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Protein-based coatings on peanut to minimise oil migration

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ABSTRACT

Soy protein isolate (SPI) or calcium caseinate (CC)-based coatings were applied on peanuts as a barrier of oil migration, and their efficiency was evaluated. These coatings induced a decrease of oil migration as compared to the uncoated peanuts. Subsequently, three polysaccharides were individually added to the coating solution to prevent oil migration further. The incorporation of carboxymethylcellulose (CMC) with SPI and CC coating solutions significantly ($p \leqslant 0.05$) decreased the migration of oil as compared to the coatings without CMC. The addition of pectin seemed unfavourable because it enhanced the migration of oil. Starch in SPI-based coating solution helped to decrease the oil migration, but it did not affect the migration level in samples treated with CC solution. The results from size-exclusion chromatography showed strong cross-links by glutaraldehyde and transglutaminase treatments in SPI and CC-based coating solutions. A strong cross-linking in protein-based coating correlated closely with a reduction in oil migration. For both proteins, the cross-linking by transglutaminase was most efficient for improving the effectiveness of coating. This study demonstrated that our protein-based coatings can be effectively implemented to food industry for better quality of peanut products.

1. Introduction

The average annual peanut consumption is 2.7 kg per person in Canada (PBC., 2005). More than 70% of the peanuts consumed in Canada were imported from the United States in 2005. In the US, peanuts are consumed at an approximate level of 798 million kg, and the average peanut consumption is 2.7 kg per person in 2007 (NASS, 2008). Peanuts contain about 40–55% of lipids depending on cultivar and seasonal growing conditions (Ahmed & Young, 1982). When peanuts are enrobed by other confectionery product like chocolate, the lipid as a form of oil from peanut itself migrates to the outer chocolate layer over time (Brake & Fennema, 1993). The elements of these oils most likely to migrate are fatty acids with short chains or unsaturated fatty acids, and they have the lowest melting point and greatest fluidity, respectively (Nelson & Fennema, 1991). The oil migration from peanuts would result in a quality loss of confectionery products during the storage. For example, the oil migration to the outer chocolate layer can soften the texture of the chocolate layer, and reduce flavour by oxidation of fatty acids (Brake & Fennema, 1993). The absorption of moisture in the air and incompatibility of lipids are two important factors that make chocolate soft and sticky. In addition, the migrated oil can change the crystalline structure of chocolate, and it causes a fatty oil bubble to the surface of chocolate, so-called fat bloom. Chocolate surface can become dry over time, and thereby it changes the taste of products (Beckett, 1990; Cakebread, 1972). All of these phenomena would reduce consumers' acceptability of products.

The amount of oil that migrates depends on several factors, including the composition of fatty acids, storage time and temperature (Shuleva, 1989; Wootton, Weeden, & Munk, 1970). First, the migration of oil decreases with an increase in the solid fraction of the lipid (Wacquez, 1975). Secondly, during the early days of storage, the migration of oil occurs quite rapidly, and decreases over time (Wacquez, 1975; Wootton et al., 1970). Lastly, an increase of the storage temperature accelerates the rate of oil migration (Wootton et al., 1970). Confectionery products are usually stored at room temperature (17–23 °C) and even at the ambient temperature, and thus the degree of oil migration is quite significant (Wacquez, 1975).

Previous studies have demonstrated that the quality of chocolate-enrobed peanut products could be improved by applying an edible coating layer between chocolate and peanut. Dried cellulose films were efficacious as a lipid barrier, but these fine films were sensorially detectable in the mouth (Fennema & Kester, 1993). The migration of lipids could be reduced by both hydroxypropylated starch coating with high amylose content (70%) (Jockay, Nelson, & Powell, 1967) and hydrocolloid coating consisting of high methoxyl pectin (Brake & Fennema, 1993). A confectioner's glaze on peanuts helped to inhibit lipid migration (Minson, 1990). Whey protein-based coatings on roasted peanuts were highly effective against lipid oxidation due to the excellent oxygen barrier

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characteristics (Lee, Trezza, Guinard, & Krochta, 2002; Maté & Krochta, 1996).

As an extension of these studies, we have developed a dairy or a soy protein-based edible coating solution with different treatments, such as an addition of polysaccharides and heat, chemical and enzymatic treatments, which could be an effective barrier to migration of lipids. It has been noted that preventing oil migration from peanut is significant to control the quality of peanut products. These treatments could affect the polymer network in coating solution, and therefore we focused on how the change of polymer network could affect the properties of polymer coating to prevent oil migration from peanut. As an intervening layer, it should adhere easily to peanut, and should not crack or develop undesirable sensory characteristics during the storage. The specific objectives were to (i) determine the effect of SPI and CC coatings on migration of oil from the peanut; (ii) determine the effect of polysaccharide addition, such as CMC, pectin, and starch, to the protein-based coating solutions; and (iii) evaluate physical (heating), chemical (glutaraldehyde) and enzymatic (transglutaminase) treatments on cross-linking of SPI and CC coating solutions, and their barrier property against the migration of oil.

2. Materials and methods

2.1. Preparation of coating solution

The formulation established by Ressouany, Vachon, and Lacroix (2000) served as a model to prepare different coating solutions. Their coating solution was composed of 5% (w/v) CC, 2.5% (w/v) glycerol, and 0.25% (w/v) CMC, solubilised in distilled water under stirring. In our study, we modified the composition of coating solution to improve the performance of coating against the migration of oil. First, two proteins, namely CC (93% protein content, w/w; New Zealand Milk Product Inc., Santa Rosa, CA, USA.) and SPI (90% protein content, w/w; Supro 500E Dupont, Wilmington, DE, USA.), were individually dissolved in distilled water at 5% (w/v, final) concentration. Then, 0.5% (w/v, final) of CMC (medium viscosity 400–800 cps, Sigma, St. Louis, MO, USA.) was added to the protein solution to evaluate its effect. Also, two other polysaccharides, pectin (apple pectin, Sigma, St. Louis, MO, USA.) or starch (potato starch, Sigma, St. Louis, MO, USA.), were added to the mixture at 0.25% (w/v, final) concentration under stirring. Finally, 10% (w/v, final) of glycerol (Laboratory Mat, QC, Canada) was added to the protein coating solution as a plasticiser. The pH of solution was adjusted to 7 with 1 M Na_2CO_3. The solution was degassed using a vacuum for about 1–2 h depending on their viscosity.

In addition, we prepared a sugar-based coating formula as a positive control, which could be commercially used for peanut coatings to compare its effectiveness with the protein-based coatings. This sugar-based coating solution was composed of 40% (w/v, final) sugar (Glucose 43, Nealanders International Inc., Mississauga, ON, Canada) and 10% (w/v, final) gum arabic (Alland & Robert, Port-Mort, France), which were dissolved in distilled water at room temperature (21 °C) with gentle stirring. The solution was degassed using a vacuum for about 1–2 h.

2.2. Treatments on coating solution

2.2.1. Heating treatment

Aqueous protein coating solution composed of 5% (w/v) CC or SPI was heated to 90 °C allowing the rearrangement of protein structure. Coating solutions were heated on a hot plate with stirring at a medium speed for 30 min, and then cooled down rapidly by placing on an ice bath for 30 min. The volume of evaporated water by heating was adjusted to keep the initial concentration of the components. Thereafter, CMC at a concentration of 0.5% (w/v) and other polysaccharides (pectin or starch) at a concentration of 0.25% (w/v) were added to the coating solution. Finally, the glycerol at a concentration of 10% (w/v) was added and mixed completely with agitation at a medium speed. The pH of solution was adjusted at 7 with 1 M Na_2CO_3 and degassed using a vacuum for about 1–2 h.

2.2.2. Chemical treatment

The chemical process was characterised by adding glutaraldehyde (CAS Number: 111-30-8, 25% aqueous solution, Sigma, St. Louis, MO, USA.) to establish cross-linking by a covalent bond between the protein chains. Glutaraldehyde is unlikely to be employed as a food ingredient, but commonly used in cross-linking proteins. Glutaraldehyde has GRAS (generally recognised as safe) approval for use as a food additive in the USA (FDA., 2006). Once the protein solution was treated with heating as previously described above, the temperature was lowered to 50 °C to accelerate the effect of chemical agent. The pH was adjusted to 7.0 with 1.0 M NaOH, and the glutaraldehyde was added to the protein solution at a concentration of 2.5% (w/v) with agitation at medium speed for 30 min. Finally, the solution was degassed using a vacuum for about 1–2 h.

2.2.3. Enzymatic treatment

The enzymatic treatment on protein was carried out using transglutaminase (EC Number: 2.3.2.13) according to Motoki, Aso, Seguro, and Nio (1987). Transglutaminase used in all treatments was Activa TG–TI enzyme (99% maltodextrin and 1% microbial transglutaminase, Ajinomoto USA, Inc., Teaneck, NJ, USA). The activity of transglutaminase was determined by the manufacturer as approximately 100 units/g transglutaminase. Once the heating treatment on protein solution was completed as described above, the temperature was lowered to 50 °C to optimise the catalytic reaction of enzyme. The pH was stabilised at 7.0 with 1.0 M NaOH, and then transglutaminase was added to the solution at a ratio of 4 units/g of protein. The enzyme was inactivated after 2 h by placing the solution in a water bath at 85 °C for 20 min. The solution was degassed using a vacuum for about 1–2 h.

2.3. Study of oil migration from peanut

The migration of oil was measured based on the method described by Brake and Fennema (1993). Unsalted peanuts (kernels) were purchased from a local grocery store. Peanuts were weighed before and after being coated using protein-based (SPI or CC) or sugar-based (glucose) solutions. The coating procedure of peanuts was as follows. Peanut attached to a needle was dipped 3 times in the coating solution and dried using a blow dryer for 1 h. When the last dipping was complete, the needle was removed from the peanut and the hole by the needle was filled with a drop of coating solution. Thereafter, peanuts were left at least 24 h at room temperature for a uniform drying of the solution. Once peanuts were completely dried, they were weighed and inserted by 5 pieces in a 5 ml amber bottle. In this bottle, 3 g of tricaprylin (97–98% saturated oil, Sigma, St. Louis, MO, USA.) was added, which was sufficient to cover the 5 peanuts. The bottles were purged with nitrogen gas, sealed hermetically and stored at 30 °C for up to 12 weeks. Bottles were prepared in triplicate for each storage time. The storage temperature was modified at 30 °C to accelerate the oil migration for 12 weeks of storage period. At the same time, a peanut without coating was also prepared as a control.

In order to determine the degree of the oil migration from peanuts, the presence of linoleic acid migrated from peanuts in tricaprylin was analysed by the AOAC (1990) method. Linoleic acid is an unsaturated fatty acid which is a main element of oils migrated from peanut (Nelson & Fennema, 1991). This method determined the presence of methylene-interrupted cis,cis-unsaturated fatty

acids (nonconjugated). Lipoxygenase which catalyses the oxidation of unsaturated fatty acids to form a product with conjugated unsaturation was used to convert linoleic acids into two double bonds conjugated. The product was detected by using an UV-spectrophotometer (DMS 200, Varian Co., Walnut Creek, CA, USA.) at a wavelength of 234 nm.

In this study, 2.5 g of tricapryline was removed from the bottle stored with peanuts, and diluted 100 folds with hexane. Thereafter, 1 ml was withdrawn and evaporated to dry with a stream of nitrogen. Fatty acids were saponified with 1.0 M potassium hydroxide (Mat Laboratory, Beauport, QC, Canada) for 5 h under the darkness at room temperature. Then, fatty acids were diluted 100 folds in a 1.0 M borate buffer. Three-ml of this solution was taken into 4 different bottles. In the first 2 bottles, 0.1 ml of the solution containing active lipoxygenase was added to 50,023 units/ml. In the other 2 bottles, they represented as the blanks by adding inactive lipoxygenase heated at 90 °C for 5 min. Samples were incubated at room temperature for 30 min under agitation and measured the absorbance at 234 nm in each storage day. A standard curve, linear between 2 and 12 μg linoleic acid per ml sample, was constructed using known amounts of trilinolein (Sigma, St. Louis, MO, USA.), which is equivalent to linoleic acid. The amount of migration of oil from peanut was expressed as μg linoleic acid per g peanut after considering dilution factors.

2.4. Size-exclusion chromatography

All of the protein solutions were filtered through 0.45 μm nylon membrane filters (WVR, Mississauga, ON, Canada). Prior to filtration, protein solutions were treated by heating, chemical agent or enzyme, as described previously. Nontreated protein solution served as a control. To determine the changes in molecular weight of proteins, a size-exclusion chromatography was performed with soluble protein fractions using a Varian Vista 5500 HPLC at 25 °C. Protein was detected using a standard UV detector set at 280 nm. Two Supelco Progel TSK PWH and GMPW columns (Supelco, Bellefonte, PA, USA) followed by two Waters Ultrahydrogel columns 2000 and 500 (Waters, Mississauga, ON, Canada) were used to determine the molecular weight of the cross-linked proteins after different treatments. The limit of maximum molecular weight was determined as 25×10^3 kDa based on linear polyethylene glycol (PEG). The eluant (80% v/v aqueous and 20% v/v acetonitrile) was flushed through the columns at a flow rate of 0.8 ml/min. The aqueous portion of the eluant was 0.02 M tris buffer (pH = 8.0) and 0.1 M NaCl. The molecular weight of protein solutions were determined by the comparison between the elution times of the solutions based on their molecular weight. The molecular weight calibration curve was established using a series of protein molecular weight markers (MW-GF-1000 Sigma, St. Louis, MO, USA) ranging from 2×10^3 to 29 kDa.

2.5. Statistical analysis

Statistical analyses were performed using SPSS software (Version 10.1). The average rate of oil migration for each solution was compared with the One-way ANOVA test (Duncan and LSD). All comparisons were performed in triplicate and two samples per replication were analysed.

3. Results and discussion

3.1. Effect of protein-based coatings on oil migration

The SPI and CC coatings on peanuts induced a significant ($p \leqslant 0.05$) decrease of the oil migration by 22.3% (from 99.22 to 77.08 μg linoleic acid/g peanut) and 16.0% (from 99.22 to 83.31 μg linoleic acid/g peanut), respectively, as compared to the uncoated peanuts (Fig. 1). To increase further the capacity of protein coating to decrease peanut oil migration, protein cross-linking methods were used. Protein cross-linking can be achieved mainly in three different ways such as physical (heat or radiation), chemical (cross-linking agent like glutaraldehyde) and enzymatic (transglutaminase) treatments (Gerrard, 2002). First, we investigated whether physical cross-linking of the protein by heating affected the rate of oil migration from peanuts which were stored in the bottles with nitrogen gas and sealed hermetically for 12 weeks at 30 °C. As a barrier of the oil migration, a slight increase in efficiency was observed after 12 weeks when the protein solutions were treated with heating (Fig. 1). Heating treatment improved slightly the efficiency of SPI and CC coatings in preventing oil migration. Heating treatment decreased oil migration by 6.7% in SPI coating (from 77.08 to 71.94 μg linoleic acid/g peanut) and 3.4% in CC coating (from 83.31 to 80.51 μg linoleic acid/g peanut), respectively. Moreover, heating treatment on coating solutions induced a significant ($p \leqslant 0.05$) decrease of the oil migration by 27.5% (from 99.22 to 71.94 μg linoleic acid/g peanut) for SPI coating and 18.9% (from 99.22 to 80.51 μg linoleic acid/g peanut) for CC coating, respectively, as compared to the uncoated peanuts.

According to McHugh, Aujard, and Krochta (1994), heating treatment would cause the deformation (denaturation), rearrangement, and aggregation of proteins, and thus promotes the formation of either covalent or noncovalent intra/inter-molecular cross-links. In specific, heat-induced cross-linking by cysteine sulphydryl groups is called as thermocondensation. Heat treatment allows the formation of intra/inter-molecular disulphide bonds in protein chains by thiol-disulphide interchange and thiol oxidation reactions. The SPI has several cysteines in its structure, but the CC has few of this amino acid which provides thiol groups necessary for cross-linking. Cross-links in SPI have been demonstrated by Hoffman, Sala, Olieman, and De Kruif (1997). They achieved the cross-linking of the proteins by heating at 90 °C for 30 min for SPI. Meanwhile, heat treatment was less effective on CC to bring about cross-links, but CC was cross-linked when treated by irradiation (Letendre, 1999). From the results, it is reasonable to assume that protein cross-link induced by disulphide bond formation plays a role to prevent oil migration in case of SPI. Likewise, increased barrier property after heat treatment for CC might be explained

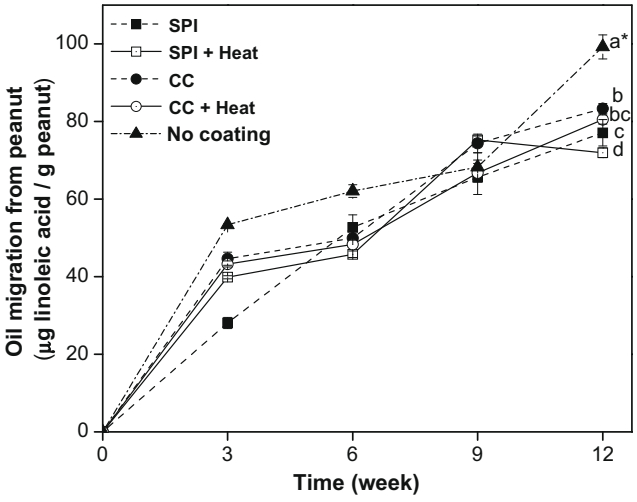

Fig. 1. Effect of protein-based coatings and heating treatment (90 °C) on oil migration. Protein concentration was 5% (w/v), and glycerol concentration was 10% (w/v). Storage temperature was 30 °C. *Means at one time with different letters are significantly different ($p \leqslant 0.05$).

by other structural changes like heat induced denaturation, rather than disulphide bond formation.

3.2. Effect of CMC addition to protein-based coatings on oil migration

Polysaccharides are the most commonly used molecules to produce biodegradable films due to their ability to form stable and rigid gels. Polysaccharide films or coatings can preserve sensory attributes of food, retard lipid oxidation, and suppress the release/migration of oil (Kester & Fennema, 1986; Krochta & De Mulder-Johnson, 1997). To enhance the barrier property of protein coating against oil migration, we added one of the polysaccharides, CMC into the protein solution after heat treatment for physical cross-link. Addition of CMC (0.5% w/v) in SPI- and CC-based coating solutions was strongly efficient in improving barrier property, as compared to the cases with heat treatment only (Fig. 2). Result showed that the presence of CMC caused a significant ($p \leqslant 0.05$) decrease of oil migration by 27.5% (from 71.94 to 52.18 μg linoleic acid/g peanut) in SPI coating and 26.5% (from 80.51 to 59.16 μg linoleic acid/g peanut) in CC coating, respectively. Moreover, the migration of oil decreased by 47.4% (from 99.22 to 52.18 μg linoleic acid/g peanut) and 40.4% (from 99.22 to 59.16 μg linoleic acid/g peanut) in SPI and CC coating solutions, respectively, as compared to the uncoated control peanuts. As a result, the addition of a cationic polysaccharide like CMC in protein-based coating solution was highly favourable as compared to the protein-based coating solution without CMC, improving a barrier property against oil migration.

This improved barrier property by the addition of polysaccharide in protein coating solution can be explained by its cationic characteristic. In protein, some of the amino acids have either positive or negative charges, so the intermolecular electrostatic interactions among protein molecules attribute to maintain a compact network, restricting the spaces required for the passage of oil. However, it is important to note that both SPI and CC have mainly negative charges at neutral pH because the isoelectric point (pI) of SPI is between pH 5 and 7, and the pI of CC is ≈4.6. On the other hand, CMC is partially charged in solution by the presence of cationic carboxyl groups. Consequently, further ionic interactions between proteins (SPI or CC) and cationic CMC can stabilise the protein network in this condition (Hidalgo & Hansen, 1971).

3.3. Effect of pectin addition to protein-based coatings on oil migration

In addition to heat treatment for physical cross-linking and CMC addition, we added pectin to the protein coating solution for further improvement. The pectin-based films play an important role in preventing the loss of moisture, as well as the migration of lipid in food (Kester & Fennema, 1986; Krochta & De Mulder-Johnson, 1997). The addition of pectin (0.25% w/v) in SPI or CC coating solution containing CMC after heating, significantly increased the migration of oil during storage at 30 °C for 12 weeks (Fig. 3). Result showed that the presence of pectin caused a significant ($p \leqslant 0.05$) increase of oil migration by 34.5% (from 52.18 to 70.20 μg linoleic acid/g peanut) in SPI coating and 17.3% (from 59.16 to 69.40 μg linoleic acid/g peanut) in CC coating, respectively, as compared to the coatings without pectin. It did not show any additional barrier property to oil migration. Nevertheless, these values with pectin (70.20 μg linoleic acid/g peanut for SPI and 69.40 μg linoleic acid/g peanut for CC) were lower than the control sample without protein coating (99.22 μg linoleic acid/g peanut).

Pectin is a linear polysaccharide with a repetitive unit of α-(1 → 4)-linked D-galacturonic acid bound to form the pectin-backbone. This polysaccharide is particularly abundant in fruits and is predominantly found in cell membranes and intercellular material of plants. The presence of polar groups such as methoxyl-, carboxyl- or hydroxyl groups in the D-galacturonic acid would improve networks in protein-based solutions (Imeson, Ledward, & Mitchell, 1977). However, it is known that pectin is negatively charged at pH > 3.5, and therefore it does not adsorb on to caseinate-coated film because of electrostatic repulsion (Liu, Verespej, Alexander, & Corredig, 2007). In our case, both SPI and CC have a net negative charge at pH 7, thus an electrostatic repulsion between the protein and pectin could allow more space for the migration of oils and reduce the barrier properties to oil migration.

3.4. Effect of starch addition to protein-based coatings on oil migration

Opposite to pectin, the addition of starch (0.25% w/v) in SPI-based coating solution containing CMC after heat treatment significantly ($p \leqslant 0.05$) lowered the migration of oil by 10.8% (from 52.18 to 46.52 μg linoleic acid/g peanut) after 12 weeks (Fig. 4). It seemed to improve the network in the case of SPI-based coating

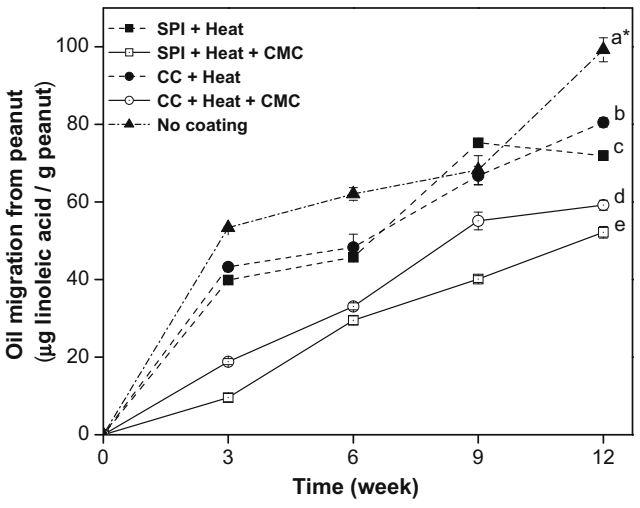

Fig. 2. Effect of CMC addition (0.5% w/v) to protein-based coatings on oil migration. Protein concentration was 5% (w/v), and glycerol concentration was 10% (w/v). Heating temperature was 90 °C. Storage temperature was 30 °C. *Means at one time with different letters are significantly different ($p \leqslant 0.05$).

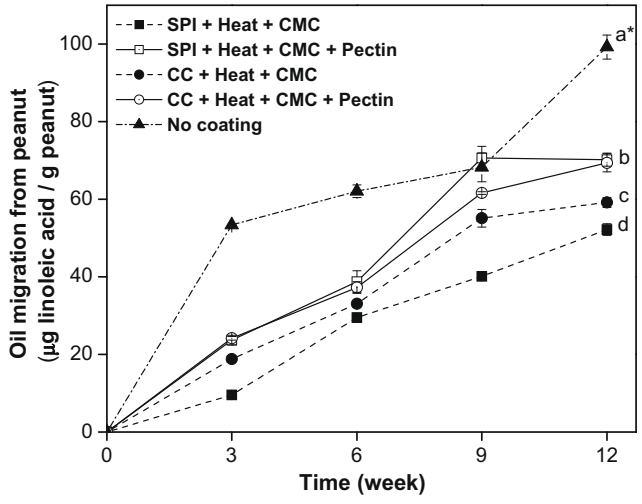

Fig. 3. Effect of pectin addition (0.25% w/v) to protein-based coatings on oil migration. Protein concentration was 5% (w/v), and glycerol concentration was 10% (w/v). CMC concentration was 0.5% (w/v). Heating temperature was 90 °C. Storage temperature was 30 °C. Means at one time with different letters are significantly different ($p \leqslant 0.05$).

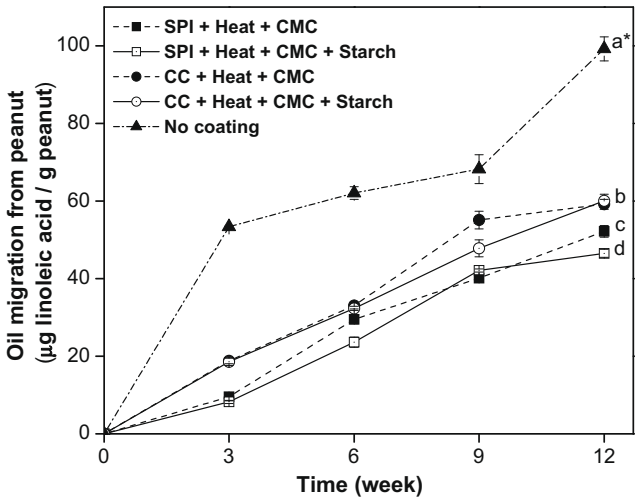

Fig. 4. Effect of starch addition (0.25% w/v) to protein-based coatings on oil migration. Protein concentration was 5% (w/v), and glycerol concentration was 10% (w/v). CMC concentration was 0.5% (w/v). Heating temperature was 90 °C. Storage temperature was 30 °C. *Means at one time with different letters are significantly different ($p \leqslant 0.05$).

solution. However, the addition of starch to CC-based solution barely ($p > 0.05$) affected the migration of oil, as compared to the solution without starch. Starch did not adversely affect the network formed by proteins and CMC. Moreover, the starch addition to coating solutions induced a significant ($p \leqslant 0.05$) decrease of the oil migration by 53.1% (from 99.22 to 46.52 μg linoleic acid/g peanut) and 39.5% (from 99.22 to 60.06 μg linoleic acid/g peanut) in SPI and CC solutions, respectively, as compared to the uncoated control peanuts.

Starch is a polysaccharide carbohydrate connected by the glycosidic bonds of glucose monosaccharide units. Two predominant components of starch are amylose (20%) and amylopectin (80%). In general, starch-based films are rigid, opaque and susceptible to water (Krochta & De Mulder-Johnson, 1997). Starch has hydroxyl polar groups which induce the formation of nonelectrostatic interactions with proteins or CMC, improving the network in the coating solutions. Unlike pectin, the presence of starch in SPI coating solution significantly decreased the migration of oil. This improvement may be due to a large amount of nonelectrostatic interactions between SPI and starch in solution. Starch may compensate for the loss of interactions between proteins and CMC, and thus preserving the integrity of coating. Starch was not efficient for CC-based coating solution, but it was significantly beneficial for SPI-based coating solution.

3.5. Effect of cross-links in protein-based coatings on oil migration

The formation of intermolecular interactions in protein-based solutions induced by heating slightly improved the barrier property against migration of oil when applied to peanut coating (Fig. 1). Here, we utilised other protein cross-linking methods, such as addition of chemical (glutaraldehyde) or enzymatic (transglutaminase) reagent, in combination with heating treatment.

Glutaraldehyde has been used extensively as a cross-linking agent for proteins. Glutaraldehyde polymerises through a Schiff base, coupling two amino groups from two amino residues: methylene bridges are formed in the process (Wong, 1991). It mainly reacts with the ε-amino group of lysine and N-terminal of polypeptides, but also reacts with other nucleophilic groups in proteins, such as the sulfhydryl group of cysteine, the imidazole ring of histidine, and the phenolic hydroxyl group of tyrosine (Lundblad, 2005). Park, Bae, and Rhee (2000) reported that soy protein cross-linked by glutaraldehyde had a higher tensile strength and greater elongation than the native soy protein. Also, mechanical properties of protein-based plastics were improved by using glutaraldehyde (Zhang, Chen, Huang, Yang, & Zheng, 2003). Wang, Mo, Sun, and Wang (2007) improve the water resistance of soy protein adhesive by introducing cross-linking between amino groups of amino acid residue.

On the other hand, transglutaminase catalyses the formation of intermolecular and intramolecular ε-(γ-glutamyl)lysine cross-links in most food proteins, promoting an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors) including the ε-amino group of lysine residues in certain proteins (Motoki & Seguro, 1998). Enzymatic polymerisation of proteins through the formation of ε-(γ-glutamyl)lysine bonds has attracted much attention to modify the functional properties of soy or milk proteins (Kurt & Rogers, 1984; Motoki & Nio, 1983; Tanimoto & Kinsella, 1988).

Fig. 5 shows an increase in efficiency of coating solutions treated by several curing methods after 12 weeks of storage at 30 °C. Treatments with both glutaraldehyde and transglutaminase com-

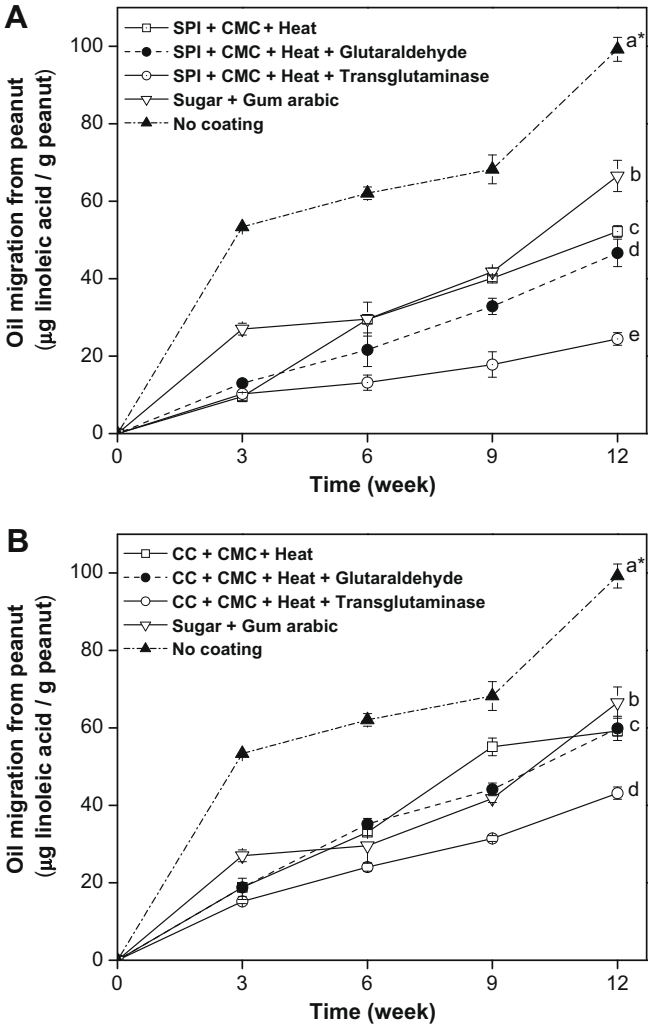

Fig. 5. Effect of 3 different curing methods (heating, glutaraldehyde, and transglutaminase) of protein-based coatings on oil migration: (A) SPI coating on peanut, and (B) CC coating on peanut. Protein concentration was 5% (w/v), and glycerol concentration was 10% (w/v). CMC concentration was 0.5% (w/v). Heating temperature was 90 °C. Storage temperature was 30 °C. *Means at one time with different letters are significantly different ($p \leqslant 0.05$).

bined with heating appeared to be beneficial against the migration of oil in SPI and CC coating solutions. Especially, when the coating solution was treated with transglutaminase in combination with heating, it improved the barrier effectiveness to oil migration for SPI and CC coatings by 53.2% (from 52.18 to 24.44 μg linoleic acid/g peanut) and 27.1% (from 59.16 to 43.15 μg linoleic acid/g peanut), respectively, as compared to heat treatment alone. Moreover, it decreased the migration of oil by 75.4 and 56.5% in SPI and CC coatings, respectively, as compared to the uncoated control peanuts. Therefore, the transglutaminase treatment combined with heating seemed to give the most degree of cross-linking to obtain an optimum efficiency.

Glutaraldehyde treatment in combination with heating significantly ($p \leqslant 0.05$) improved the barrier effectiveness to oil migration for SPI coating by 10.6% (from 52.18 to 46.65 μg linoleic acid/g peanut) but less pronounced for CC coating, as compared to heat treatment alone. Nevertheless, glutaraldehyde treatment lowered the migration of oil by 53.0 and 39.6% in SPI and CC coatings, respectively, as compared to the uncoated control peanuts.

Lastly, we compared these barrier properties of SPI- and CC-based coating solutions with the sugar-based coating (a positive control) composition with 40% (w/v) sugar and 10% (w/v) gum arabic. Both protein-based coatings tested in this experiment were more effective than sugar-based coating after 12 weeks. For example, the SPI- and CC-based coatings treated by transglutaminase in combination with heating decreased the oil migration by 63.3 and 35.1%, respectively, as compared to the sugar-based coating. Also, the SPI and CC coatings treated by glutaraldehyde in combination with heating significantly lowered oil migration by 29.9 and 10.0%, respectively, as compared to the sugar-based coating formula.

3.6. Size-exclusion chromatography study

To examine the degree of protein cross-linking, size-exclusion chromatograph was employed. The size-exclusion chromatography can separate proteins depending on their size. Cross-linking is a specific chemical modification by which two molecular components can be joined together by a covalent bond. Any protein cross-linked by treatments should show an increase of the molecular size or weight, which caused an elution time shift toward shorter in size-exclusion chromatography profile. Fig. 6A shows the elution profiles of SPI obtained for nontreated (control), heated, and combined treatment (heat and transglutaminase). The profile in Fig. 6A shows the presence of two peaks for SPI, corresponding to around 1500 kDa, and 40 kDa, respectively. In SPI control (nontreated), a peak at 40 kDa was predominant over a peak at 1500 kDa. When SPI was treated with heating, a peak at 40 kDa decreased, whilst a peak at 1500 kDa increased slightly. Strikingly, after SPI treated with glutaraldehyde, a peak at 40 kDa considerably decreased and a peak at 1500 kDa apparently increased, implying an increase in molecular weight of SPI caused by glutaraldehyde treatment. A similar result was shown in SPI after treated with transglutaminase. With combined treatments on SPI by heating and transglutaminase, the profile of SPI was highly close to the result in transglutaminase treatment alone. Also, with combined treatments on SPI by heating and glutaraldehyde, the profile was very similar to the result in glutaraldehyde treatment alone (data not shown).

Fig. 6B shows the elution profiles of CC obtained for nontreated (control), heated, and combined treatment (heat and transglutaminase). It is clear that CC was less heterogeneous than SPI (Fig. 6A and B). The profile displays a presence of main peak for CC (nontreated) at around 35 kDa. When CC was treated by heating, a shift of main peak to a molecular weight around 1000 kDa was observed. A peak at 35 kDa decreased and a new peak at approximately 1000 kDa appeared after heat treatment. On the other hand, a peak at 35 kDa almost disappeared after glutaraldehyde or transglutaminase treatment, showing a new, broad main peak at 1000 kDa. With combined treatments on CC by heating in combination with transglutaminase (or glutaraldehyde), the profile was very similar to the result in transglutaminase (or glutaraldehyde) treatment alone (data not shown for heating with glutaraldehyde treatment). As a result, it was clear that both SPI and CC were strongly cross-linked in the presence of glutaraldehyde or transglutaminase. Moreover, an increase of cross-linking corresponded to an improvement in the efficiency of protein-based coating against migration of oil.

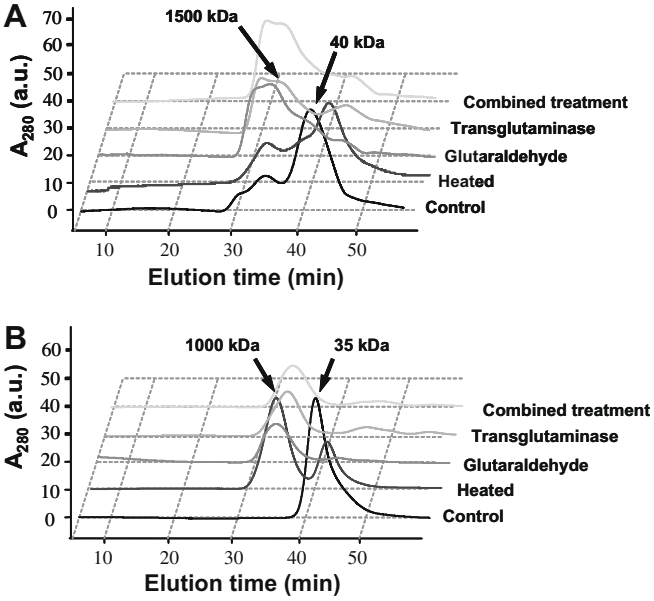

Fig. 6. Profiles of size-exclusion chromatography of proteins: (A) SPI, and (B) CC. Proteins were treated by different curing methods: native (nontreated), heating (90 °C), glutaraldehyde (2.5% w/v, at 50 °C for 30 min), transglutaminase (4 units/g protein, at 50 °C for 120 min), and combined treatments (heat + transglutaminase).

The size-exclusion chromatography data showed a slight cross-linking of SPI when treated with heating. We already described that the cross-link of SPI protein by thermocondensation helped to improve the effectiveness of the coating against the migration of oil. This improvement is probably due to the formation of a network of interactions among the proteins by forming new intermolecular disulphide linkages (Gennadios & Weller, 1991; Sabato et al., 2001). For CC protein, size-exclusion chromatography data indicated a low degree of cross-linking after heat treatment. This is consistent with the rate of oil migration data after heat treatment, demonstrating that the heat-induced cross-linking by disulphide interaction seems not enough to be able to block the migration of oil. The cross-linking in SPI and CC proteins by both glutaraldehyde and transglutaminase treatments was clearly effective to increase the molecular weight of the proteins more than heat treatment. As long as the degree of cross-linking of these proteins increased, the effectiveness of the coatings against the migration of oil improved.

In conclusion, applying a dairy or soy protein-based coating to the surface of peanut would be an effective barrier to the migration of oil. Addition of a polysaccharide like CMC in protein solutions improved the effectiveness of the coating. This enhanced barrier property is probably caused by the presence of charge groups on the surface of this polysaccharide, enable to improve the polymer networks or act as a repulsive agent to oils. Also, the formation of electrostatic or nonelectrostatic interactions can help increase the tightness of polymer networks in film, resulting in reduced

oil passage. The degree of cross-linking in proteins was verified by the change of molecular weight using a size-exclusion chromatography. In SPI- and CC-based coating solutions, a slight degree of cross-linking was observed after heating, whereas both glutaraldehyde and transglutaminase treatments induced strong cross-link. As desired, oil migration test showed that strong cross-linking of these proteins by glutaraldehyde and transglutaminase improved the effectiveness of the coating. The SPI- or CC-based coatings treated with heating in combination with transglutaminase gave the best results compared to the coatings treated by other curing methods. This study demonstrated that our protein-based coatings can serve as a barrier of oil migration from peanut, and they can be applied for the commercial coating solution in industry with potential applications in the chocolate-enrobed peanut products.

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