin and Na₂EDTA concentrations on rate of bacterial growth (dBG) during days 0-6 of storage at 12°C

<table>
<thead>
<tr>
<th>dBG 0-6</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.275573</td>
<td>0.00931383</td>
<td>$4.54376e-011$</td>
<td>0.016881</td>
</tr>
<tr>
<td>Alg</td>
<td>-0.0506657</td>
<td>0.014243</td>
<td>$0.00520514$</td>
<td>0.025815</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.00373545</td>
<td>0.0091667</td>
<td>0.692226</td>
<td>0.0166144</td>
</tr>
<tr>
<td>Nis</td>
<td>-0.00281916</td>
<td>0.0091667</td>
<td>0.764739</td>
<td>0.0166144</td>
</tr>
<tr>
<td>EDT</td>
<td>-0.0486817</td>
<td>0.014243</td>
<td>$0.00657007$</td>
<td>0.025815</td>
</tr>
<tr>
<td>Nis*Nis</td>
<td>0.0100514</td>
<td>0.00871231</td>
<td>0.275449</td>
<td>0.0157908</td>
</tr>
<tr>
<td>Alg*Bee</td>
<td>-0.0432419</td>
<td>0.0186093</td>
<td>$0.0425066$</td>
<td>0.0337289</td>
</tr>
<tr>
<td>Alg*Nis</td>
<td>-0.0330624</td>
<td>0.0119769</td>
<td>$0.0201118$</td>
<td>0.0217077</td>
</tr>
<tr>
<td>Bee*Nis</td>
<td>0.0156646</td>
<td>0.0119769</td>
<td>0.220172</td>
<td>0.0217077</td>
</tr>
<tr>
<td>Bee*EDT</td>
<td>-0.061182</td>
<td>0.0186093</td>
<td>$0.0081817$</td>
<td>0.0337289</td>
</tr>
<tr>
<td>Nis*EDT</td>
<td>-0.0170316</td>
<td>0.0119769</td>
<td>0.185446</td>
<td>0.0217077</td>
</tr>
</tbody>
</table>

N = 21  Q2 = 0.637  Cond. no. = 3.9327
DF = 10  R2 = 0.793  Y-miss = 0
          R2 Adj. = 0.586  RSD = 0.0339
          Conf. lev. = 0.90

Figure 5.26. Main effect of alginate on bacterial growth rate (dBG) during days 0-6 of storage at 12°C
The concentration of Na₂EDTA in the coating significantly decreased the rate of bacterial growth at the 1% level (Table 5.22). Thus, Na₂EDTA can significantly slow down the rate of bacterial growth on mushrooms during the early days of storage. The main effect of Na₂EDTA is shown graphically in Figure 5.27. The concentrations of nisin and beeswax did not show a significant effect on the rate of bacterial growth (Table 5.22).

![Figure 5.27. Main effect of Na₂EDTA on bacterial growth rate (dBG) during days 0-6 of storage at 12°C](image)

The interaction between alginate and nisin had a significant effect on lowering the rate of bacterial growth (p<0.05) when the concentration of alginate was kept at a constant high level and the concentration of nisin was increased (Figure 5.28). Similarly when the concentration of nisin was kept at a constant high level and the concentration of alginate was increased, the rate of bacterial growth was decreased (Figure 5.29).
Figure 5.28. Interaction between alginate and nisin on bacterial growth rate (dBG) during days 0-6 of storage at 12°C

Figure 5.29. Interaction between nisin and alginate on bacterial growth rate (dBG) during days 0-6 of storage at 12°C
The interaction between alginate and beeswax had a significant effect on lowering the rate of bacterial growth (p<0.05) when the concentration was high for alginate and beeswax concentration was increased (Figure 5.30), this interaction is highly confounded with the linear effect of Na$_2$EDTA (correlation = 0.77) which is significant (p<0.01) so it is difficult to conclude if the effect is due to the main effect of Na$_2$EDTA or this interaction.

Figure 5.30. Interaction between alginate and beeswax on bacterial growth rate (dBG) during days 0-6 of storage at 12°C

The interaction between beeswax and Na$_2$EDTA had a significant effect on lowering the rate of bacterial growth (p<0.01) when the concentration of beeswax was at the high level and Na$_2$EDTA was increased (Figure 5.31). However, this interaction is highly confounded with the linear effect of alginate (correlation = 0.77) which is significant in the model (p<0.01) so it is difficult to speculate if the effect is due to the main effect of alginate or this interaction.
Figure 5.31. Interaction between beeswax and Na$_2$EDTA on bacterial growth rate (dBG) during days 0-6 of storage at 12°

For days 6-16 of storage the regression model fitted to the data was significant (p<0.01) with no significant lack of fit and a coefficient of determination ($R^2$) of 0.80 (Table 5.23).

The concentration of alginate and beeswax in the coating significantly decreased the rate of bacterial growth at the 1% and 5% level respectively (Table 5.24). Thus, in the later days of storage time alginate and beeswax can significantly slow down the rate of bacterial growth. The main effect of alginate and beeswax are shown graphically in Figure 5.32 and Figure 5.33. The concentrations of nisin and Na$_2$EDTA did not show a significant effect on the rate of bacterial growth between days 6-16 of the storage (Table 5.24).
Table 5.23: ANOVA table for rate of bacterial growth (dBG) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>dBG 6-16</th>
<th>DF</th>
<th>SS</th>
<th>MS (variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>22</td>
<td>1.09679</td>
<td>0.049854</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>1.08139</td>
<td>1.08139</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total Corrected | 21 | 0.0153987 | 0.000733273 |       |       | 0.027079 |
| Regression      | 9  | 0.0123728 | 0.00137475   | 5.45184 | 0.004  | 0.0370777 |
| Residual        | 12 | 0.00302596| 0.000252163  |       |       | 0.0158796 |

| Lack of Fit (Model Error) | 7  | 0.000166201| 2.3743e-005  | 0.0415123 | 1.000  | 0.00487268 |
| Pure Error (Replicate Error) | 5  | 0.00285976 | 0.000571951  |       |       | 0.0239155 |

- N = 22
- Q2 = 0.693
- Cond. no. = 4.3382
- DF = 12
- R2 = 0.803
- Y-miss = 0
- Comp. = 4
- R2 Adj. = 0.656
- RSD = 0.0159

Table 5.24. Regression coefficients of alginate, beeswax, nisin and Na<sub>2</sub>EDTA concentrations on rate of bacterial growth (dBG) during days 6-16 of storage at 12°C

<table>
<thead>
<tr>
<th>dBG 6-16</th>
<th>Coeff. SC</th>
<th>Std. Err.</th>
<th>P</th>
<th>Conf. int(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.215165</td>
<td>0.00488544</td>
<td>1.22201e-014</td>
<td>0.00870712</td>
</tr>
<tr>
<td>Alg</td>
<td>-0.023695</td>
<td>0.00667658</td>
<td>0.00400421</td>
<td>0.0118994</td>
</tr>
<tr>
<td>Bee</td>
<td>-0.0094215</td>
<td>0.00429701</td>
<td>0.0487823</td>
<td>0.00765837</td>
</tr>
<tr>
<td>Nis</td>
<td>0.00491091</td>
<td>0.00544695</td>
<td>0.385014</td>
<td>0.00970787</td>
</tr>
<tr>
<td>EDT</td>
<td>-0.00754197</td>
<td>0.00667658</td>
<td>0.280713</td>
<td>0.0118994</td>
</tr>
<tr>
<td>Block(1&amp;2)</td>
<td>0.00862885</td>
<td>0.00374099</td>
<td>0.0397218</td>
<td>0.00666738</td>
</tr>
<tr>
<td>Bee*Bee</td>
<td>-0.00646567</td>
<td>0.00409332</td>
<td>0.14019</td>
<td>0.00729534</td>
</tr>
<tr>
<td>Nis*Nis</td>
<td>0.0222097</td>
<td>0.00575688</td>
<td>0.0022771</td>
<td>0.0102602</td>
</tr>
<tr>
<td>Alg*Bee</td>
<td>-0.024481</td>
<td>0.00872337</td>
<td>0.0158558</td>
<td>0.0155473</td>
</tr>
<tr>
<td>Bee*EDT</td>
<td>-0.0331347</td>
<td>0.00872337</td>
<td>0.00253724</td>
<td>0.0155473</td>
</tr>
</tbody>
</table>

- N = 22
- Q2 = 0.693
- Cond. no. = 4.3382
- DF = 12
- R2 = 0.803
- Y-miss = 0
- R2 Adj. = 0.656
- RSD = 0.0159
- Conf. lev. = 0.90
**Figure 5.32.** Main effect of alginate on rate of bacterial growth (dBG) during days 6-16 of storage at 12°C

**Figure 5.33.** Main effect of beeswax on rate of bacterial growth (dBG) during days 6-16 of storage at 12°C
The interaction between alginate and beeswax had a significant effect on decreasing the rate of bacterial growth ($p<0.05$) when the concentration was high for alginate and beeswax was increased (Figure 5.34), this interaction is highly confounded with the main effect of Na$_2$EDTA (correlation $= 0.77$), but in this case the main effect of Na$_2$EDTA is not significant.

![Graph](image)

**Figure 5.34.** Interaction between alginate and beeswax on rate of bacterial growth (dBG) during days 6-16 of storage at 12°C

The interaction between beeswax and Na$_2$EDTA had a significant effect on decreasing the rate of bacterial growth ($p<0.01$) when the concentration was high for beeswax and Na$_2$EDTA was increased (Figure 5.35). However, this interaction is highly confounded with the main effect of alginate (correlation $= 0.77$) and the main effect of alginate is significant at the 1% level.
Figure 5.35. Interaction between Na$_2$EDTA and beeswax on rate of bacterial growth (dBG) during days 6-16 of storage at 12°C

The concentration of alginate in the coating significantly decreased the rate of bacterial growth at the 1% level throughout storage. As explained in section 5.3.2, alginate significantly increased weight gain after coating (p<0.05) this is translated in a thicker coating, which would release more nisin and Na$_2$EDTA onto the mushroom surface. Thus, alginate can significantly slow down the rate of bacterial growth on mushrooms during the early days of storage.

The concentration of Na$_2$EDTA in the coating significantly decreased the rate of bacterial growth at the 1% level in the early days of storage. Indigenous microflora of fresh mushrooms includes 54% of fluorescent Pseudomonas and 10% Flavobacterium, these are both gram-negative bacteria. Nisin is an effective antimicrobial against gram-positive bacteria but has limited activity against gram-negatives. However, gram-negative bacteria can become vulnerable to nisin by exposure to chelating agents such as Na$_2$EDTA (Delves-Broughton and others 1996). Na$_2$EDTA removes cations Mg$^{+2}$ and Ca$^{+2}$, from the cell wall of gram negatives disrupting the phospholipid and lipoprotein
structure of the wall and making it more permeable to antimicrobial agents, this mechanism is shown in Figure 5.36 (Delves-Broughton and others 1996). Increasing the concentration of Na$_2$EDTA would increase the vulnerability of mushroom microflora to nisin and that explains why increasing the concentration of Na$_2$EDTA significantly decreases the rate of bacterial growth on the early days of storage.
Figure 5.36. Mode of action of nisin and Na₂EDTA on gram negative bacteria
It is likely that the main effect of Na$_2$EDTA was not significant in the later days of storage because the diffusion from the coating might have already reached equilibrium by this time. EDTA incorporated at 400ppm in an in vitro study has shown to increase the antimicrobial activity of hydrogen peroxide, octanoic and dodecanoic acids, monocarpynin, ascorbyl decanoate and ascorbyl dodecanoate against *Pseudomonas* isolated from fresh mushrooms. Moreover, when EDTA was incorporated at 1000ppm together with hydrogen peroxide at 10,000ppm in a washing solution for fresh mushrooms, purple blotch disease was inhibited and color was preserved for up to 6 days (McConnell 1991).

The concentration of beeswax did not show a significant effect on the rate of bacterial growth on the early days of storage but it was significant at the 1% level in the latter days of storage. The concentration of beeswax in the coating also reduced the weight gain and increased the weight loss of coated mushrooms (see sections 5.3.2 and 5.3.3). Mushrooms with higher weight loss have lower moisture. Moisture accumulation in the cap has been found to favor growth of *Pseudomonas tolaasii* (Barber and Summerfield 1990), overwatering during growing also increases bacterial populations (Chikthimmah and Beelman 2006). Mushrooms with lower moisture would have less water available for bacterial growth. Fresh mushrooms packaged with 15 g pouches of sorbitol to control humidity of the package have shown reduction in total plate count (p<0.05) (Roy and others 1995b).

The interaction between alginate and nisin had a significant effect on lowering the rate of bacterial growth in the early days of storage. A high concentration of alginate provides a thicker coating which would have a higher capacity to hold the antimicrobial agent, so an increase in the concentration of nisin would decrease the rate of bacterial growth.
The interaction between alginate and beeswax had a significant effect on lowering the rate of bacterial growth throughout storage, this interaction is highly confounded with the linear effect of Na$_2$EDTA (correlation = 0.77) which is significant in the early days of storage but not in the later storage time. Reducing polarity of films reduces the cohesive strength and increases permeability to solutes (Kester and Fennema 1986), an increase in beeswax concentration would reduce polarity of the coating. Alginate is negatively charged and nisin is positively charged, a reduction in polarity of the coating would reduce the electrostatic interaction between alginate and nisin allowing for more nisin to be released from the coating matrix which would reduce the rate of bacterial growth. In preliminary work a 6.5% increase in inhibition zone was observed in agar diffusion bioassay with *Pseudomonas fluorescens*, when the concentration of beeswax was increased from 0.2% to 0.4% in the alginate film containing 750 IU/mL nisin and 5mg/ml of Na$_2$EDTA.

The interaction between beeswax and Na$_2$EDTA had a significant effect on lowering the rate of bacterial growth (p<0.01) throughout the storage time, this interaction is highly confounded with the linear effect of alginate (correlation = 0.77) which is significant in the model (p<0.01) in the early and later days of storage. Nonetheless, this interaction could be explained similarly as the previous interaction; an increase in beeswax concentration would decrease the polarity of the coating and increase the permeability to solutes (Kester and Fennema 1986) allowing more Na$_2$EDTA to be released from the coating and increase vulnerability of gram-negative bacteria to nisin (Delves-Broughton and others 1996) further reducing bacterial growth.

5.3.6. Interpretation and visualization of the model

The models developed in Sections 5.3.1 - 5.3.5 are summarized in Table 5.25.
### Table 5.25. Regression models: effect of coating components on quality parameters of fresh mushrooms

<table>
<thead>
<tr>
<th>Regression</th>
<th>P-value</th>
<th>Slope ΔL* value 0-6</th>
<th>Slope ΔL* value 6-16</th>
<th>Slope ΔE* value 0-6</th>
<th>Slope ΔE* value 6-16</th>
<th>Slope weight loss</th>
<th>% WG</th>
<th>(Slope MI)² 0-6</th>
<th>(Slope MI)² 6-16</th>
<th>BG 0-6</th>
<th>BG 6-16</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Block</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alginate</td>
<td>Coeff</td>
<td>-0.704a</td>
<td>0.115b</td>
<td>0.104b</td>
<td>0.067c</td>
<td>0.033</td>
<td>0.672a</td>
<td>-0.024a</td>
<td>-0.006a</td>
<td>0.009b</td>
<td></td>
</tr>
<tr>
<td>Beeswax</td>
<td>Coeff</td>
<td>-0.122</td>
<td>-0.177a</td>
<td>-0.130b</td>
<td>-0.171a</td>
<td>0.049c</td>
<td>0.585b</td>
<td>0.001</td>
<td>-0.001</td>
<td>-0.004</td>
<td></td>
</tr>
<tr>
<td>Nisin</td>
<td>Coeff</td>
<td>-0.294c</td>
<td>-0.046</td>
<td>-0.162b</td>
<td>-0.026</td>
<td>0.056b</td>
<td>-0.289</td>
<td>-0.026a</td>
<td>0.002</td>
<td>-0.003</td>
<td>0.005</td>
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<tr>
<td>EDTA</td>
<td>Coeff</td>
<td>-0.682b</td>
<td>0.021</td>
<td>-0.165c</td>
<td>0.065</td>
<td>-0.037</td>
<td>0.371</td>
<td>0.002</td>
<td>0.002</td>
<td>-0.049a</td>
<td>-0.008</td>
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<tr>
<td>Alg*BW</td>
<td>Coeff</td>
<td>-0.949a</td>
<td>-0.285b</td>
<td></td>
<td></td>
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<td></td>
<td>-0.043b</td>
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</tr>
<tr>
<td>Alg*Nis</td>
<td>Coeff</td>
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<td>-0.126a</td>
<td>-0.017</td>
<td>0.003</td>
<td>-0.033b</td>
<td></td>
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</tr>
<tr>
<td>Alg*EDTA</td>
<td>Coeff</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Conf BW</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW*Nis</td>
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<td>0.104</td>
<td>-0.095</td>
<td></td>
<td></td>
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<td>0.016</td>
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</tr>
<tr>
<td>BW*EDTA</td>
<td>Coeff</td>
<td>-0.131</td>
<td>-0.171c</td>
<td></td>
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<td></td>
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<td>-0.061a</td>
</tr>
<tr>
<td>Conf Alg</td>
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<td></td>
<td></td>
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<td>-0.033a</td>
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</tr>
<tr>
<td>Nis*EDTA</td>
<td>Coeff</td>
<td>0.131b</td>
<td>0.159b</td>
<td>-0.093a</td>
<td>0.902a</td>
<td>-0.017</td>
<td>0.006a</td>
<td>-0.017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alg²</td>
<td>Coeff</td>
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<td></td>
<td>0.045c</td>
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</tr>
<tr>
<td>BW²</td>
<td>Coeff</td>
<td>0.037</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td></td>
<td></td>
<td>-0.006</td>
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</tr>
<tr>
<td>Nis²</td>
<td>Coeff</td>
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<td></td>
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<td>0.003</td>
<td>0.010</td>
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<tr>
<td>EDTA²</td>
<td>Coeff</td>
<td>0.288c</td>
<td>0.090</td>
<td></td>
<td></td>
<td></td>
<td>0.003c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a, b and c superscripts denote statistical significance at the 1, 5 and 10% levels respectively. †Model would not be significant if this point was not taken out. †† Sample dried out and last measurement was inaccurate.
5.3.6.1. ΔL* Value

From the models developed in section 5.3.1 (see Table 5.25) the contour plots were obtained for each block during the early (0-6) and later (6-16) days of storage. Figure 5.37 and Figure 5.38 show the effect of the concentrations of alginate, beeswax, nisin and Na$_2$EDTA on d ΔL* value on days 0 to 6 of storage for blocks 1 and 2 respectively. In these plots the lower contours mean less change in ΔL* value which is desirable. The highly significant effect of the block can be observed by comparing the values of the contours for both figures, this indicates the high variability between different mushrooms flushes. Even though the value of the rate of change is different, the same trend can be observed in both figures. The significant effect of Na$_2$EDTA can be seen in both plots, as Na$_2$EDTA is increased the slope of ΔL* value decreases. The effect of nisin barely shows up in the plots given that nisin is only significant at the 10% level.

Figure 5.39 and Figure 5.40 show the effect of the concentrations of alginate, beeswax, nisin and Na$_2$EDTA on dΔL* value on days 6 to 16 of storage for blocks 1 and 2 respectively. In these plots the lower contours mean less change in ΔL* value which is desirable. The variability between mushrooms flushes shows up again which changes the values of the contours when comparing both figures but, again, the same trend can be observed. The significant effect of beeswax can be seen in both plots, as beeswax is increased the slope of ΔL* value decreases. The interaction between nisin and Na$_2$EDTA shows up as the saddle points observed in these plots, as explained in Section 5.3.1.1, when the concentration of Na$_2$EDTA is low, an increase in nisin concentration decreases the change in ΔL* value. However, when the concentration of Na$_2$EDTA is high, nisin slightly increases change in ΔL* value.
Figure 5.37. Contour plots for absolute slope of slope of ΔL* value (Block 1) during days 0-6 of storage at 12°C.
Figure 5.38. Contour plots for absolute slope of slope of ΔL* value (Block 2) during days 0-6 of storage at 12°C
Figure 5.39. Contour plots for absolute slope of slope of ΔL* value (Block 1) during days 6-16 of storage at 12°C
Figure 5.40. Contour plots for absolute slope of slope of ΔL* value (Block 2) during days 6-16 of storage at 12°C
In preliminary experiments as shown in Chapter 4, uncoated control stored at 12°C and 60% R.H. reached the Retailer Acceptability Value (RAV) ΔL* of 11 between days 6 and 8 of storage. Our goal was to extend that to 16 days. To calculate the predicted shelf life from the contour plots, one contour was chosen for each storage period. Each contour is a slope which means a specific change in ΔL* value units per day. The contour of 0.5 was chosen for the early days of storage and 0.725 for the later days of storage and the shelf life was predicted as follows:

\[
\frac{0.5}{\text{day}} \cdot 6 \text{ days} = 3 \text{ units in } \Delta L \text{ value}
\]

\[
\frac{0.725}{\text{day}} \cdot 10 \text{ days} = 7.25 \text{ units in } \Delta L \text{ value}
\]

3 units + 7.25 units = 10.25 units in 16 days
5.3.6.2. Maturity index

From the models developed in section 5.3.4 the contour plots were obtained for each block during the early (0-6) and later (6-16) days of storage.

Figure 5.41 and Figure 5.42 show the effect of the concentrations of alginate, beeswax, nisin and Na$_2$EDTA on the slope of maturity index on days 0 to 6 of storage for blocks 1 and 2 respectively. In these plots the lower contours mean less change in maturity index which is desirable. The effect of the block can be observed by comparing the values of the contours for both figures. Even though the value of the rate of change in maturity index varies between flushes, the same trend can be observed in both figures.

Figure 5.43 and Figure 5.44 show the effect of the concentrations of alginate, beeswax, nisin and Na$_2$EDTA on the slope of maturity index on days 6 to 16 of storage for blocks 1 and 2 respectively. In these plots the lower contours mean less change in maturity index which is desirable. The variability between mushrooms flushes can be observed again which changes the values of the contours when comparing both figures. However, the same trend can be observed. The interaction between nisin and Na$_2$EDTA and the interaction between beeswax and Na$_2$EDTA shows up as saddle points and changes in direction of the contours as the concentrations are increased. As explained in Section 5.3.4, when the concentration of Na$_2$EDTA is low, an increase in nisin concentration decreases the change in maturity index. In contrast, when the Na$_2$EDTA is at a high level and beeswax concentration is increased the slope of maturity index decreases.
Figure 5.41. Contour plots for slope of maturity index (Block 1) during days 0-6 of storage at $12^\circ$C
Figure 5.42. Contour plots for slope of maturity index (Block 2) during days 0-6 of storage at 12°C
Figure 5.43. Contour plots for slope of maturity index (Block 1) during days 6-16 of storage at 12°C
Figure 5.44. Contour plots for slope of maturity index (Block 2) during days 6-16 of storage at 12°C.
In preliminary experiments as shown in Chapter 4, uncoated control stored at 12°C and 60% R.H. reached a maturity index of 4 (more than half of the veil partially broken) between days 6 and 8 of storage. Our goal was to extend that to 16 days. To calculate the predicted shelf life from the contour plots one contour was chosen for each storage period. Each contour is a slope which means a specific change in maturity index units per day. The contour of 0.45 was chosen for the early days of storage and 0.16 for the later days of storage and the shelf life was predicted as follows:

\[
\frac{0.45}{\text{day}} \cdot 6 \text{ days} = 2.7 \text{ units in MI}
\]

\[
\frac{0.16}{\text{day}} \cdot 10 \text{ days} = 1.6 \text{ units in MI}
\]

2.7 units + 1.6 units = 4.3 units in 16 days
5.3.6.3. Standard plate count

From the models developed in section 5.3.5 the contour plots were obtained for each block during the early (0-6) and later (6-16) days of storage.

Figure 5.45 shows the effect of the concentrations of alginate, beeswax, nisin and Na$_2$EDTA on the slope of bacterial growth on days 0 to 6 of storage. In this plot the lower contours mean less bacterial growth which is desirable. In the early days of storage the variability between the flushes in bacterial growth was not high enough for the block to be significant. In Figure 5.45 the significant effects of alginate and Na$_2$EDTA can be observed, as the concentration of this components is increased the slope of bacterial growth decreases. The interactions between alginate and nisin, alginate and beeswax and beeswax and Na$_2$EDTA, can be observed as changes in direction in the contours. As explained in Section 5.3.5, the rate of bacterial growth was lowered when the concentration of alginate was kept at a high level and the concentration of nisin was increased, similarly when alginate was kept at a high level and beeswax was increased. Finally when beeswax was at a high level and Na$_2$EDTA was increased the slope of bacterial growth was also decreased.

Figure 5.46 and Figure 5.47 show the effect of the concentrations of alginate, beeswax, nisin and Na$_2$EDTA on the slope of bacterial growth on days 6 to 16 of storage for blocks 1 and 2 respectively. In these plots the lower contours mean less bacterial growth which is desirable. The variability between mushrooms flushes shows up again which changes the values of the contours when comparing both figures. However, the same trend can be observed. The significant effect of alginate and beeswax can be seen in both plots, as the concentration of these components is increased the slope of bacterial growth decreases. The interactions between alginate and beeswax, and beeswax and Na$_2$EDTA, can be observed as saddle points and changes in direction in the contours. As explained in Section 5.3.5, the rate of bacterial growth was decreased when alginate was
at a high concentration and beeswax was increased, similarly when the concentration of beeswax was high and Na$_2$EDTA was increased the bacterial growth was slowed down.
Figure 5.45. Contour plots for slope of bacterial growth rate (dBG) during days 0-6 of storage at 12°C
Figure 5.46. Contour plots for slope of bacterial growth rate (dBG) (Block 1) during days 6-16 of storage at 12°C
Figure 5.47. Contour plots for slope of bacterial growth rate (dBG) (Block 2) during days 6-16 of storage at 12°C.
In preliminary experiments as shown in Chapter 4, uncoated control stored at 12°C and 60% R.H. had a bacterial growth of 1.75 Log CFU/g in the first 3 days of storage. Our goal was to extend that to 10 days. To calculate the predicted shelf life from the contour plots one contour was chosen for each storage period. Each contour is a slope which means a specific change in bacterial growth per day. The contour of 0.175 was chosen for both the early and later days of storage and the shelf life was predicted as follows:

\[
\frac{0.175 \text{ Log}}{\text{day}} \cdot 6 \text{ days} = 1.05 \text{ Logs}
\]

\[
\frac{0.175 \text{ Log}}{\text{day}} \cdot 4 \text{ days} = 0.7 \text{ Logs}
\]

1.05 Logs + 0.7 Logs = 1.75 Logs in 10 days
5.3.7. Identification of optimum conditions

The contours chosen in the previous section were superimposed to identify the appropriate combination of alginate, beeswax, nisin and Na$_2$EDTA. Figure 5.48, represents the region of high concentration of alginate and beeswax of Figure 5.37, Figure 5.41 and Figure 5.45, which belong to the first block during days 0-6 of storage.

![Graph showing contours of dΔL*, dMI, and dBG at +1 level of alginate and beeswax for Block 1 days 0-6.](image)

**Figure 5.48.** Contour lines of slopes of ΔL* value (dΔL), maturity index (dMI) and bacterial growth (dBG) at +1 level of alginate and beeswax for Block 1 days 0-6. Arrows indicate direction of desired region, shaded area indicates overlapping of all contours.
Figure 5.49, represents the region of high concentration of alginate and beeswax of Figure 5.38, Figure 5.42 and Figure 5.45, which belong to the second block during days 0-6 of storage.

**Figure 5.49.** Contour lines of slopes of $\Delta L^*$ value ($d\Delta L$), maturity index ($dMI$) and bacterial growth ($dBG$) at +1 level of Alginate and Beeswax for Block 2 days 0-6. Arrows indicate direction of desired region, shaded area indicates overlapping of all contours.
Figure 5.50, represents the region of high concentration of alginate and beeswax of Figure 5.39, Figure 5.43 and Figure 5.46, which belong to the first block during days 6-16 of storage.

**Figure 5.50.** Contour lines of slopes of ΔL* value (dΔL), maturity index (dMI) and bacterial growth (dBG) at +1 level of Alginate and Beeswax for Block 1 days 6-16. Arrows indicate direction of desired region, shaded area indicates overlapping of all contours.
Figure 5.51 represents the region of high concentration of alginate and beeswax of Figure 5.40, Figure 5.44 and Figure 5.47 which belong to the second block during days 6-16 of storage.

**Figure 5.51.** Contour lines of slopes of ΔL* value (dΔL), maturity index (dMI) and bacterial growth (dBG) at +1 level of Alginate and Beeswax for Block 2 days 6-16. Arrows indicate direction of desired region, shaded area indicates overlapping of all contours.
From each superimposed plot, the region where all contours overlapped was taken and plotted in Figure 5.52. The region with high level of Na$_2$EDTA and medium level of nisin was identified as the one where all the desired contours overlapped. Thus, the desired conditions explained in the previous section were met when alginate, beeswax and Na$_2$EDTA were at a high level (2.49%, 0.82% and 8.18mg/mL respectively) and when nisin was at a medium level (4000 IU/mL).

**Figure 5.52.** Superimposed contour lines from days 0-6 and 6-16 Block 1 and 2, gray shading indicates overlapping of contours.
5.3.8. Validation study

After identifying the desired concentrations of each component of the coating, a validation study was performed where the optimal coating (OC) was used to coat mushrooms. In this study the combination that belonged to the center point of the experimental design (CC) and a combination identified as a sub-optimal coating (SC) were also used (see Table 5.26).

Table 5.26. Composition of coatings for validation study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium Alginate</th>
<th>Beeswax</th>
<th>Nisin</th>
<th>Na\textsubscript{2}EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coded</td>
<td>Actual (%)</td>
<td>Coded</td>
<td>Actual (%)</td>
</tr>
<tr>
<td>Optimal Coating (OC)</td>
<td>+1</td>
<td>2.49</td>
<td>+1</td>
<td>0.82</td>
</tr>
<tr>
<td>Sub-optimal Coating (SC)</td>
<td>-1</td>
<td>1.01</td>
<td>+1</td>
<td>0.82</td>
</tr>
<tr>
<td>Center Point of Design (CC)</td>
<td>0</td>
<td>1.75</td>
<td>0</td>
<td>0.55</td>
</tr>
<tr>
<td>Uncoated washed (UW)</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Uncoated unwashed (UU)</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

The ΔL* value of each coating treatment was compared to that predicted by the model. Figure 5.53 shows that overall, the OC fell within the area delimited by the model for ΔL* value. Figure 5.53 also compares the OC to uncoated washed (UW) and unwashed uncoated (UU) mushrooms.

The model predicted that mushrooms coated with the OC would have a shelf life of 21 days, defined by a retailer acceptability value (RAV) ΔL* of 11 (See Figure 5.53). The lowest shelf life predicted by the model was 13 days, and the highest shelf life was above 25 days (See Figure 5.53). However, the model was developed using data of 16 days of storage. Thus, the shelf life prediction of 21 days obtained with the model was an extrapolation. It is only possible to predict a shelf life above 16 days, and not the exact days of shelf life after that period of time.
The mushrooms coated with the OC reached a ΔL* value of 11 close to day 14 of storage. Uncoated mushrooms reached this threshold between days 6 and 8 of storage. The OC extended shelf life of mushrooms by 7 days when stored at 12°C, which represented temperature abuse (See Figure 5.53).

The model was developed using first and second flush mushrooms. The high variability between flushes resulted in a model which gave a broad range of shelf life prediction. The validation study was performed only on second flush mushrooms to reduce the variability obtained when data was collected from different flushes. This can explain why the shelf life obtained with the validation study was closer to the lower limit predicted by the model.

**Figure 5.53.** ΔL* value predicted by model and experimental validation. Each data point is the average of three replications, error bars represent standard deviation. Dotted line represents ±2 standard deviations of the model.
The maturity index of each coating treatment was compared to that predicted by the model. Figure 5.54 shows that overall, the OC fell within the area delimited by the model for maturity index. Figure 5.54 also compares the OC to uncoated washed (UW) and unwashed uncoated (UU) mushrooms.

Figure 5.54. Maturity index predicted by model and experimental validation. Each data point is the average of three replications, error bars represent standard deviation. Dotted line represents ±2 standard deviations of the model

Shelf life of mushrooms coated with the OC was nearly doubled compared to that of the uncoated control. Further shelf life extension might be achieved in mushrooms stored at 4°C. Visual appearance of uncoated mushrooms and mushrooms coated with the optimal coating at the beginning and end of shelf life is shown in Figure 5.55.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Optimal coating</td>
<td><img src="image1" alt="Image" /></td>
</tr>
<tr>
<td>Washed control</td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>Unwashed control</td>
<td><img src="image7" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 5.55.** Appearance of mushrooms coated with optimal coating, washed-uncoated mushrooms and unwashed-uncoated mushrooms.
The results for the rates of change in quality parameters obtained in the validation experiment and the ones predicted by the model are shown in Table 5.27. The slopes for ΔL* value and maturity index provided by the three coatings (OC, CC, SC) utilized in the validation study did not show a significant difference from those predicted by the model (p>0.05) except for the slope of maturity index from days 6-16 of mushrooms coated with OC. However, on days 0-6 of shelf life the model overestimated the rate of change in maturity index of these mushrooms as can be observed in Figure 5.54, so on the later days of shelf life the rate of cap opening was faster.

The model accurately predicted change in ΔL* value over storage for mushrooms coated with CC. However, for the SC the model overestimated the change in ΔL* value over storage and for the OC the model underestimated the change. Given that the replications of the experimental design were done at the center point it was expected that the model would be more accurate at predicting the performance of the coating that belonged to the center point of the design.

The model was accurate at predicting change in maturity index of mushrooms coated with CC, and the rate of change in maturity index in the early days of storage for the OC and SC. However, for the later days of storage the model overestimated the rate of cap opening for the SC and underestimated the rate of cap opening for the OC. As explained before it was expected that the model would be more accurate at predicting the rate of change in quality parameters at the center point of the design.

The effect of the OC and the SC on ΔL* value and maturity index is compared to the WU and UU in Figure 5.56 and Figure 5.57 respectively.
<table>
<thead>
<tr>
<th>Coating</th>
<th>Composition (coded values)</th>
<th>Time Period (Days)</th>
<th>dΔL</th>
<th>dMI</th>
<th>p-value</th>
<th>Model Predicted</th>
<th>Experimental Validation</th>
<th>p-value</th>
<th>Model Predicted</th>
<th>Experimental Validation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>+1 +1 0 +1</td>
<td>0-6</td>
<td>0.315 ± 0.339</td>
<td>0.612 ± 0.121</td>
<td>0.226</td>
<td>0.415 ± 0.121</td>
<td>0.329 ± 0.043</td>
<td>0.309</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-16</td>
<td>0.587 ± 0.428</td>
<td>1.182 ± 0.130</td>
<td>0.083</td>
<td>0.137 ± 0.031</td>
<td>0.213 ± 0.022</td>
<td>0.027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0 0 0 0</td>
<td>0-6</td>
<td>0.658 ± 0.339</td>
<td>0.757 ± 0.143</td>
<td>0.665</td>
<td>0.404 ± 0.121</td>
<td>0.333 ± 0.022</td>
<td>0.376</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-16</td>
<td>0.881 ± 0.433</td>
<td>1.023 ± 0.285</td>
<td>0.662</td>
<td>0.163 ± 0.031</td>
<td>0.195 ± 0.023</td>
<td>0.232</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>-1 +1 -1 -1</td>
<td>0-6</td>
<td>1.161 ± 0.339</td>
<td>0.812 ± 0.139</td>
<td>0.175</td>
<td>0.409 ± 0.121</td>
<td>0.375 ± 0.046</td>
<td>0.668</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-16</td>
<td>1.085 ± 0.428</td>
<td>1.023 ± 0.226</td>
<td>0.836</td>
<td>0.232 ± 0.031</td>
<td>0.187 ± 0.009</td>
<td>0.072</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.56. Effect of coatings on $\Delta L^*$ value during storage. (Data represents average of three replications; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)

Figure 5.57. Effect of coatings on maturity index during storage. (Data represents average of three replications; error bars represent standard deviation; different letters within each day are significantly different by Duncan's Multiple Range Test at 5% probability level)
The OC significantly reduced the change in ΔL* value compared to the SC from days 2 through 8 of storage (p<0.05). After day 12 no significant difference was found between the OC and SC. The OC significantly reduced change in ΔL* value compared to UW and UU throughout the 16 days of storage (p<0.05). The SC significantly reduced change in ΔL* value compared to UW after day 4 of storage and after day 6 of storage compared to UU.

The OC significantly reduced change in maturity index compared to UU and UW from days 6 to 16 of storage (p<0.05). No significant differences were found in maturity index between mushrooms coated with the OC and the SC. The SC significantly reduced change in maturity index compared to UW after day 6 of storage and after day 8 of storage compared to UU.
5.4. Summary

Mushrooms flushes were shown to have high variability indicated by significance of the block. Alginate concentration increased coating weight and reduced the rate of bacterial growth throughout storage. Beeswax reduced overall color change, indicated by $d\Delta E^*$ value, increased weight loss throughout storage and reduced change in $d\Delta L^*$ value and bacterial growth in the later days of storage. Nisin decreased $d\Delta E^*$, $d\Delta L^*$ values, and maturity index on the early days of storage. Nisin also increased weight loss but significant interactions indicated that at high concentrations of Na$_2$EDTA and alginate, this effect was minimal. The concentration of Na$_2$EDTA in the coating reduced $d\Delta E^*$, $d\Delta L$ values, and bacterial growth in the early days of storage.

Several interactions were significant, among them, the interaction between nisin and Na$_2$EDTA was found significant in several of the quality parameters. Increasing nisin concentration at low levels of Na$_2$EDTA increased the rate of weight loss, reduced color change and maturity index. In some cases this interaction was found significant even when the main effects of nisin and Na$_2$EDTA were not.

A better understanding of these results could be obtained by performing additional work. The effect of alginate, beeswax, nisin and Na$_2$EDTA concentration on physical properties of the coating and the respiration rate of mushrooms could be evaluated. These results could later be related to the results obtained in this study.

The effect of the coating on the microbial quality of mushrooms could be better understood by using a different method to evaluate the microbial quality of mushrooms. Standard plate count gives the total aerobic microflora, and even if nisin was suppressing the bacteria responsible for browning, some other bacteria could be growing in its place. The microbial quality of mushrooms should be evaluated by performing an inoculation study with fluorescent Pseudomonas prior to coating.
The OC contained 2.49% alginate, 0.82% beeswax, 8.18mg/mL Na$_2$EDTA and 4000IU/mL nisin. $\Delta L^*$ and MI of mushrooms with OC were compared to those of the C. The OC significantly reduced $\Delta L^*$ and MI, extending the shelf life by 7 days. These results indicate that the shelf life of fresh mushrooms coated with the OC was nearly doubled. Moreover, further shelf life extension might be obtained if mushrooms were stored at 4°C.

This coating has shown to improve quality parameters of mushrooms and increase shelf life. Work is yet to be done to evaluate the effect of the coating on the sensory attributes of fresh mushrooms. In this research project only two hurdle technologies were evaluated: edible coatings and refrigeration. Later on these technologies could be combined with antimicrobial wash to find if further shelf life extension can be obtained.
5.5. Conclusions

The concentration of alginate in the coating significantly increased coating weight (p<0.05) and reduced the rate of bacterial growth (p<0.01) throughout the 16 days of storage. Alginate concentration did not have a significant (p>0.10) effect on color, weight loss or maturity index.

Beeswax concentration in the coating significantly reduced overall color change (p<0.05), indicated by Δ𝐸* value, increased weight loss (p<0.10) throughout the 16 days of storage and significantly reduced change in Δ𝐿* value (p<0.01) and bacterial growth (p<0.05) in the later days of storage (6-16).

Nisin significantly decreased Δ𝐸* (p<0.05), Δ𝐿* (p<0.10) values, and maturity index (p<0.01) on the early days of storage. Nisin also significantly (p<0.05) increased weight loss. However, significant (p<0.01) interactions between nisin and Na₂EDTA and nisin and alginate indicated that at high alginate or high Na₂EDTA nisin did not increase weight loss.

The concentration of Na₂EDTA in the coating significantly reduced Δ𝐸* (p<0.10), Δ𝐿 (p<0.05) values, and bacterial growth (p<0.01) in the early days of storage.

Increasing nisin concentration at low levels of Na₂EDTA during the later days of storage significantly increased the rate of weight loss (p<0.01), reduced color change (p<0.05) and reduced maturity index (p<0.01).

The optimum coating obtained by response surface methodology contained 2.49% alginate, 0.82% beeswax, 8.18mg/mL Na₂EDTA and 4000IU/mL nisin. The experimental validation showed that the optimal coating fell within the predicted limits obtained from the model for Δ𝐿* value and maturity index.
No significant (p>0.05) differences were obtained when comparing the slopes of ΔL* value and maturity index predicted by the model to the ones obtained from the experimental validation except for the slope of maturity index from days 6-16 of mushrooms coated with optimal coating. However, on days 0-6 of shelf life the model overestimated the rate of change in maturity index of these mushrooms, so on the later days of shelf life the rate of cap opening was faster.

The optimal coating significantly (p<0.05) reduced the change in ΔL* value compared to the sub-optimal coating from days 2 through 8 of storage and compared to washed and unwashed control throughout the 16 days of storage.

The optimal coating significantly (p<0.05) reduced change in maturity index compared to uncoated controls washed and unwashed from days 6 to 16 of storage. No significant differences (p>0.05) were found in maturity index between mushrooms coated with the optimal coating and the sub-optimal coating.

Shelf life of mushrooms coated with optimal coating was extended from 7 to 14 days when stored at 12°C and 60% relative humidity.
References


Chapter 6

SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

6.1. Summary

Sodium alginate concentration in the coating significantly reduced the rate of bacterial growth (p<0.01) throughout storage and increased weight gain (p<0.05) after coating. Alginate concentration did not have a significant (p>0.10) effect on color, weight loss or maturity index.

Beeswax concentration in the coating significantly reduced overall color change (p<0.05), indicated by dΔE* value, increased weight loss (p<0.10) throughout the 16 days of storage and significantly reduced change in dΔL*value (p<0.01) and bacterial growth (p<0.05) in the later days of storage (6-16).

Nisin significantly decreased dΔE* (p<0.05), dΔL* (p<0.10) values, and maturity index (p<0.01) on the early days of storage. Nisin also significantly (p<0.05) increased weight loss, but significant interactions indicated that at high concentrations of Na₂EDTA and alginate, this effect was reduced. Nisin concentration did not have a significant (p>0.10) effect on bacterial growth.

The concentration of Na₂EDTA in the coating significantly reduced dΔE* (p<0.10), dΔL (p<0.05) values, and bacterial growth (p<0.01) in the early days of storage. Na₂EDTA concentration did not have a significant (p>0.10) effect on weight loss or maturity index.
Increasing nisin concentration at low levels of Na\textsubscript{2}EDTA during the later days of storage significantly increased the rate of weight loss (p<0.01), reduced color change (p<0.05) and reduced maturity index (p<0.01).

The optimum coating obtained by response surface methodology (2.49% alginate, 0.82% beeswax, 8.18mg/mL Na\textsubscript{2}EDTA and 4000IU/mL nisin) significantly (p<0.05) reduced the change in ΔL* value compared to the sub-optimal coating from days 2 through 8 of storage and compared to washed and unwashed control throughout the 16 days of storage. This coating also reduced change in maturity index significantly (p<0.05) compared to uncoated controls washed and unwashed from days 6 to 16 of storage. No significant (p>0.50) differences were found in maturity index between mushrooms coated with the optimal coating and the sub-optimal coating.

The shelf life of fresh mushrooms coated with optimal coating was extended from 7 to 14 days when stored at 12°C and 60% relative humidity
6.2. Suggestions for future research

- Study the effectiveness of alginate based coatings on maintaining the microbial quality of mushrooms by doing a challenge study with fluorescent *Pseudomonas*.

- Determine the effectiveness of alginate based coatings on maintaining the microbial safety of mushrooms by doing a challenge study with *Listeria monocytogenes*, and *Staphylococcus aureus*.

- Study the effect of alginate, beeswax, nisin and Na$_2$EDTA concentration on physical properties of the films: water vapor permeability, oxygen permeability, tensile strength and elongation at break; at high relative humidity.

- Determine the effect of alginate based coatings on the respiration rate of mushrooms and relate it to effect on maturity index and color.

- Determine the effect of an antimicrobial wash together with alginate based coatings on the quality and shelf life of fresh mushrooms.

- Evaluate the effect of alginate based coatings on sensory attributes of fresh mushrooms.

- Study the effect of alginate based coatings on the quality and shelf life of sliced mushrooms.