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Whey protein isolate coating and concentration effects on egg shelf life

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Abstract: The influences of three different concentrations (6, 12 and 18%) of whey protein isolate (WPI) coatings on shelf-life enhancements of the fresh egg quality (weight loss, pH, Haugh unit, yolk index and colors) and the shelf life were evaluated at room temperature. All coated eggs showed lower weight loss than uncoated eggs. Less weight loss (2.46 for 12% WPI and 2.38 for 18% WPI) was observed in WPI-coated eggs. Haugh units (HU) indicated that coated eggs remained in grade 'A' during 3 weeks storage period, whereas uncoated (UC) changed from grade 'A' to 'B' after 1 week of storage. The HU and yolk-index (YI) values of all WPI-coated eggs were significantly higher than those of UC. Among the coated eggs, there were no significant differences in HU, but 12 and 18% WPI coated had higher YI than WPI 6% coated and UC. The albumen pH of the UC eggs was significantly higher than that of coated eggs. Yolk lightness (*L) and (*b**); shell (*a**) and (*b**) of coated eggs were not different from UC after 4 weeks. Performance of WPI coatings depended on the concentration up to 12% but not between 12 and 18%. Results also indicated that WPI coatings served as protective barrier for shelf life of the eggs.**

Keywords: eggshells; coating; whey protein isolate; shelf life; Haugh unit; yolk index

INTRODUCTION

Interest in edible films and coatings has been gaining ground because of consumer demands for higher quality foods with fresh appearance and easy access to materials.[1] Edible films and coatings are an attractive addition to current packaging. They provide a semi-permeable barrier against gases and moisture, thereby reducing respiration, water loss and oxidation reaction rate. When applied to food, they enhance shelf life and improve the quality of enrobed products without any environmental burden.[1–5]

Whey protein (WP) films are one of the most widely studied edible films. They provide an effective way to utilize excess WP, a by-product of the dairy industry. Substantial progress has been made in understanding the basic chemical and structural properties of WP.[6] Formulation technology and application of both WP fraction and isolate films have been extensively investigated.[7–10] The films have several desirable characteristics for flexible packaging. They are flexible and transparent.[10] Because of the high content of hydrogen bonds in WP, they have an excellent gas barrier function under dry condition.[11] The protein concentration in the solution casting of whey protein films is important for the final quality.[10,11] Oxygen permeability of these films was comparable with that of ethylene–vinyl alcohol copolymer (EVOH) at low or intermediate RH.[7,10]

Food industries continually seek cost-effective solutions to extend shelf life and improve the quality of the product delivered to the end user. The use of edible whey protein films to coat the surface of foods may effectively solve storage and transportation problems in addition to reduction of food quality losses due to damage and extension of shelf life.[12]

Eggs are a common, inexpensive, versatile and nutritious part of human diet.[13–19] Economic losses of eggs during storage and handling was estimated to be in excess of ten million dollars annually.[15] During storage, eggs can rapidly lose their quality as albumen, yolk, weight and pH change.[13–19] Tiny pores on eggshells cause mass transfer, mainly carbon dioxide and moisture, resulting in change in egg yolk and albumen as well as weight loss. In order to slow down the change in egg quality, the pores on eggshells need to be sealed. Even a small percentage improvement in the overall quality could result in significant economic savings to the egg industry.[15]

A process such as coating could increase the available storage time for shell eggs. Efforts are made to reduce economical loss of eggs by using coating of oil and various food grade materials. The coatings delay the interior quality deterioration rate of egg

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and improve mechanical properties of eggshells.[15–21] Wong *et al*[15] reported the effect of various coatings (mineral oil, soy protein, wheat gluten or corn zein) can enhance mechanical properties of shell eggs and interior quality. Chitosan coating is effective in preserving the interior quality of eggs.[16] Herald *et al*[20] studied the quality of eggs coated with wheat gluten solution. Xie *et al*[21] showed that soy protein isolate, whey protein isolate (WPI) and wheat gluten coating can enhance mechanical properties of shell eggs and minimize egg microbial contamination. Protecting egg surfaces with coatings (chitosan, WPI and shellac) offered improved sensory attributes and longer shelf life. Coating made by WP had the best consumer perception among the three materials[13] because of high gloss and transparency of WP coating.[8]

Since external appearance is an important parameter to consumer perception and purchasing decision, WP has high potential to be used as a coating on egg shell.[13] However, to date little or no work has been done on the effect of coating formulation on the quality of eggs. The present study aimed at investigating three concentrations (6, 12 and 18%) of WPI coatings on shelf-life enhancements of the fresh egg quality (weight loss, pH, Haugh unit and yolk index) during 4 weeks and yolk and shell color after 4 weeks of storage at ambient conditions.

MATERIALS AND METHODS

Preparation of whey protein isolate coating solution

Whey protein isolate coating solutions were prepared with 6, 12 and 18% (w/w protein) by dissolving the WPI (Davisco Foods International Inc, Eden Prairie, MN) in distilled water. Glycerol was then added to give plasticizer: protein ratios of (w/w) (WPI:glycerol (2.5:1)). The glycerol was added in solution while the solution was stirred continuously on a magnetic stirrer at 80 °C for 30 min under neutral pH.[3,10,13]

Eggshell coating

A batch of fresh grade chicken eggs was supplied by local producer in Canakkale. After washing with water dried eggs were immersed in the coating solutions by hand for 1 min, repeated one more time and than dried at ambient temperature for 1 day. The eggs were dried before being placed on a mold-pulp container, and then stored at ambient laboratory conditions during the experiment (around 25 °C for 4 weeks).

Samples were divided into four groups, one control and three with coatings. Ten separate eggs for each group (control, 6, 12 and 18%) were drawn at each storage interval for evaluation.

Determination of moisture loss

Weight loss of eggs was calculated as the reduction in weight expressed as a percentage of the original weight.[16] Ten eggs for each treatment were measured and recorded to within 0.001 g. The average weight loss was calculated.

Haugh unit and yolk index

Haugh unit was calculated by the following formula:[13,16]

$$\text{Haugh unit} = 100 \log(H - 1.7\, G^{0.37} + 7.6)$$

where H is the height of the thick albumen in mm and G is the mass of the whole egg in g.

The parameter H was estimated by averaging three measurements carried out in different points of thick albumen at the distance of 10 mm from the yolk using a digital caliper (CD-15CP, Mitutoya Ltd, Hampshire, UK). AA, HU above 72; A, HU 71 to 55; B, 54 to 31; C, below 30.

Yolk index was calculated as yolk height/yolk width. Yolk width was measured with digital caliper (CD-15CP, Mitutoya Ltd, UK).[6] A fresh, good-quality egg has a yolk index of around 0.45.

pH measurement

After albumen height (mm) was measured, albumen was separated from yolk. The volumes (ml) of firm and thin albumen were homogenized for 20 s by a Waring Blender Model 32 BL 80 (Waring Com, Torrington, Connecticut) and then measured by a pH 210 meter (Hanna Inst, Woonsocket, RI).

Shell and yolk color

The color of shell[15] and yolk[16] was measured with Minolta Chroma Meter Model CR-300 (Minolta Co Ltd, Osaka, Japan) equipped with a DP-300 data processor. CIE L^* a^* b^* color space was used to determine the color of the shell and yolks. Five eggshells were crushed so that the coated shell surface could be evenly distributed inside the measurement cup. The shell[15] and yolk[16] were scanned at four different locations and averaged. Results were expressed as $L - a - b$ values (lightness, redness, yellowness). A single numerical value, ΔE^*_{ab} was used to indicate the size of color differences when compare to control and was calculated by the following equation:[7]

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where $\Delta L^* = L_{\text{Coating}} - L_{\text{Control}}$; $\Delta a^* = a_{\text{Coating}} - a_{\text{Control}}$; $\Delta b^* = b_{\text{Coating}} - b_{\text{Control}}$

Chroma and Hue angle were also calculated by the following equations:

$$\text{Chroma} = (a^{*^2} + b^{*^2})^{1/2}$$

$$\text{Hue angle} = \tan^{-1}\left[\frac{b^*}{a^*}\right]$$

Data analysis

This study evaluated the combined effect of coating and storage time on the egg properties of interest.

Analysis of variance was carried out on all the measured parameters between the control and WPI-coated eggs. Every treatment was performed twice. Statistical procedures were done using least-square means (LSM-PROG GLM) of the statistical analysis software program.[21] p-Values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

Weight loss

The weight loss during storage is caused mainly by evaporation of water and loss of carbon dioxide from the albumen trough the pores of shells. This is one of the important measurements to monitor the changes in quality of fresh shell eggs during storage. The overall weight loss of eggs coated with 18% WPI were similar to those of 12% WPI, and had lower weight loss than control and 6% of WPI treatment. Compared with the uncoated eggs (5.66%), significantly lower weight loss reduction (3.63%) was observed for eggs coated with 18% of WPI (Table 1). Eggs coated with 6, 12 and 18% of WPI did not exhibit a significant moisture loss until 3 weeks of storage. After 4 weeks of storage, eggs coated with 12 and 18% of WPI had a 3.63 and 3.72% moisture loss, whereas, WPI 6% and the control had losses of 4.17 and 5.66%, respectively. During the 4-week storage period, significant differences in weight loss were not observed between WPI 12% and WPI 18% coated eggs and served as the most effective in reducing water diffusion through the eggshell (Table 1).

Caner[13] reported that the lowest weight loss (0.526%) was observed in shellac-coated eggs. Coated eggs with chitosan and whey protein isolate also had a statistically significant lower weight loss than uncoated (UC) eggs. Wong *et al*[15] reported that corn zein exhibited the least moisture loss, while wheat gluten, soy protein isolate, egg albumen and mineral oil had also less moisture loss than uncoated eggs. Different coating material can enhance protective barrier properties of shell eggs, and minimize weight loss, thus helping to extend shelf life.

Table 1. Effect of coating on LSMEAN values of the weight loss

Coating	1 week	2 weeks	3 weeks	4 weeks
Control	1.91^{a}	3.01^{b}	4.28^{c}	5.66^{d}
WPI 6%	1.30^{e}	2.19^{a}	3.07^{b}	4.17^{c}
WPI 12%	1.24^{e}	2.03^{a}	2.85^{b}	3.72^{f}
WPI 18%	1.11^{e}	1.99^{a}	2.78^{b}	3.63^{f}
Standard error	(0.1135)	(0.1135)	(0.1135)	(0.1135)

LSMEAN (SE: standard error) in the table followed by different letters differ significantly.

Haugh unit

Changes in the Haugh unit (HU) (used for measurement of the albumen quality) of uncoated and WPI coated eggs are shown in Table 2. The HU of the uncoated eggs were significantly lower than that of coated eggs (Table 2). After 1-week storage period, the uncoated and coated eggs exhibited significant differences in HU, whereas, no significant decreases in HU were observed between 6, 12 and 18% WPI coated eggs during 4 weeks (Table 2). Overall, the HU significantly decreased with increasing storage periods from 69.72 to 41.45 (Table 2). The HU of the uncoated eggs were decreased more rapidly than coated eggs (Table 5). These results were in agreed with Bhale *et al*[16] and also Wong *et al*.[15]

The Haugh unit values indicated that uncoated eggs changed in quality from grade 'A' (HU >55) to grade 'B' after 1 week of storage (Table 3). While eggs coated with the WPI 6% remain in grade A over 2 weeks, eggs coated with WPI 12% and 18% remain grade A over 3 weeks of storage (Tables 2 and 3).

Caner[13] reported that among the coated eggs, the shellac eggs had the highest value of HU during the storage. Chitosan and shellac effectively maintained eggs at grade A over 3 weeks and WPI over 2 weeks. Bhale *et al*[16] reported that chitosan-coating can maintain grade A during a 2-week storage period, and grade B during 5 weeks of storage. Wong *et al*[15] reported that uncoated eggs changed from grade A to B after 1 week of storage and coated with different coating materials (soy, corn) were still in grade B after 28 days of storage. Shell eggs coated with wheat gluten

Table 3. Grade of coated eggs during 4 weeks storage based on the HU

Coating	0 week	1 week	2 weeks	3 weeks	4 weeks
Control	A	A	B	B	B
WPI 6%	A	A	A	B	B
WPI 12%	A	A	A	B/A	B
WPI 18%	A	A	A	A/B	B

The grade A, or B is given an egg based upon interior and exterior quality, not size.
A grade >55, B range from 31 to 54; C <30.

Table 2. Effect of coating on LSMEAN values of the HU

Coating	0 week	1 week	2 weeks	3 weeks	4 weeks
Control	69.70^{a,h} (2.65)	57.73^{c,g} (1.87)	51.09^{h} (1.53)	46.09^{l} (1.53)	41.45^{m} (1.32)
WPI 6%	69.70^{a} (2.65)	63.53^{b} (1.53)	55.88^{c,d} (1.53)	51.89^{d,k,h} (1.53)	48.79^{e,h,k,l} (1.41)
WPI 12%	69.70^{a} (2.65)	65.77^{a,b} (1.67)	57.94^{c} (1.67)	54.88^{d,c,h} (1.41)	51.97^{d,e,h} (1.53)
WPI 18%	69.70^{a} (2.65)	65.25^{a,b} (1.87)	58.16^{c} (1.67)	55.77^{c,d} (1.41)	52.81^{d,e,g,h} (1.87)

LSMEAN (SE: standard error) in the table followed by different letters differ significantly.

solutions resulted in maintaining quality grade A for 30 days at room temperature.[21]

Yolk index

The yolk index (YI) of the uncoated eggs was significantly lower than all coated eggs. Overall, the YI significantly decreased with increasing storage periods (Table 4). After 2 weeks of storage, YI values of uncoated eggs decreased from 0.37 to 0.25. While the YI value of 6, 12 and 18% WPI coated eggs after 4 weeks of storage were 0.26, 0.28 and 0.29, respectively; YI of the uncoated was 0.22. The YI values of coated eggs decreased by 0.084 to 0.108 after 4 weeks of storage, whereas YI values of the control eggs decreased by 0.155 (Table 4). These YI values at 4 weeks were similar to control values at 2 weeks (Table 4). According to these results WPI coating has significant preservative potential effects on the YI values during the storage period. The coatings with 12 and 18% WPI were able to preserve the yolk quality for at least 2–3 weeks longer than control at room temperature.

pH measurement in albumen

The pH of albumen is an important factor in quality retention. An egg initially contains about 30 ml of dissolved carbon dioxide (CO_2) existing in the carbonate form, all in the white (albumen). Moisture and carbon dioxide in the white evaporate through the pores, allowing more air to penetrate the shell. The pH of albumen is initially about 7.6–8 but, as the egg ages and carbon dioxide escapes through the pores of shell, increasing alkalinity, the pH eventually increases to 8.9–9.4.[22,23] It is with this loss of CO_2 that the pH of the egg becomes more basic and structural changes take place in the albumen and result in a thinning of the albumen.[24,25] The albumen pH measures primarily the freshness of the egg.[25] The WPI coating had significant effect controlling the pH of the coated eggs after 1 week. Coated eggs had significantly lower pH value than uncoated eggs after 1 week (Table 5). Eggs coated with 12 and 18% WPI exhibited significantly lower pH, which is an indication of a lower loss of CO_2 in albumen, than 6% WPI and uncoated eggs after 1 week of storage. Overall, the pH of the all eggs albumen significantly increased with storage periods. During storage periods, the egg ages, the CO_2 escapes which significantly increases the pH from 7.65 to 9.56 (Table 5). After 2 weeks of storage, the average pH of the albumen in uncoated increased and reached 9.4 which is an indication of a greater loss of CO_2. WPI 18% and 12% exhibited similar pH in albumen and significantly lower pH than WPI 6% and uncoated eggs (Table 5).

According to these result, eggshell coating decreases carbon dioxide permeation through the egg shell. The WPI coatings act as barrier and help diffuse gases less rapidly through the shell. The results agree with those of Caner[13] and of Scott and Silversides.[26]

Shell color

A functional property such as color and visual appearance is one of the main factors responsible for the consumers decision to purchase products. Discoloration of products may lead to dissatisfaction for consumers. The L^* values, an indication of lightness or brightness of the shell, ranged from 90.73 to 92.11, indicating very bright shell. All coated eggs have a higher L^* values because of the glossier surface. The (a^*) and b value ranges for all treatments were in gray region (Table 6).

Table 6. Color parameters as lightness (L), greenness ($-a$), yellowness (b), ΔE^*_{ab}, Chroma and Hue values of uncoated and coated eggs shell after 4 weeks of storage at room temperature

Coating	L^* value	a^* value	b^* value	ΔE^*_{ab}	Chroma	Hue
Control	90.03^{a}	-0.93^{a}	4.94^{a}	–	5.02	79.45
WPI 6%	$92.11^{b,c}$	-0.94^{a}	3.67^{a}	2.43	3.79	75.62
WPI 12%	$91.67^{b,c}$	-0.77^{a}	3.90^{a}	1.94	3.93	78.83
WPI 18%	$90.70^{a,c}$	-0.84^{a}	4.13^{a}	1.05	4.22	78.47

Table 4. Effect of coating on LSMEAN values of the yolk index

Coating	0 week	1 week	2 weeks	3 weeks	4 weeks
Control	0.376^{a} (0.014)	$0.292^{b,h}$ (0.009)	$0.27^{c,k}$ (0.007)	0.252^{d} (0.008)	0.221^{e} (0.007)
WPI 6%	0.376^{a} (0.014)	$0.340^{f,l,j}$ (0.009)	$0.306^{b,g,h}$ (0.008)	$0.288^{b,c}$ (0.007)	$0.268^{d,k}$ (0.007)
WPI 12%	0.376^{a} (0.014)	0.346^{f} (0.009)	$0.323^{g,l}$ (0.008)	$0.307^{b,g}$ (0.007)	$0.287^{b,c,k}$ (0.008)
WPI 18%	0.376^{a} (0.014)	0.353^{f} (0.009)	$0.321^{g,j}$ (0.008)	$0.313^{g,h}$ (0.008)	$0.292^{b,h}$ (0.009)

LSMEAN (SE: standard error) in the table followed by different letters differ significantly.

Table 5. Effect of coating on LSMEAN values of the pH

Coating	0 week	1 week	2 weeks	3 weeks	4 weeks
Control	7.65^{a} (0.049)	8.14^{b} (0.034)	9.16^{c} (0.028)	9.42^{d} (0.026)	9.66^{e} (0.024)
WPI 6%	7.65^{a} (0.049)	7.99^{f} (0.040)	8.98^{k} (0.028)	9.35^{l} (0.026)	9.55^{l} (0.026)
WPI 12%	7.65^{a} (0.049)	$7.95^{f,j}$ (0.034)	8.77^{g} (0.028)	9.26^{h} (0.026)	$9.41^{d,l}$ (0.028)
WPI 18%	7.65^{a} (0.049)	7.87^{j} (0.034)	8.83^{g} (0.028)	$9.22^{c,h}$ (0.026)	$9.40^{d,l}$ (0.031)

LSMEAN (SE: standard error) in the table followed by different letters differ significantly.

Table 7. Color parameters as lightness (*L*), greenness (-*a*), yellowness (*b*), ΔE^*_{ab}, Chroma and Hue values of uncoated and coated eggs yolk after 4 weeks of storage at room temperature

Coating	L^* value	a^* value	b^* value	ΔE^*_{ab}	Chroma	Hue
Control	60.51^{a}	7.84^{a}	25.57^{a}	—	26.69	72.91
WPI 6%	61.46^{a}	8.41^{a,b}	25.49^{a}	1.10	26.85	71.74
WPI 12%	59.72^{a}	9.63^{a,b}	24.46^{a}	2.22	26.29	68.51
WPI 18%	58.31^{a}	11.88^{b}	26.62^{a}	4.73	29.15	65.94

The L, a^* and b^* values measured were converted to total color difference ΔE^*_{ab} values as reference. Although ΔE^*_{ab} of the coated eggs increased with the WPI concentration up to 12%, but 18% WPI had the lowest ΔE^*_{ab} value. The coated eggs had ΔE^*_{ab} values of 1.05–2.43, depending on the concentration used (Table 6). However, it is known that ΔE^* values less than 3.0 cannot be easily detected by the naked human eye.[8]

The chroma value indicates the degree of saturation of color and is proportional to the strength of the color. Little change was found in chroma between coated and uncoated egg shell. The response surface for chroma showed a similar trend to that of the b value (Table 7). This is expected as chroma depends mainly on b^* value because a^* values have smaller magnitudes in the present experiments. Hue angle value describes a dimension of color when we look at color and is defined as starting at $+a$ axis. It is expressed in degrees 0° (red), 90° (yellow). The Hue angle values also ranged from about 75.62 to 79.34.

Yolk color

The desired yolk color varies with geographical location and customer, but, in general, a fairly light color is preferred.[16] Color is mainly dependent upon yolk carotenoids (lutein, zeaxanthin, β-cryptoxanthin and other) content. Carotenoids can be degraded by oxidative process, changing yolk pigmentation during storage. The L^* values for uncoated, 6, 12 and 18% WPI-coated eggs yolk were 60.52, 61.46, 59.72 and 58.31, respectively after 4 weeks of storage and are presented in Table 7. The a^* values varied between +7.84 and 11.88 indicating ($+a^*$) redness. The b^* ranged form 25.57 to 26.62 indicating yellowness.

The ΔE^*_{ab} values of coated eggs yolk varies between 1.10 and 4.73 (Table 7). Overall, ΔE^*_{ab} increased with WPI concentration used. The only ΔE^*_{ab} values of 18% WPI-coated egg yolk were more than 3.0 that can be easily detected by the naked eye.[8] Chroma values for the yolk of the 18% WPI-coated eggs were higher than others. This indicated that at the end of the experiment, the yolk of coated eggs with the 18% WPI had more intense color than others. The Hue angle values also ranged from about 65.94 to 72.91. As the WPI concentration increased, Hue values decreased.

CONCLUSIONS

Coating with different percentages of WPI in solution, especially 12 and 18% WPI, were effective in preserving the interior quality of eggs. WPI has been successfully used for extending shelf life of the fresh egg quality when stored at room temperature. As percentage concentration increased until 12%, performance of coating on the interior quality of shell eggs increased. Coating with 12 and 18% WPI maintained quality of the fresh shell eggs during storage. These facts may help industry in decreasing economic losses during storage.

It may be desirable to apply antimicrobial and antioxidant coating to minimize egg microbial contamination. Effect of coating on the mechanical properties (puncture and impact strength) of eggshell needs also to be performed with a view to reducing the percentage breakage of eggshells.

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