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Effect of Different Cations in Pickling Solution on FTIR Characteristics of Pidan White and Yolk in Comparison to the Fresh Duck Egg

(Kesan Kation Berbeza dalam Larutan Jeruk ke atas Ciri FTIR Putih dan Kuning Pidan Berbanding Telur Itik Segar)

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ABSTRACT

Fourier transform infrared (FTIR) study of pidan white and yolk treated with different cations were investigated in comparison to the fresh duck egg. FTIR study of PbO_2 and $ZnCl_2$ treated pidan white and yolk at a level of 2 g kg^{-1} had different spectra to those of fresh egg. The amide B with wavenumbers of 3083 and 3084 cm^{-1} was observed for fresh and PbO_2 treated pidan white at a level of 2 g kg^{-1}. Higher wavenumber of 3084 cm^{-1} was noticeable for PbO_2 treated pidan white at a level of 2 g kg^{-1}. Scanning electron microscopic study showed that the more ordered network was found in PbO_2 treated pidan white at a level of 2 g kg^{-1}, compared with $ZnCl_2$ treated counterpart. Thus cations in the pickling solution affected the FTIR characterestics of pidan white and yolk.

Keywords: CLSM; FTIR; pidan; SEM

ABSTRAK

Penyelidikan transformasi Fourier inframerah (FTIR) putih dan kuning pidan yang dirawat dengan kation berbeza berbanding telur itik segar dikaji. Kajian FTIR PbO_2 dan $ZnCl_2$ putih dan kuning pidan pada tahap 2 g kg^{-1} mempunyai bilangan spektrum yang berbeza dengan telur segar. Amida B dengan gelombang nombor 3083 dan 3084 cm^{-1} diperhatikan pada PbO_2 putih pidan yang dirawat pada tahap 2 g kg^{-1}. Gelombang nombor yang lebih tinggi daripada 3084^{-1} adalah ketara bagi putih pidan dirawat dengan PbO_2 pada tahap 2 g kg^{-1}. Kajian microskopi imbasan elektron menunjukkan bahawa rangkaian lebih tersusun dilihat pada putih pidan PbO_2 yang dirawat pada tahap 2 g kg^{-1}, berbanding dengan yang dirawat oleh $ZnCl_2$. Oleh itu kation dalam larutan jeruk memberi kesan kepada ciri FTIR putih dan kuning pidan.

Kata kunci: CLSM; FTIR; pidan; SEM

INTRODUCTION

Fourier transform infrared (FTIR) spectroscopic study is one of the powerful technique to study the intramolocular interaction of the food proteins in various foods (Boye et al. 1995). The intra molecular interaction of the proteins by thermally induced gelation of whey protein was studied using FTIR. Few researchers also reported the intermolecular interactions of fish proteins that involves in gelation and film formation using FTIR (Nagarajan et al. 2013; 2012). However, there was no report up to date regarding the FTIR study on the alkaine induced aggregation of duck egg protein. Pidan or alkaline treated egg have been known as preserved egg, consumed widely in south-east Asia. Pidan white is generally amber brown in color with the gelly texture, whereas pidan yolk is solidified with greenish brown color. Under alkaline pH used for pidan production, electrostatic repulsion extensively opposes protein-protein interactions. The addition of cations in pickling solution enhances the penetration of those cations into egg, thereby diminishing the repulsive forces and protein–protein association occurs, forming a self-supporting gel (Ganasen & Benjakul 2011a, 2011b; 2010). Lead has been used in pidan for gel stabilisation but it is toxic for consumption (Chen & Su 2004). Alternatively pidan is produced by soaking duck eggs in NaOH (42 g kg^{-1}), NaCl (50 g kg^{-1}) and divalent $ZnCl_2$ (2 g kg^{-1}) or $CaCl_2$ (2 g kg^{-1}) solution at room temperature (30°C) for 3 weeks and ageing for another 3 weeks (Ganasen & Benjakul 2011a, 2011b; 2010).

Alkaline treatment also causes the exceptional formations of lysinoalanine, racemisation of amino acids, aminoacids degradation and Maillard reactions during the processing of pidan (Chang et al. 1999). The formation of certain compounds in the Maillard reaction of pidan white is very complicated and the classes of compounds are more or less known. However, the characteristic texture and brown color of pidan white makes the product more consumable. Although the impact of cation types on the properties of pidan white and yolk has been elucidated (Ganasen & Benjakul 2011a, 2011b; 2010). There was no information regarding the FTIR spectra analysis and microstructure of pidan white and yolk induced by selected cations has been reported. Therefore, the objective of this study was to characterize FTIR of pidan white and yolk during pickling in comparison to the fresh duck egg.

Materials and Methods

Chemicals

Lead oxide (PbO_2), zinc chloride ($ZnCl_2$), sodium hydroxide and sodium chloride were purchased from Lab-Scan (Bangkok, Thailand). Glutaraldehyde, ethanol and silver nitrate were obtained from Merck (Darmstadt, Germany). Nile blue A was procured from Merck (Darmstadt, Germany). Purity of all chemicals used was greater than 99%.

Duck Egg Collection

Fresh duck eggs (*Anas platyrhucus*) with the weight range of 65–75 g were obtained within 1 day of laying from a farm in Rathabhum, Songhkla province, Thailand. Duck eggs were cleaned and checked for any crack prior to pickling.

Preparation of Pidan

Clean duck eggs were soaked in a pickling solution containing NaOH (42 g kg^{-1}), NaCl (50 g kg^{-1}) and divalent $ZnCl_2$(2 g kg^{-1}). Traditionally prepared pidan using PbO_2 at a level of 2 g kg^{-1} was used as the control. Sixty eggs were soaked in different pickling solutions (6 L) at room temperature (30-32°C) for 3 weeks. Pidan were removed and coated with white clay paste (clay: water, 4:1 (w/v)) to obtain a thickness of 2-3 mm. Coated eggs were left at room temperature for another three weeks for ageing. The samples were then taken for analyses.

Fourier Transform Infrared (FTIR) Spectroscopy

Prior to FTIR analysis the pellets containing 2 mg freeze dried samples and approximately 100 mg potassium bromide (KBr) were prepared. All spectra were recorded using Bruker Model Vector 33 FTIR spectrometer (Bruker Co., Ettlingen, Germany) from 4000 to 400 cm^{-1} at a data acquisition rate of 4 cm^{-1} per point. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker Co., Ettlingen, Germany).

Scanning Electron Microscopy (SEM) of Pidan White

Pidan white samples were broken into liquid nitrogen with an approximate size of 0.5 × 0.5 cm^2 and fixed at room temperature in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH7.2) for 2 h. Fixed samples were rinsed with distilled water three times to remove the salt. The samples were dehydrated in graded series of ethanol (50, 70, 80, 90 and 100 g kg^{-1}) and then were mounted on SEM stubs using a double backed cellophane tape. The samples were coated with gold and examined using a scanning electron microscope (JEOL JSM-5800LV, Tokyo, Japan).

Confocal Laser Scanning Microscopy (CLSM) of Pidan Yolk

Microstructures of pidan yolk treated samples were examined with a confocal laser scanning microscopy (Olympus, FV300, Tokyo, Japan) following the modified method of Mineki and Kobayashi (1997). Yolk of fresh and all pidans was suspended in 0.1 g kg^{-1} Nile blue A solution at a ratio of 1:10 (w/v) and manually stirred until the uniformity was obtained. Fifty μL of suspension was smeared on the microscopy slide. CLSM was operated in the fluorescence mode at the excitation wavelength of 533 nm and the emission wavelength of 630 nm using a Helium Neon Red laser (HeNe-R) for lipid analysis and at the excitation wavelength of 488 nm and the emission wavelength of 540 nm using a Helium Neon Green laser (HeNe-G) for protein analysis.

Statistical Analysis

All experiments were run in triplicate. The experimental data were subjected to analysis of variance (ANOVA) and the differences between means were evaluated by Duncan's new multiple range test (Steel & Torrie 1980). Data analysis was performed using a SPSS package (SPSS 14.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results and Discussion

FTIR Spectra of White and Yolk of Fresh Egg and Pidan

FTIR spectra of egg white from fresh egg and pidan white are depicted in Figure 1(a). The absorption bands in the spectra of all white samples were situated in the amide band region such as amide I (1600-1700 cm^{-1}), amide II (1500-1600 cm^{-1}), amide III (1200-1300 cm^{-1}), amide A (3293-3306 cm^{-1}), amide B (2920-2922 cm^{-1}) and S-S (400-500 cm^{-1}). For the spectrum of fresh egg white, the characteristic absorption bands at wavenumbers of 3303, 3083, 1651, 1542, 1250 and 457 cm^{-1} were observed, whereas pidan treated with PbO_2 and $ZnCl_2$ at a level of 2 g kg^{-1} showed the main absorption bands at wavenumbers of 3305, 3084, 1651, 1542, 1239 and 465 cm^{-1} and 1652 1548 and 1242 cm^{-1}. The amide I bands are originated from CO stretching vibrations coupled to N–H bending vibrations, CN stretch and CCN deformation. For amide I band, fresh egg white had the lowest wave number, indicating the interaction of CO with the adjacent chains; while $ZnCl_2$ treated pidan white at a level of 2 g kg^{-1} had the highest wave numbers. The amide II bands of all samples were obtained at different wavenumbers, representing N–H bending vibrations coupled to C–N stretching vibrations. Generally, the lower wavenumber showed the existence of hydrogen bonds, which were found in fresh egg white and PbO_2 treated pidan white at a level of 2 g kg^{-1}. The other bands, arising from the stretching vibrations of N–H group,

appeared at 3303 and 3305 cm^{-1}, corresponding to amide A, which occurs commonly in the range of 3280-3300 cm^{-1}. The amide B with wavenumbers of 3083 and 3084 cm^{-1} was observed for fresh and PbO_2 treated pidan white at a level of 2 g kg^{-1}. Higher wavenumber of 3084 cm^{-1} was noticeable for 0.2% PbO_2 treated pidan white. Amide B corresponds to asymmetric stretch vibration of C–H as well as $–NH_3$. The S-S with wavenumber 400-500 cm^{-1} was observed for fresh and 0.2% PbO_2 treated pidan white. Thus alkali penetration and cation binding of proteins in egg resulted in the changes in the structure of protein.

FTIR spectra of fresh egg yolk, PbO_2 and $ZnCl_2$ treated pidan yolk at a level of 2 g kg^{-1} are illustrated in Figure 1(b). The FTIR spectra shows different samples consisted of several peaks with different wavenumbers. Decrease in the absorbance band at 3600-3200 cm^{-1} was observed in $ZnCl_2$ treated pidan yolk at a level of 2 g kg^{-1}, indicating the loss of hydroperoxide, compared with that of fresh yolk. The result suggested that the decomposition of hydroperoxide took place, yielding the secondary lipid oxidation products during pidan production. Van de Voort et al. (1994) reported that the absorbance at 3800–3100 cm^{-1} in the ATR/FTIR spectra, referred to as the OH stretching region. Hydroperoxide moieties exhibit characteristic absorption bands between 3600 and 3400 cm^{-1} due to their –OO–H stretching vibrations. Pidan treated with PbO_2 at a level of 2 g kg^{-1} had no marked changes in this region, suggesting that lipid oxidation occurred at a low extent. Two stretched bands at wavenumbers of 2854 and 2924 cm^{-1} were due to the methylene asymmetrical and symmetrical stretching vibration, respectively (Guillen & Cabo 1997). Both the methylene asymmetrical stretching bands at approximately 2925 cm^{-1} and the methylene symmetrical stretching band near 2854 cm^{-1} were obviously present in most of the lipid samples (Guillen et al. 2004). The bands associated with the fingerprint region observed between 1500 and 1000 cm^{-1} were not different between the fresh and pidan yolk. Carbonyl absorption of the triglyceride ester linkage was observed at 1746 cm^{-1} (Setiowaty et al. 2000). Yolk lipid mainly contained triglycerides with very low amounts of free fatty acids.

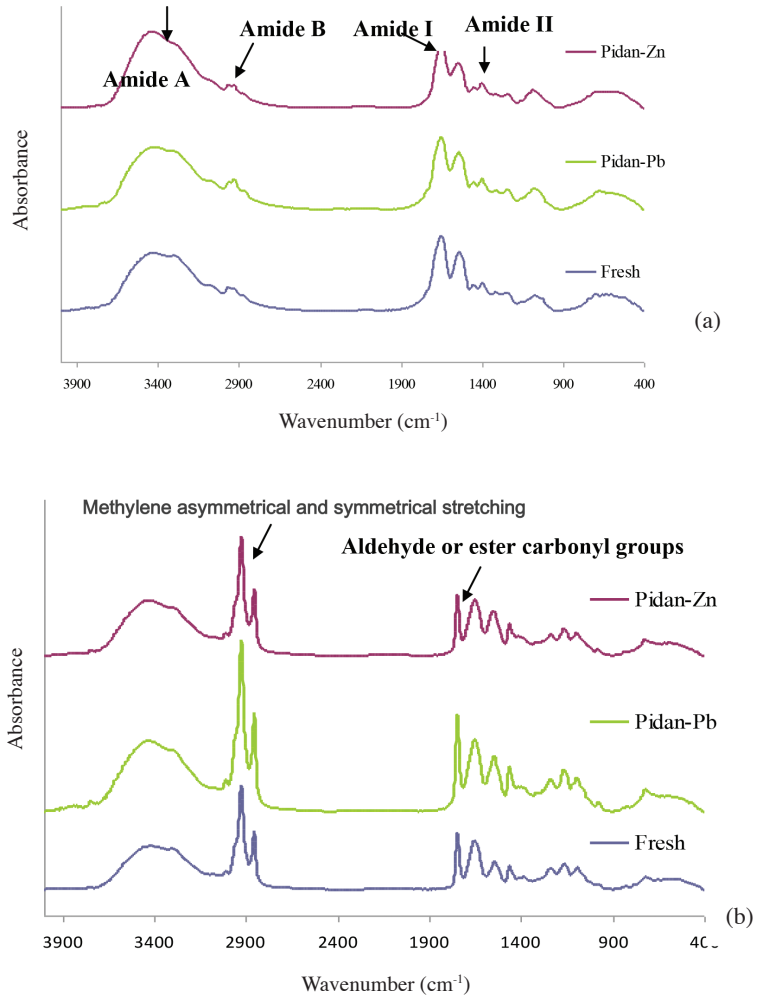

FIGURE 1. Fourier-transform infrared spectra of white and yolk of fresh egg and pidan Fresh: Fresh egg yolk, Pidan-Pb: PbO_2 treated pidan, Pidan-Zn: $ZnCl_2$ treated pidan, pidan white (a) and pidan yolk (b)

MICROSTRUCTURE OF WHITE AND YOLK OF PIDAN

Microstructures of pidan white treated with PbO_2 and $ZnCl_2$ at a level of 2 g kg^{-1} visualised by SEM are shown in Figure 2(a). Heterogeneous aggregates with the cracks were observed in pidan treated with $ZnCl_2$. Pidan gels had more compact structure without the gap or void in the network when treated with 0.2% PbO_2. Rough surface of pidan was observed in pidan treated with 0.2% $ZnCl_2$ (Pidan-Zn) whereas highly cross-linked network with fine strands was found in pidan white treated with PbO_2 (Pidan-Pb) at a level of 2 g kg^{-1}. Woodward and Cotterill (1987) reported that egg white gel examined with SEM was very coarse with large irregularly shaped voids. Nevertheless, ovalbumin gels showed the homogeneous microstructure (Heertje & Van Kleef 1986). The ordered structure of PbO_2 treated pidan white at a level of 2 g kg^{-1} indicated that Pb cation somehow involved in the formation of gel network with high stability (Figure 2(a)). Alkaline conditions are known to unfold protein molecules (Creighton 1993). Those unfolded proteins could be cross-linked to form protein networks, particularly in the presence of cation via salt bridge mechanism. Thus, the appropriate cations most likely played an essential role in ion-induced gelation of pidan white.

The confocal laser scanning microscope (CLSM) micrographs of pidan yolk using a two channel technique, in which both protein and lipid were stained, are illustrated in Figure 2(b). Proteins in yolk were organized into micellar and granular structures together with polar and non-polar lipid molecules (Kiosseoglou 2003). When the penetration of alkali proceeded to interior yolk, 3 layers of pidan yolks were formed. Irregular shapes of both lipid and protein were found in both pidan yolk, irrespective of treatments. Nevertheless, the amount of lipid released and alteration of lipid shape were varied with the types of cation used. The greater release of free lipid from lipoprotein of

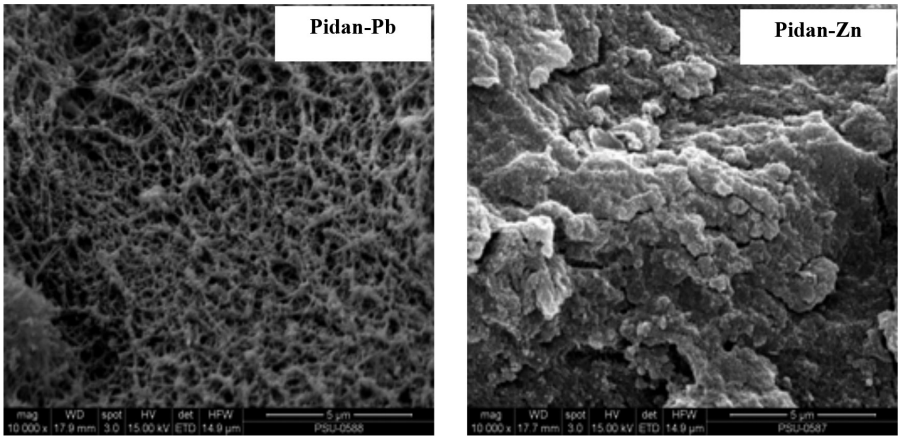

FIGURE 2(a). Scanning electron microscopic photograph of pidan white Magnification: 10000× Pidan-Pb: PbO_2 treated pidan white, Pidan-Zn: $ZnCl_2$ treated pidan white

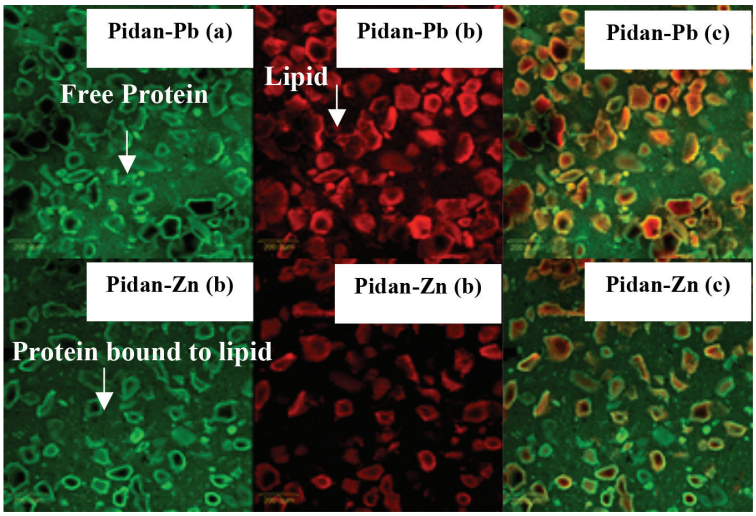

FIGURE 2(b). Confocal laser scanning microscope (CLSM) micrographs of pidan yolk Pidan-Pb: PbO_2 treated pidan yolk, Pidan-Zn: $ZnCl_2$ treated pidan yolk Magnification: 200× (zoom ×2.5) protein distribution (a) and lipid distribution (b) and combined image of protein and lipid (c)

pidan yolk was obtained when PbO_2 at a level of 2 g kg^{-1} was used as indicated by the denser lipid granules appeared in the combined image (Pidan-Pb(C)). In the presence of alkali, saponified lipid was postulated to bind protein, leading to the formation of shielding surface. As a result, the dehydration was lowered and soft yolk pidan was obtained. CLSM micrograph of pidan treated with $ZnCl_2$ at a level of 2 g kg^{-1} suggested that binding of lipid to protein yielded the hard aggregated yolk, whereas PbO_2 at a level of 2 g kg^{-1} treatment rendered the soft yolk containing more free lipids with less association with proteins.

Conclusion

This is the first report on the FTIR study of pidan white and yolk treated with the different cations and found to be different bands which in turn associate with the gelling behavior of the pidan. Interactions with the cations increase the wavenumber and confirms the binding of protein. PbO_2 treated pidan white contained higher cross linking of protein than that produced with $ZnCl_2$ treatment at a level of 2 g kg^{-1}. Thus cations in the pickling solution affected FTIR characteristics and microstructure of both pidan white and yolk.

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