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Research Article

Measurement of Total Sodium Alginate in Restructured Fish Products Using Fourier Transform Infrared Spectroscopy

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Abstract

A novel method was developed using Fourier transform infrared spectroscopy (FTIR) to measure sodium alginate in a restructured fish meat product. Sodium alginate was added into tilapia paste at different concentrations (0, 0.5, 1.0, 2.0, and 5.0%). There were 2 procedures for sample preparation: direct oven-drying to remove moisture and an extraction method that included several steps. These steps were fat removal by acetone extraction, enzymatic protein degradation, ethanol precipitation of polysaccharides, centrifugation, and air-drying for 30 min. FTIR was then used at room temperature to directly quantify the amount of sodium alginate present in the fish. Partial least squares (PLS) and principal component analysis (PCA) were used to develop quantitative and qualitative models for hydrocolloid analysis. The results show that FTIR can be used for the rapid measurement of sodium alginate in restructured fish and other meat products.

Keywords: *Sodium Alginate; PLS, PCA, FTIR, Restructured Fish*

Practical Application

Sodium alginate possesses considerable water-absorbing and water-retaining properties. The USDA (U.S. Department of Agriculture) regulates the maximum usage rate for different types of meat products. In order to efficiently monitor the levels of sodium alginate, quantitative methods must be available. The new method described here can be used to determine the sodium alginate content in various types of meat samples.

Introduction

The function of fat is to provide unique flavor and to contribute to the texture of meat products. Reducing the fat content in a meat product can result in a firmer, more rubbery, less juicy product with dark color and a higher cost [1]. Removing fat from meat or a meat product requires a fat substitute to re-adjust the lost flavor, texture, and mouthfeel, and to mimic traditional meat samples. Alginate is one of the most popular hydrocolloid fat substitutes due to its high water-binding capacity. Using alginate as the fat replacement can make up for the juice lost from fat removal and can provide a creamy meat texture [2].

Sodium alginate is the sodium salt of alginic acid, a natural polyuronide constituent of certain brown algae. Alginates and alginic acid are widely used in the food industry, biotechnology, and pharmaceuticals because of their gel-forming capacity. Alginic acid is a linear, 1, 4-linked copolymer of β-D-mannuronic acid (M unit) and its C5 epimer, α-l-guluronic acid (G unit), as seen in figure 1 [3-5]. The G and M units are joined together in homopolymeric and heteropolymeric sequentially alternating blocks that affect the strength of the gel formed in restructured food products. The carboxyl groups present at C-6 of the G and M uronic acid units stabilize the glycosidic bonds against acid hydrolysis [2].

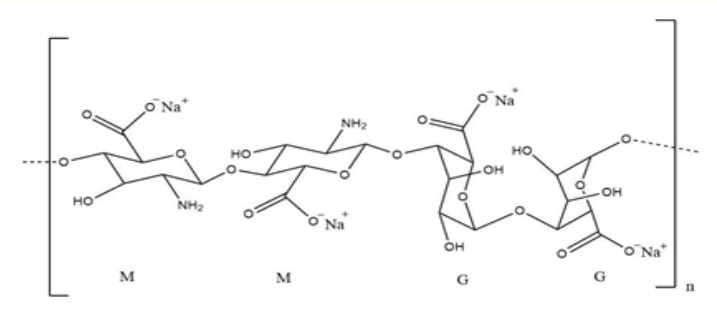

Figure 1: *The chemical structure of sodium alginate.*

Alginate system restructuring has been developed to meet the consumer demand for restructured meats. A process of making restructured meat with alginate gels was patented by scientists at Colorado State University [6]. The patent describes the process of development of restructured meat products using sodium alginate, calcium carbonate, and glucono-delta-lactone (GDL). After adding sodium alginate into meat blocks, sodium alginate is hydrated by the moisture of the meat. Calcium carbonate has low solubility, which can be used to control the rate of gel formation and thereby allow more time for meat processors to stuff the meat mixture into casings. Continuous exposure of alginate to the calcium solution can increase the firmness of the meat gel due to the calcium ion binding with the G block within the alginate structure [7]. The function of GDL is to change the pH of the meat, which causes an increase of solubility of calcium carbonate. This leads to a release of more calcium to the meat system, which causes gel formation. The alginate gel is able to glue the meat particles into larger meat blocks. Once the gel has set, the product is sliced into steaks or chops, and since the calcium alginate gel is heat-stable, the product will not fall apart during cooking. This technique can eliminate the disadvantages of the traditional methods, which use salt and phosphate along with mechanical action. This additional salt can alter meat color and cause lipid oxidation, as well as increase sodium content.

There are 2 functions of alginate application in the meat industry: fat replacement and gelling in restructured meats. The 2 applications have significantly increased the demand for this chemical. In the current market, only 15 to 20% of a carcass is used as steaks and chops, the remaining carcass is made into ground meat and sausages. The USDA limits the usage of alginate for the purpose of restructuring meats because alginates increase water retention. Hence, in order to prevent economic adulteration with alginates and excessive water content in processed meat, a quantitative method for the determination of sodium alginate in meat products is needed. Such a validated method should be able to monitor alginate content efficiently. Based on the current USDA regulations, a mixture of sodium alginate (not to exceed 1% of product formulation), calcium carbonate (not to exceed 0.2%), and calcium lactate/lactic acid (not to exceed 0.3%) is permitted for use in restructured meat food products to bind meat pieces. The entire mixture is not to exceed 1.5% of product at formulation and it must be added dry.

Current methods used for the quantification of alginate include the colorimetric or chromatographic analysis of uronic acids released from enzymatic depolymerization or chemical hydrolysis of alginate [5,8]. The disadvantage of these methods is the indirect quantification of alginate content via determination of uronic acids. Colorimetric methods are not specific to the various forms of uronic acids because they are based on an estimation of polysaccharide content from the mean uronic acid concentrations [5]. Rourke and others [9] reported using cation exchange HPLC to analyze alginate content added into pork products, which was based on the indirect quantification of analysis of uronic acids of alginate copolymers.

Other methods may be useful for the direct measurement of the total content of alginate without hydrolysis. Horn and others [10] directly determined the alginate content in brown algae by near-infrared (NIR) spectroscopy. Moore and others [11] developed and validated a method for the quantitative determination of alginic acid, which involved a preliminary separation of the alginic acid from an

antacid formulation, followed by using capillary electrophoresis wherein alginic acid was measured by a UV detector. Oztekin and others [5] concluded that micellar electrokinetic chromatography (MEKC) was a promising method to be applicable to the quality control of alginate content in antacid formulations. The solution preparation was fast without purification, except for centrifugation, and the sample matrix did not interfere with the analysis. Volpi [12] also determined the alginic acid content of both solid and liquid antacid formulations treated with alginate lyase followed by capillary electrophoresis and UV detection. Awad and Aboul-Enein [8] studied a direct method using HPLC to directly quantify the total alginate content without sample pretreatment in a medicine formulation. In 2011, the British and U.S. Pharmacopeia recommended development of a method for the assay of alginic acid and alginate as raw material for pharmaceutical applications. However, such a method is unlikely to be applicable to a finished meat product because of the complexity of the meat matrix.

Currently, there is an increasing demand for seaweed-derived polysaccharides by the food industry. Quick and reliable non-destructive methods to assess the application of polysaccharides are required. Based on the literature review, Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy could be used as suitable methods to identify the main polysaccharides in an unknown seaweed sample. These methods measure the sodium/calcium film to determine the mannuronate/guluronate (M/G) ratios when sodium alginate is immersed in a calcium chloride solution. Peak shift, peak shape, and new peak appearance was observed by FTIR techniques [13]. Pereira and others [14] combined 2 spectroscopic techniques, FTIR-attenuated total reflection (FTIR-ATR) and FT-Raman, to identify the principal different types of carrageenan colloids in ground seaweed powder, and they performed analyses based on peaks in the spectra. Gomez-Ordonez and Ruperez [15] proposed that FTIR-ATR spectroscopy could be a useful tool for the food, pharmaceutical, and cosmetics industries to check the hydrocolloid types and to differentiate sodium alginate and carrageenan by a quick and non-destructive method. Pereira and Neto [16] summarized the FTIR-ATR and FT-Raman spectroscopy peaks in main seaweed polysaccharides spectra with attributed bonds (Table 1). The seaweed polysaccharides include alginate, fucoidan, laminaran, agars, kappa-, iota- and lambda-carrageenan. Sakugawa and others [17] introduced the method to determine the M and G ratio by FTIR. Various approaches have been reported for the alginate determination in the pharmaceutical field, but there is no general procedure for their analysis in simple food systems, not to mention more complicated meat systems. Therefore, the objective of this study was to develop a valid method that can directly measure the total alginate content in restructured meat using a FTIR technique coupled with multivariate statistical tools, specifically partial least squares (PLS) and principal component analysis (PCA).

Materials and Methods

Product Preparation

Swai fillets were purchased at a local grocery store. The fish had been farm- raised and produced in Vietnam. The fillets were individually vacuum-packed and sold in the frozen state (-18°C). Before use, fish samples were thawed under refrigerated conditions overnight. The semi-thawed fillets were cut into small pieces and then blended for 2 min in a food processor (Cuisinart® Prep 9™ 9-Cup Food Processor, Model DLC-2009CHBM). Sodium alginate and (Danisco, Kansas City, KS, USA) and calcium carbonate (Imerys, Roswell, GA, USA) were sprinkled manually into the paste, a rubber spatula was used to cover the powder to avoid sticking to the processor's internal wall. The food processor was turned on again for another 3 min. Lactic acid (IFP, St. Paul, MN, USA) was added in encapsulated form because encapsulated acids are released more slowly and thereby prevent texture breakdown. After adding encapsulated acid, mixing was limited to avoid damage to the encapsulation coating. There were 5 treatments, which included the control (without sodium alginate system), 0.5%, 1.0%, 2.0%, and 5.0% sodium alginate based on meat weight. The ratio of sodium alginate, calcium carbonate, and encapsulated lactic acid was held constant to 1.0: 6.0: 1.5. The samples were allowed to set into a gel at refrigeration temperature overnight.

There were two pre-preparation methods. The first method is called direct oven drying sample preparation, which removes moisture with an oven. The meat sample are placed in an aluminum tray and dried in a vacuum oven at 80°C for 24 hours at 23 kPa and cooled to room temperature in a desiccator prior to measurement. The second method is called indirect sample preparation or an extraction method that includes several steps. The polysaccharide extraction method included 5 steps: fat extraction, protein degradation, precipitation of

polysaccharide, centrifugation, and air drying. Three samples per treatment were randomly collected from each package. Fat was removed by adding 100 mL acetone (Sigma-Aldrich, St. Louis, MO, USA) to 5g samples with or without sodium alginate. The mixture was stirred and extracted for 1h. After the extraction, the mixture was passed through Whatman® Grade 1 Qualitative filter paper. The residue was saved and placed into the original 500 mL polyethylene beaker. Next, 50 mL distilled deionized water and 1 mL of 1 N NaOH were added to the meat residues with agitation for 10 minutes. The mixture was incubated at 100°C in a water bath for 10 min to denature the protein. The mixture was allowed to cool to room temperature (24°C) and the pH was adjusted to 7.5 with 1N HCl. Protease (Sigma-Aldrich) was added to the mixture to digest the protein for 16 h in a 37°C water bath. The enzymatic reaction was then stopped and the remaining protein was coagulated by heating the mixture at 70°C for 20 min. After fat extraction and protein denaturation, the next step was polysaccharide precipitation. Polysaccharides were precipitated from previously treated samples by adding 350 mL 90% ethanol at room temperature and mixing for 2 - 3h. Excess ethanol was removed with a vacuum pipette tip and the samples were centrifuged at 10,000 rpm g for 15 min at 4°C. The solid polysaccharides were carefully removed with a small spatula and air-dried for 30 min. The samples were dried by applying tissue paper until no more water was drawn. The samples had to be sufficiently dry before FTIR analysis.

Measurements by FTIR Spectroscopy

FTIR analysis was carried out using a Thermo Nicolet 380 FTIR spectrometer (Thermo Electron Corporation, Madison, WI, USA). The FTIR spectra were measured at room temperature using attenuated transmission and an internal reflection accessory made of composite zinc selenide (ZnSe) and diamond crystals. The spectra were acquired at wave numbers of 400 - 4000 cm^{-1} in 64 scans with a resolution of 4 cm^{-1}. The software Delight Version 3.2.1 (D-Squired Development Inc., La Grande, OR, USA) was used for data analysis. Data preprocessing algorithms including polynomial subtraction and Gaussian smoothing were used to subtract the baseline shift and to eliminate high-frequency noises from the instrument. A multivariate statistical regression method (partial least squares, PLS) was used to predict the analyte concentrations in tested samples. The number of PLS latent variables was optimized based on the lowest root mean square error of prediction (RMSEP) values to avoid over-fitting of spectral data.

Results and Discussion

The FTIR spectra of commercial sodium alginate and pure alginic acid are shown in figure 2. The most remarkable difference between commercial sodium alginate and pure alginic acid was in the wavenumber range of 1550 to 1750 cm^{-1}, which is a carboxylic ester band (Figure 3). This carbonyl group showed the carboxylic acid ester form in alginic acid at C=O at 1730 cm^{-1}, and carboxylate anion form (COO^-) at 1600 cm^{-1} in sodium alginate samples, which was in agreement with Gomez-Ordonez and Ruperez [15].

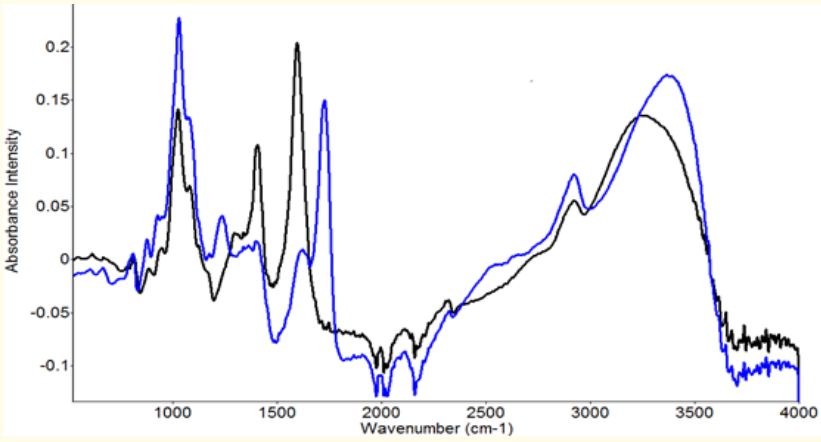

Figure 2: *Spectra of commercial sodium alginate (blue) and pure alginic acid (black).*

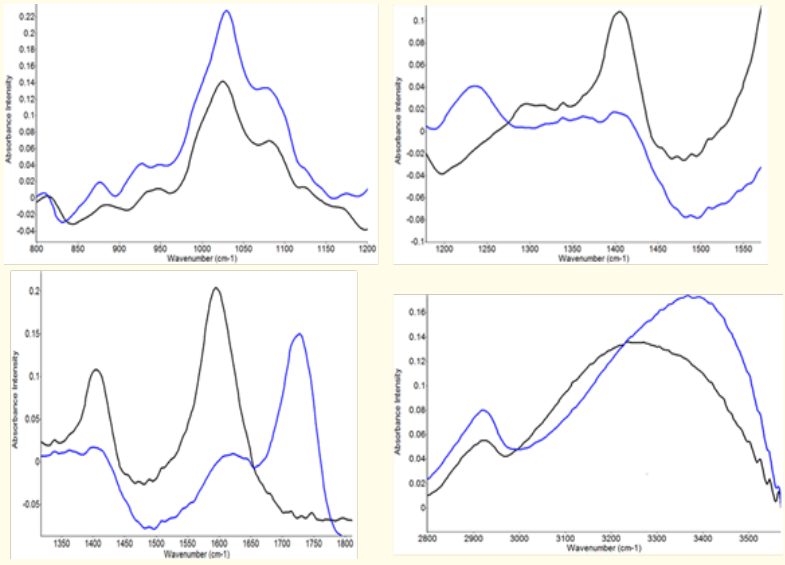

Figure 3: *The spectra of commercial sodium alginate (blue) and pure alginic acid (black) at bands ~817, 1030, 1417, 1617, 2920, and 3426 cm^{-1}.*

The band at 817 cm^{-1} is characteristic of mannuronic acid residues which showed in both sodium alginate and alginic acid samples. The band at 878 cm^{-1} is assigned to the C1-H deformation vibration of β-mannuronic acid residues. The band at 948 cm^{-1} is assigned to C-O stretching vibration of uronic acid residues. Gomez-Ordonez and Ruperez [15] concluded the same results that both alginate and alginic acid samples in the anomeric region of fingerprint from 750 - 950 cm^{-1} showed these 3 characteristic absorption bands. The ~1083 and 1024 cm^{-1} bands are assigned to C-O and C-C stretching vibrations of pyranose ring and C-O-C glycosidic bonds. Mohamed and others [18] also stated that a peak at wavenumber of ~1030 cm^{-1} is due to vibration of C-O, C-C-C, and vibrational asymmetry of the pyranose ring. Praveena and others [19] agreed that the band at 1030 cm^{-1} may be due to C-C stretching vibrations of the pyranose ring. The band at 1406 cm^{-1} was assigned to C-OH deformation vibration with O-C-O symmetric stretching vibration of carboxylate group. The weak signal at ~2926 cm^{-1} is due to C-H stretching vibrations. The broad band centered at ~3260 cm^{-1} was assigned to hydrogen bonded O-H stretching vibrations [15,19]. The strong peak was located at 3446 cm^{-1} in pure alginic acid due to O-H stretching, which was indicated at 3249 cm^{-1} in commercial sodium alginate spectra in this study.

Malesu and others [20] demonstrated the FTIR spectra of pure alginate. They addressed that the bands around 1030 cm^{-1} (C-O-C stretching) is due to the saccharide structure of sodium alginate. The bands at 1617 and 1417 cm^{-1} were assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups. Swamy and others [21] pointed out that the molecule chain of sodium alginate contains both –OH and -COO^{-} groups. The O-H stretching vibration showed at ~3388 cm^{-1}. The peaks at 1604 and 1411 cm-1 bands were assigned for the asymmetric -COO– stretching vibration and symmetric -COO^{-} stretching vibration, respectively. Table 1 summarizes FTIR spectral band assignments of sodium alginate, which showed slightly different bands and wavenumbers at the same functional groups.

Pereira and others [22] summarized that different types of alginate showed different G block and M block ratios and patterns. These differences can cause differences in the physical properties of sodium alginate. The guluronic units can be identified from a band at ~1025 cm^{-1}. The mannuronic units can be identified from a band at ~1100 cm^{-1}. The ratio of guluronic and mannuronic concentration ratio of alginate samples can be determined from the relative intensity ratio of 1025 and 1100 cm^{-1} bands. Usov [23] recommended that determination of M block to G block values in alginate can be measured with the ratio of absorption band intensity at 808 (M) and 787 (G) in the infrared (IR) spectra. The author also pointed out that a comparison of band intensities at 1320 (M) and 1290 (G) is another

good way to determine the ratio. Pereira and Neto [16] summarized that sodium alginate showed 2 characteristic bands in the IR spectra. The band at 808 cm^{-1} was assigned to M units, and 787 cm^{-1} was assigned to G units (Figure 4). However, some researchers assigned both 808 and 787 cm^{-1} bands to G units. Some studies showed the M/G ratios could be estimated from the ratio of absorbance of the bands at 1320 and 1290 cm^{-1} in the FTIR spectra. Sakugawa and others [17] addressed that polymannuronate does not have a strong interaction with divalent cations like the egg-box structure in polyglucuronate. The peaks of divalent polyglucuronate were sharper than the peaks of polyglucuronic acid. In accordance with the authors, the absorbance at 1030 cm^{-1} is reflected by the change of mannuronate concentration of calcium alginate and 1025 cm^{-1} is attributed to the OH bending of guluronate. Alginate also can be estimated from the intensity of the peak at 1030 to 1080 cm^{-1} in IR spectra. The authors suggested that samples with high content in guluronic acid showed an intense broad band centered at ~1025 cm^{-1}.

Sodium alginate	Band assignments
3388/3450/3426	O-H band stretching (Intermolecular hydrogen bonded)
2924/2926	C-H stretching
1604/1614/1617	O-C=O asymmetric stretching
1411/1420/1417/1400	O-C=O asymmetric stretching
1035/1030/1025	C-O-C stretching

Table 1: *Summarization of FTIR spectral band assignments of sodium alginate.*

**(Praveena and others 2014 [19]; Mohamed and others 2011 [18]; Pereira and Neto 2014 [16]; Pereira and others 2003 [22])*

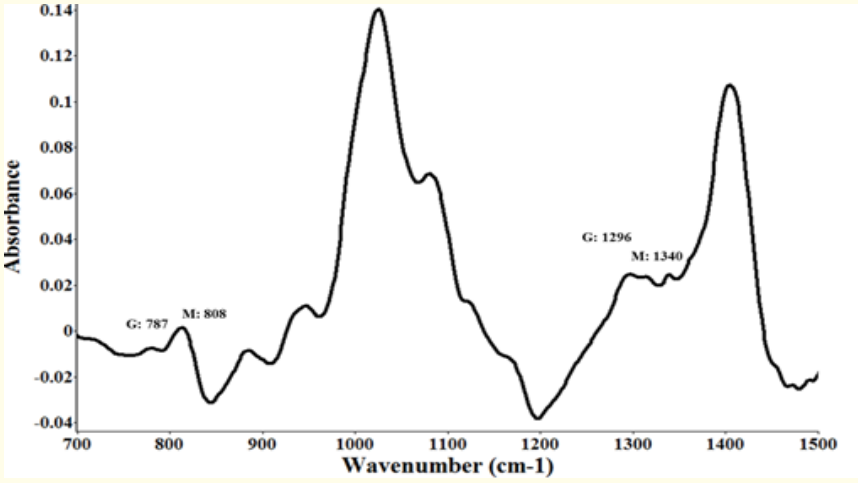

Figure 4: *Sodium alginate spectra at 525 - 4000 cm^{-1}.*

Pereira and Neto [16] pointed out that the most prominent Raman shift bands are at 950 cm^{-1}, while in FTIR the intensity at 950 cm^{-1} is very weak. Both FTIR and Raman showed strong bands at 1400 cm^{-1}, which is due to the deformation of the CH_2 group. The C-O-C and C-OH stretching modes showed in the spectral regions of 1250 - 1290 and 1000 - 1025 cm^{-1}, respectively.

Due to the fact that the medium to strong IR absorption bands at 1200 - 970 cm^{-1} were mainly due to C-C and C-O stretching in pyranoid rings and to C-O-C stretching of glycosidic bonds [15], this range is commonly used to distinguish all polysaccharides. In this study, 1200 - 800 cm^{-1} was chosen. The spectra range of 2000 - 800 cm^{-1} was also used for comparison. The second-derivatives of FTIR spectra were used to determine weak absorption bands or to improve resolution of overlapped bands to distinguish different concentrations of samples.

In this study, treatments of dry fish meat containing different concentrations of sodium alginate and calcium sources were analyzed by FTIR. Average IR spectra (n = 6) of dry meat samples are shown in figure 5. It shows that the most prominent peaks of sodium alginate and calcium alginate were at around 1030 cm^{-1}, which was present in the sodium alginate-treated meat samples, but was absent in the control dry meat sample (Figure 5). In addition, second- derivative transformation can separate the overlapped peaks, eliminate baseline effect, and increase spectral resolution ability. This has been applied to this study for analyzing the spectra. Figure 5 clearly depicts that there were different spectra at wavenumber 1030 cm^{-1}. Therefore, FTIR techniques with proper data analysis can be used to detect different amounts of sodium alginate usage in meat samples.

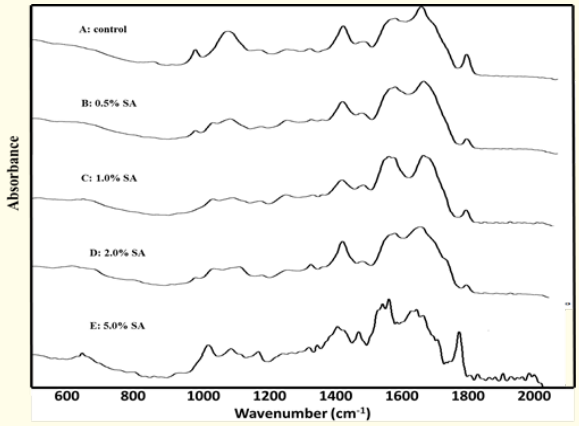

Figure 5: *Average FT-IR Spectra (n = 6) acquired from dry fish meatball samples containing different concentrations of sodium alginate by direct drying method. A: fish ball without adding sodium alginate; B: adding 0.5% sodium alginate; C: adding 1.0% sodium alginate; D: adding 2.0% sodium alginate; E: adding 5.0% sodium alginate; the ratio among sodium alginate: $CaCO_3$: encapsulated lactic acid = 6:1:1.5 for all treatments. The spectra were presents with smoothing at 5 cm^{-1} and baseline adjustment by subtracting a 2nd order polynomial function. Analysis was conducted from 525 - 2000 cm^{-1}.*

RMSEP values obtained from the PLS models with different latent variables are shown in figure 6 and figure 7. The spectral data were pre-treated with smoothing at 4 cm^{-1} and second-order polynomial subtraction in the whole spectral region. The lowest RMSEP values were achieved. The latent variable was five (Figure 8), which is the optimal value of latent variable to build a PLS model in this study. Figure 6 shows the PLS prediction results (n = 6) by plotting actual sodium alginate concentration against predicted sodium alginate concentration. The prediction result was achieved with R = 0.9993 and RMSEP = 0.066% at wavenumbers in the range of 2000 - 800 cm^{-1} (Figure 6), and R = 0.9985 and RMSEP = 0.0998 at wavenumber range of 1200 - 800 cm^{-1} (Figure 7). The results indicate that the PLS model in this study could provide satisfactory quantitative results for sodium alginate added into restructured meat. Based on USDA regulations, sodium alginate added to restructured meat product cannot exceed 1% of the product formulation. With the PLS model, very accurate quantitative results are hard to achieve if the concentration of sodium alginate is this low.

Figure 5, 9 and 10 clearly depict different spectral curves at 1030 cm^{-1} for the 5 treatments. When the treatments are compared, the control and 0.5% sodium alginate treatments have the strongest absorbance around 950 - 1000 cm^{-1}, which is associated with C-O, C-C stretching, and C-O-H and C-O-C deformation of carbohydrate [24].

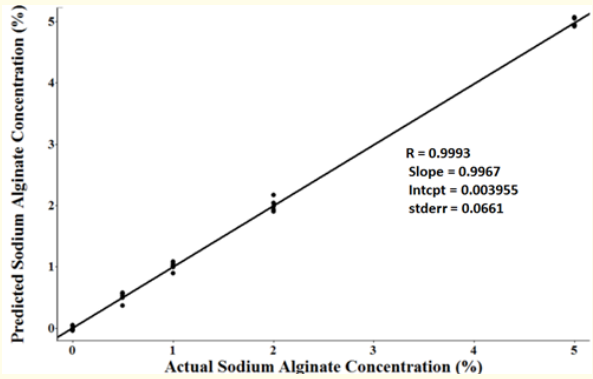

Figure 6: *Actual sodium alginate concentration (%) added into meat samples versus predicted sodium alginate concentration using the PLS model by direct drying method; smoothing 4 cm^{-1}, baseline adjustment by subtracting a 2nd order polynomial function; 7 latent variables; spectral region 800 - 2000 cm^{-1}, spectra number n = 30.*

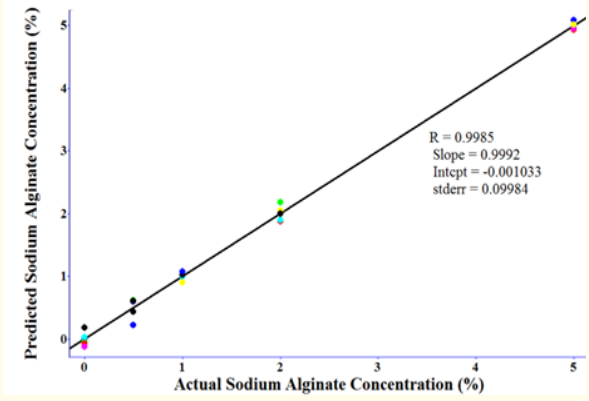

Figure 7: *Actual sodium alginate concentration (%) added into meat samples versus predicted sodium alginate concentration using the PLS model by direct drying method; smoothing 4 cm-1; baseline adjustment by subtracting a 2nd order polynomial function; 7 latent variables; spectral region 800 - 1200 cm-1, spectra number n = 30.*

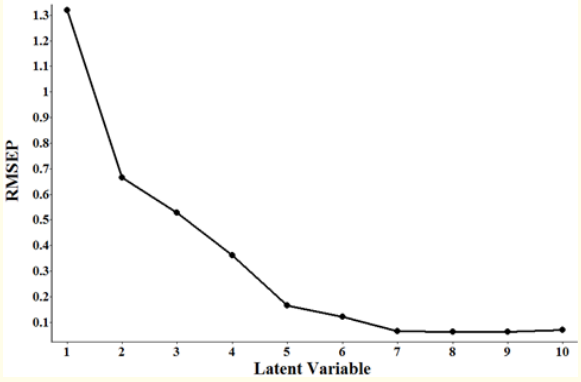

Figure 8: *Root mean square error of prediction (RMSEP) values obtained from the partial least square (PLS) models with different latent variables by direct drying method.*

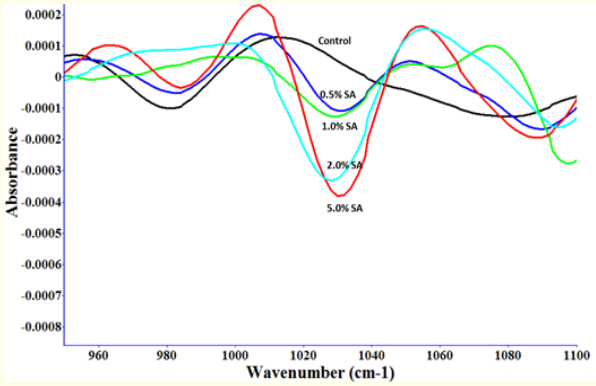

Figure 9: *Part of second derivative transformation of average FTIR spectra (n = 6) acquired from different concentration of sodium alginate by direct drying method.*

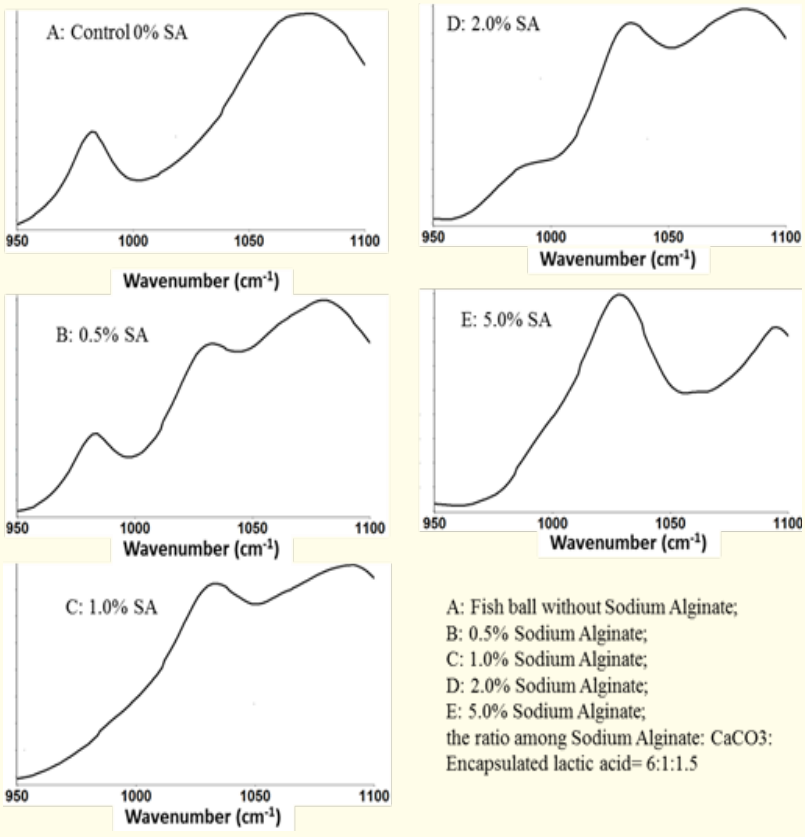

Figure 10: *Average FT-IR Spectra (n = 6) acquired from dry fish meatball samples containing different concentrations of sodium alginate by direct drying method. Spectra were presents with smoothing at 5 cm^{-1} and baseline adjustment by subtracting a 2nd order polynomial function. Analyses were conducted from 950 - 1100 cm^{-1}.*

The PCA score plot is a useful tool for quantitative analysis. Samples with the same PC scores are similar in terms of chemical composition. The samples used in this study were labeled as control, 0.5% SA, 1.0% SA, 2.0% SA, and 5.0% SA. From figure 11 it can be seen that the control treatment and sodium alginate-treated samples did not classify into the same group. There was no overlap between the control and 0.5% SA-treated samples. This may be due to the fact that even low concentrations of sodium alginate show obvious gelling and do change the meat composition structure. These results indicate that adding sodium alginate into meat samples with different concentrations could be distinguished by FTIR techniques.

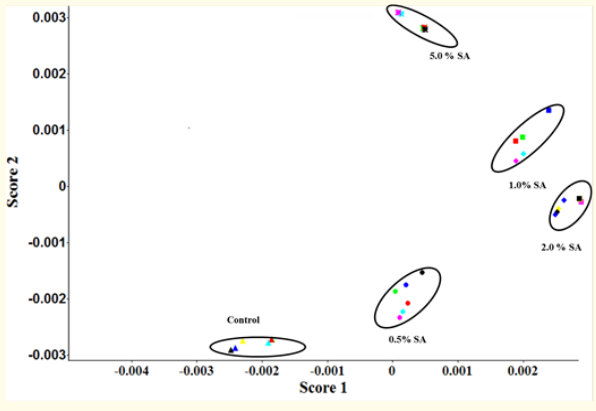

Figure 11: *Classification of sodium alginate treatments with control group using the first two principal components (PCs) at wavenumber 800 - 2000 cm-1 by direct drying method.*

The extraction method involved fat removal and a protein degradation process. However, the residues may still contain carbohydrates, residual fat, and amino acids, and minerals and vitamins. When centrifuged and air-dried for 30 minutes, the total residue is the mixture of these compounds. This explains why a peak of fats was present at 1744 cm^{-1} (Figure 12). This extraction method causes protein degradation. There was no peak around 1548 cm^{-1} [25] which indicates the IR absorption band of protein. However, the spectra of the extraction method for all treatments contain a peak at 1520 cm-1, which is an amide II band of proteins.

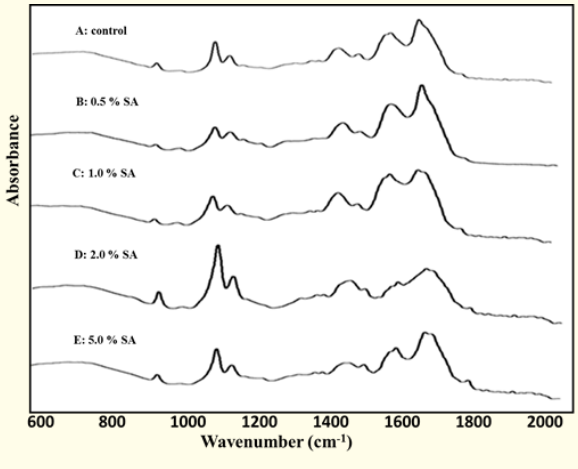

Figure 12: *Average FT-IR spectra (N = 6) acquired from dry fish meatball samples containing different concentrations of sodium alginate by extraction method. A: fish ball without adding sodium alginate; B: adding 0.5% sodium alginate; C: adding 1.0% sodium alginate; D: adding 2.0% sodium alginate; E: adding 5.0% sodium alginate; the ratio among sodium alginate: caco3: encapsulated lactic acid = 6:1:1.5 for all treatments. Spectra were presents with smoothing at 5 cm^{-1} and baseline adjustment by subtracting a 2nd order polynomial function. Analyses were conducted from 525 - 2000 cm^{-1}.*

When comparing the spectra from the 2 methods (Figure 5 and Figure 12), there was a peak at 877 cm^{-1} for all treatments, which may be due to an epoxy ring from epoxy fatty acid in fish products. The prominent peak was shifted from 1030 cm^{-1} to 1043 cm^{-1}. The 1085 cm^{-1} peak might be due to P=O symmetric stretching in DNA, RNA, and phospholipids [26]. The peak at 1400 cm^{-1} is due to C=O symmetric stretching of the COO- group in amino acids and fatty acids. The peak at 1540 cm^{-1} is due to an amide II band of proteins. The band at 1620 cm^{-1} in the control and 0.5% SA samples were sharper compared with other SA treatments. The treatments containing higher SA showed broader bands. This may be due to the chemical reaction between sodium alginate and calcium carbonate, and the development of calcium alginate compounds.

PCA was conducted based on spectra acquired from all 5 treatments to check whether PCA can recognize different treatments. The first two PCs were used to classify treatments. The results indicated that PCA is useful to discriminate between the 5 treatments (data not shown).

When comparing the 2 methods, direct oven-drying without any pretreatment and the extraction method (data not shown), there was no advantage in the extraction method. Based on spectra, there were no different peaks found when compared with control and sodium alginate-treated samples. For the direct oven-drying method, there was a peak at range 950 - 1000 cm^{-1} for the control and 0.5% sodium alginate treatment samples. As the sodium alginate concentration increased, the peak disappeared. Since sodium alginate reacts with calcium carbonate, the possible explanation is that the peak shifted from sodium alginate to calcium alginate. The spectra clearly show the different peaks at 1030 cm-1 when compared with control and sodium alginate-treated samples. The PLS model obtained a higher R-value and lower standard error at both 800 - 2000 cm-1 and 800 - 1200 cm^{-1} ranges when compared with the extraction method. The PCA model can be a good tool to distinguish sodium alginate-treated samples.

Conclusion

The FTIR spectroscopy combined with PLS and PCA methods can be used for the quantitative analysis of different concentrations of sodium alginate in meat. The R2 and RMSEC values obtained for quantification were 0.998 and 2.00%, respectively. The PCA was successfully used for the classification of lower and higher concentrations of sodium alginate. This method is simple because the only sample preparation is removing the moisture from fish samples. Further research is needed to test different types of hydrocolloid-treated meat samples and to compare the spectral features to determine the hydrocolloid type and usage amount. This method can be used to test hydrocolloids in meat samples.

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Author Contributions

Huisuo Huang designed the study, did the data collection, and drafted the manuscript. Ingolf U. Grün and Mark Ellersieck performed data analysis and interpretation. Andrew D. Clarke revised the manuscript and analyzed data, and also approved the final version of the manuscript to be published.

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