

Research Article

Prediction of Carbohydrate-Protein Interaction in Cassava Starch-Casein Blended Edible Films

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Abstract: The aim of this research was to study protein-carbohydrate interaction in edible films. Edible films were made from a blend of cassava starch and casein. Tensile Strength (TS), percentage elongation (%E) and Solubility (S) were observed to study their inter-relationships. Fourier Transform Infra Red (FTIR) Spectroscopy was used to monitor the molecular interactions. Interaction of carbohydrate and protein was studied at pH of 4, 7 and 9 and various casein percentages (0, 20, 40, 60, 80 and 100%, respectively). Increasing the casein ratio decreased %E and S but improved TS. Improving the %E decreased the TS and S when pH was increased ($p < 0.05$). FTIR spectra of the samples at 100% cassava showed there were changes in intensity at $3400-3600\text{ cm}^{-1}$ with the changes in pH and the lowest occurred at pH 7. FTIR spectra of 100% casein films showed that intensity was increased by increasing the pH and the lowest intensity was at pH 4. The FTIR spectra of films from a blend of cassava starch-casein showed an absorption band similar to cassava starch films in the range $1200-1000\text{ cm}^{-1}$ and similar to casein films in the range $1700-1500\text{ cm}^{-1}$. Highest interaction between cassava starch and casein molecules occurred at pH 9 in all cases. At $3600-2800\text{ cm}^{-1}$ it was the 60% casein films. In the region $1700-1500\text{ cm}^{-1}$ it was the 80% casein films and in the range $1200-1000\text{ cm}^{-1}$ was the 20% casein films. Presumable optimum interaction of carbohydrate-protein occurred at pH 9.

Keywords: Edible film, interaction, cassava starch, casein, tensile strength, percentage elongation, FTIR

INTRODUCTION

Besides environmental issues, the safety aspects of synthetic polymers as food packaging have been questioned, because at high temperatures, some plastic monomers decompose and contaminate the food. Therefore, it is necessary to develop a plastic that is not only biodegradable, but also safe for food packaging. The development of edible films could solve this problem. Edible films such as wax coating, sausage casings and gelatin capsules (Kroctha and De Mulder-Johnston, 1997) have been used in the food industry for many years.

Edible films can be produced from hydrocolloids such as protein and carbohydrate. Cassava starch is used as a component of edible films because of its transparency and brightness. Unfortunately, cassava starch films have poor mechanical properties, especially tensile strength. Adding protein to cassava starch films can improve these properties (Lindriati *et al.*, 2014). Casein has been used in the production of plastics, coating of paper and paperboard (Southward, 1989).

Casein, being a protein, can form hydrogen bonds, electrostatic interactions and random coil structures. This makes casein suitable for the development of edible films. Composite films consisting of a combination of carbohydrates and proteins have good mechanical characteristics (Rhim *et al.*, 1999; Coughlan *et al.*, 2004; Lindriati *et al.*, 2014).

Only limited information is available about films consisting of various blends of cassava starch and protein and little is available about the interaction between the two biopolymers. The interaction between carbohydrate and protein maybe segregative (the biopolymers dispel each other causing them to form separate layers) or associative (the biopolymers mix and form a stable mixture). The interaction of very dilute protein and polysaccharide solution are associative and they are co-soluble. Increasing the concentration of the biopolymers usually intensifies segregativity and is dependent on the type of interaction between them (DeKruif and Tuinier, 2001).

FTIR has been used by many researchers (Turhan *et al.*, 2001; Vicentini *et al.*, 2005; Li *et al.*, 2006;

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Henrique *et al.*, 2007) to study edible films because of its ease of use. Sample preparation is simple and analysis is rapid. FTIR can be used to analyse complex mixtures without prior separation. Guerrero *et al.* (2014) studied the interaction of protein-polysaccharides in extruded blends using FTIR by observation of absorption band intensity.

The aim of this research was to study protein-carbohydrate interaction in edible films of cassava starch-casein blends. FTIR was used to study the interaction through observation of changes in the intensity and shape of the spectra. The effect of the interaction on tensile strength, elongation and solubility were observed. The study was under taken at different pHs and variety of casein percentages.

MATERIALS AND METHODS

Material: Commercial cassava starch and casein were obtained from local suppliers. The macro-nutrient composition of cassava starch and casein was analyzed according to the method described in AOAC (1995). The results were as follows: tapioca starch had a protein content of 0.6%, carbohydrate 88.2%, lipid 0.3%, ash 0.3%, water 10.6%, whereas casein had a protein content of 73.3%, carbohydrate 12.2%, lipid 2.1%, ash 1.9% and water 10.5%. Technical grade glycerol was purchased from a local supplier and was used as plasticizer.

Film preparation: The edible film solution was prepared using distilled water and the pH was adjusted to 4, 7 and 9 using 0.2 M HCl and 0.2 M NaOH. Sample weights of 10 g of material consisting of cassava starch and casein blends of 0, 20, 40, 60, 80 and 100%, respectively and 2.5 g glycerol were dissolved in distilled water. The gels were formed by heating the solution in a water bath for 20 min. This was done with continuous stirring. The gel was cast on 10×10 cm² coated porcelain tiles. The films were dried at 50°C for 20 h in a temperature regulated oven. The dry films were peeled from the porcelain tiles then wrapped in high density polyethylene and stored at room temperature.

Tensile Strength (TS) and percentage elongation to breaking point (%E): TS and %E were tested using a Universal Testing Machine (Modified ASTM D638-94, Chang *et al.* (2000)). Film samples, 15×50 mm² were clamped in the machine. Initial grip space was defined as 50 mm and crosshead speed was defined at 250 mm/min. TS was calculated by dividing the maximum load (Newton) with the cross-sectional area of the sample (m²). The %E was calculated by dividing the change in length with an initial length (50 mm) and expressed as a percentage. TS and %E measurement were replicated five times for each type of film.

Solubility (S): S was calculated as the percentage of soluble matter to initial dry matter of sample (Gontard *et al.*, 1993). Samples of 2.5×5 cm² were dried at 105°C for 24 h to measure the amount of dry matter. The dry films were then soaked in distilled water (30 mL) at 25°C for 24 h. Undissolved dry matter filtered from the water, then dried 105°C for 48 h. Samples was weighed using a 0.0001 g electronic balance (Ohaus). The weight of solubilized matter was obtained by subtracting the weight of undissolved dry matter from weight of the initial dry matter.

FTIR spectroscopy analysis: Samples for FT-IR spectroscopy were prepared by drying films at 60°C then pulverised into a fine powder. Two milligrams of powder were mixed with 200 mg KBr (FTIR grade) and formed into a pellet. The pellet was mounted in the sample holder of the FTIR machine (Shimadzu) and spectra were then observed in the range 4000-450 cm⁻¹.

Statistical analysis: The experimental designed had two independent variable factors, casein percentage and pH. There were three replications. Anova (Analysis of Variance) at the 0.05 level was used to detect the interaction of casein percentage with different pH. A Tukey test was used as a multiple comparison test of significance. Statistical analysis was performed using MINITAB 14.2. Statistical analysis was used to study TS, %E and S

RESULTS AND DISCUSSION

Tensile strength: TS of cassava starch-casein blended films showed that TS increased after adding casein (Fig. 1). The increase of TS from 0% to 80% casein was not significantly different ($p < 0.05$) but significantly different when casein contents increased from 80% to 100%. Films of 100% casein have TS value greater than 0% casein or 100% cassava starch films.

In general, the tensile properties of protein films were better than starch films and our study agreed with the results of Lindriati *et al.* (2014). The reason for this is because starch is a homopolymer where as protein has a complex structure that contains more than 20 hydrogen bonds in different monomers. Protein therefore has large number of interactive sites, especially for forming intermolecular bonds. Increasing the number of intermolecular bonds decreases the ability of glycerol to plasticize edible films. Usually edible protein films have good tensile strength, but less plasticity and poor elongation properties (Coughlan *et al.*, 2004).

From Fig. 1 it can be seen that the TS of cassava starch-casein blended edible films slightly decreased after increasing the pH of the solvent. Lowering of TS was not significantly different ($p < 0.05$). These results

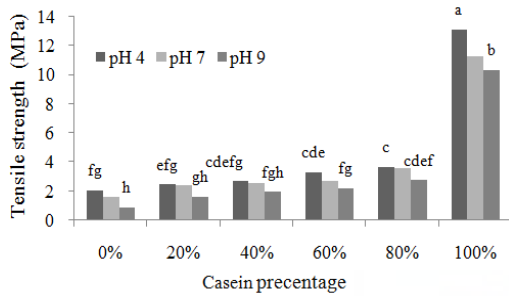


Fig. 1: Tensile strength of cassava starch-casein blended films with varied of casein percentage and pH level

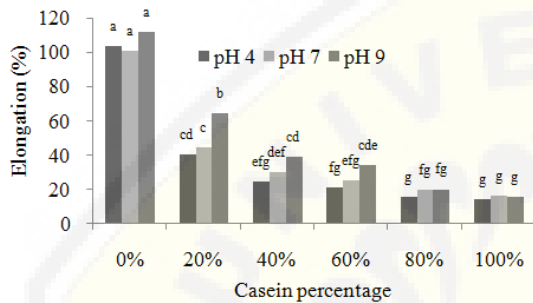


Fig. 2: Percentage elongation of cassava starch-casein blended films with varied of casein percentage and pH level

agree with Nemet *et al.* (2010) that the pH value had no significant influence on TS. The highest value of TS (13.13 Mpa) was obtained from film with 100% casein at pH 4. The TS value was in the range of casein films used by Chick and Ustunol (1998), that produced films with TS value 0.42-15.12 Mpa. The lowest value of TS (0.89 Mpa) was obtained from a film with 100% cassava starch at pH 9. A study by Parra *et al.* (2004) showed the TS value of edible films from cassava starch were 0.069-0.473Mpa which was lower than from the films in our research.

Percentage Elongation (%E): Figure 2 shows the variation in %E of cassava starch-casein blended films decreased after casein was added to the blend and the reduction was significantly different ($p < 0.05$). In general, lowering of %E was often accompanied with increasing TS. Increasing solvent pH generally increased %E but at 100% casein films, there were a reduction in the %E value when pH was increased. When pH increased from 4 to 9, protein solubility increased because of the strong electrostatic repulsion of ionized groups in a film-forming solution. Solubilization of protein molecules affects the mechanical properties of the films (Hamaguchi *et al.*, 2007). A significant effect of pH variation was observed with %E but not on TS.

The highest value of %E (112.06%) was obtained from films with 0% casein and pH 9. The lowest value was 14.5%, from films with 100% casein and pH 9. Chick and Ustunol (1998) produced films from casein

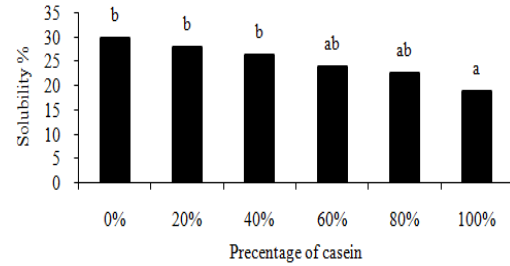


Fig. 3: Solubility of cassava starch- casein blended films with varied casein percentages

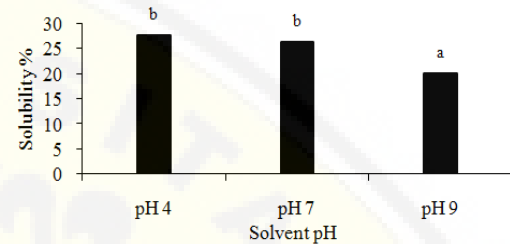


Fig. 4: Solubility of cassava starch- casein blended films with varied of pH values

with glycerol as plasticizer, their value of %E was 121.4-253.6% which is higher than the %E from our research. Parra *et al.* (2004) studied edible film from cassava starch combined with different polysaccharides, their value of %E was 9.86-72.90% which was lower than their %E from our research.

Solubility (S): S in water was measured because it is an important property used to measure hydrophobicity. Figure 3 shows that S of cassava starch-casein blended films decreased when casein percentage increased ($p < 0.05$). Unfolding of hydrophobic and cysteine amino acid residues in casein molecule occurs during solution heating and causes a decrease in S. Anova analysis results showed there was no interactive effect between the percentage of casein and pH value of edible film S.

Figure 4 show that S decreased when solvent pH increased. The reduction was significantly different at the change of pH from 7 to 9 ($p < 0.05$). Increasing the pH value increased protein solubility (Nnadozie *et al.*, 2015). Higher protein solubility probably increased protein-carbohydrate cosolubility and interaction. In this situation, stable and less soluble films can be produced.

Nemet *et al.* (2010) studied S of edible films with pH variation. Their films had been prepared from myofibrillar proteins isolated from chicken breast muscles in distilled water. The result showed increases of S by reducing the pH. The result was not similar with our study, perhaps because edible films in our research was made from a blend of protein and carbohydrate that probably interacted at the intermolecular level.

FTIR spectrum of cassava starch films: The resulting spectrain Fig. 5 show absorption bands which

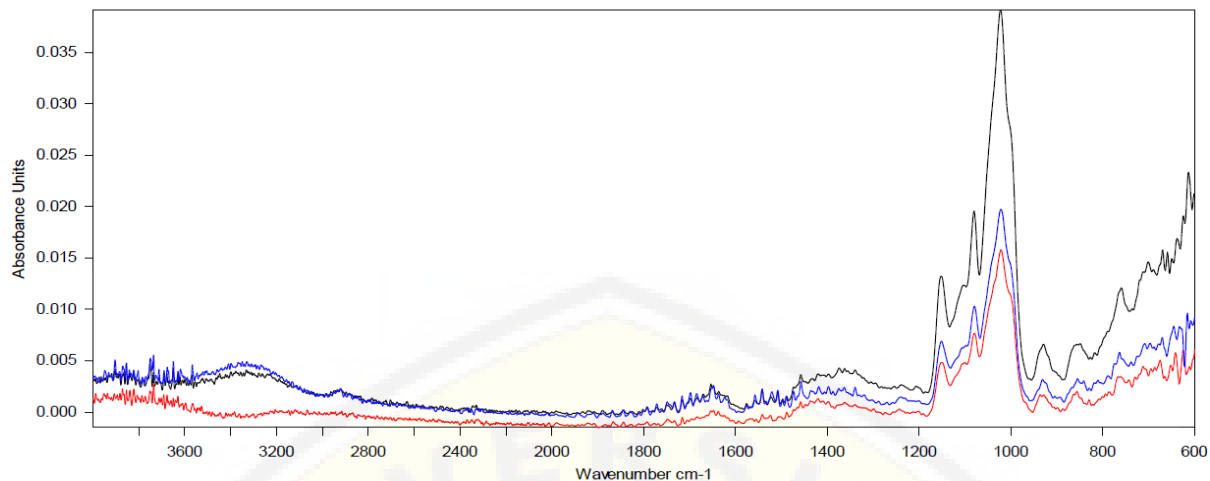


Fig. 5: Spectral comparison of edible film from 0% casein films (100% cassava starch) at pH 4, 7 and 9 (note: blue = pH 4; red = pH 7; black pH 9)

represents the fingerprint of the sample that is similar to that in a study by Henrique *et al.* (2007). According to Huang *et al.* (2006) the absorption band in the area lower than 800 cm^{-1} indicates the pyranosaring from the glucose monomer, the band in the region $1080\text{--}1158\text{ cm}^{-1}$ indicates C-OH stretching and in the region $990\text{--}1030\text{ cm}^{-1}$ indicated C-O-C stretch. The absorption band in the area $1700\text{--}1600\text{ cm}^{-1}$ indicates C = O stretch, in the region $3000\text{--}2800\text{ cm}^{-1}$ indicates CH stretch and in the area $3400\text{--}3200\text{ cm}^{-1}$ indicates OH stretch (Lindriati, 2011).

Figure 5 show there were differences in intensity of absorption band with the change of pH. In the area of $3400\text{--}3200\text{ cm}^{-1}$, when pH changed from 4-7 the intensity decreased and increased when pH changed from 7-9. Henrique *et al.* (2007) studied water vapor permeability quantification of cassava starch films by determining the band in the area of OH stretch. The difference in intensity in this area indicates differences in O-H stretch related to hydrogen bonding in the films. Edible film from cassava starch prepared using a pH 7 solution probably had the lowest ability to form hydrogen bonding.

In the area $1200\text{--}600\text{ cm}^{-1}$, the lowest intensity was obtained from the films at pH 7 and the highest was from films at pH 9. Increase in pH from 7 to 9 probably developed the ability of cassava starch molecules to interact with each other in the C-OH stretch site in the edible film matrix. The interaction between cassava starch macromolecules did not affect elongation but significantly affected tensile strength when pH changed from 4 to 9 (Fig. 1 and 2).

FTIR spectrum of casein films: The FTIR spectra of protein have nine characteristics, that is amide A, B and amide I-VII. The amide A (3300 cm^{-1}), B (3100 cm^{-1}), I ($1600\text{--}1690\text{ cm}^{-1}$) and II ($1480\text{--}1575\text{ cm}^{-1}$) are the most prominent vibrational bands of the protein backbone.

The spectra describes NH stretching, C = O stretching, CN stretching and NH bending. The most sensitive region for protein secondary structure is the amide I band ($1600\text{--}1690\text{ cm}^{-1}$) (Kong and Yu, 2007).

Figure 6 show that the intensity of absorption band increased with a pH change from 4 to 9. The highest intensity was obtained from films made from solution at pH 9. Increasing the pH made protein molecules more soluble (Nnadozie *et al.*, 2015). Probably the increase in solubility allowed the casein molecules to interact at the intermolecular level in the edible film matrix. The molecular interaction of the casein molecule had noeffect on elongation but decreased tensile strength and solubility significantly (Fig. 1 to 4).

FTIR spectrum of films from the blend of cassava starch-casein: Spectral comparison of cassava starch-casein blended films at different casein percentage can be seen in Fig. 7 to 9. The FTIR spectra of cassava starch-casein blended films show an absorption band similar to cassava starch films in the range $1200\text{--}1000\text{ cm}^{-1}$ representative of C-OH stretch and C-O-C stretch, similar to casein films in the range $1800\text{--}1500\text{ cm}^{-1}$ representative of ring aromatic stretch; C=C stretch; NH bend, NH_2 bend; ring aromatic stretch and C=O stretch. In these areas, the band intensity of films from the blend was higher than 100% casein or 100% cassava starch (0% casein) films.

In the range $3600\text{--}2800\text{ cm}^{-1}$, the spectra of cassava starch-casein blended films have a higher intensity than spectra from 100% casein films and 100% cassava starch films. According to Henrique *et al.* (2007) spectra in the area $3750\text{--}2400\text{ cm}^{-1}$, are related to the stretching of-OH that is responsible for hydrogen bonding, enhancement of intensity in these regions is presumably related to hydrogen bond formation. In this area, interaction in the carbohydrate-protein edible films matrix was a result of hydrogen bonding.

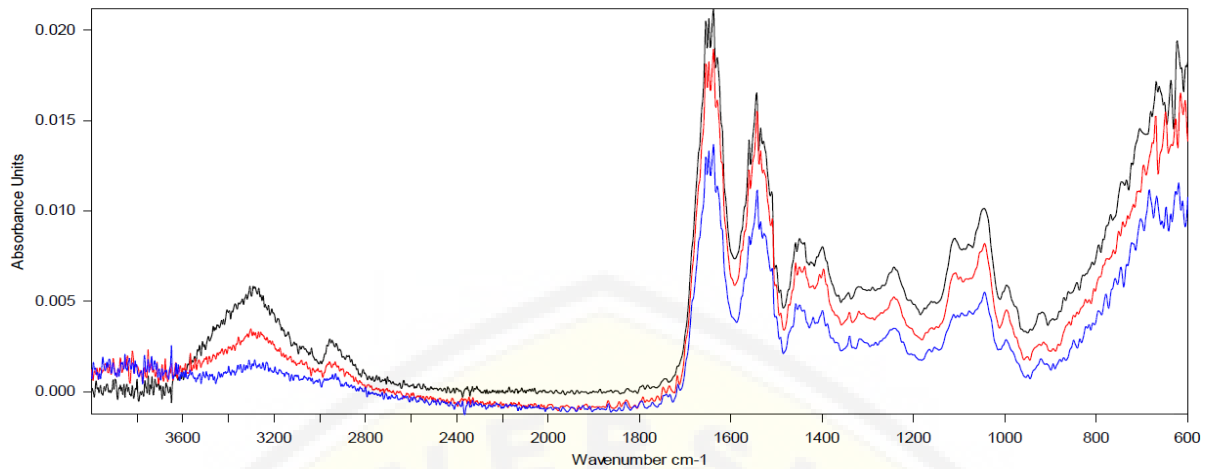


Fig. 6: Spectral comparison of edible film from 100% casein at pH 4, 7 and 9. (note: blue = pH 4; red = pH 7; black pH 9)

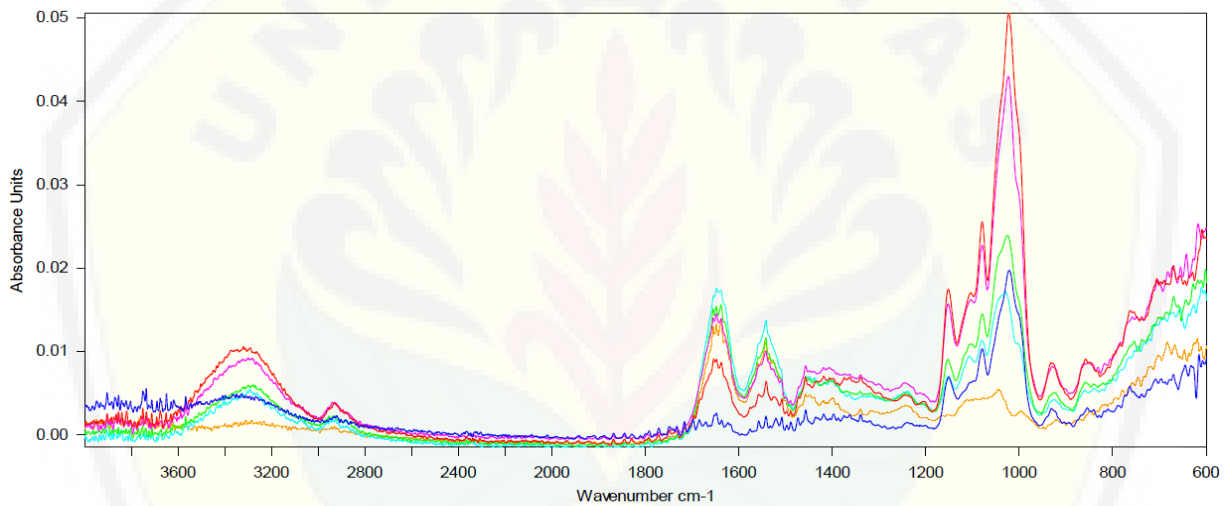


Fig. 7: Spectral comparison of edible films from cassava starch-casein blend at pH 4 with different level of casein ratio (Note: orange = casein 100%; toscablue = casein 80%; green = casein 60%; magenta = casein 40; red = casein 20; blue = cassava starch 100%)

Figure 7 show at the range 3600-2800 cm^{-1} the highest intensity was obtained in the 20% casein films followed with 40%, 60% and the lowest value was for 80% casein films. At pH 4, 20% the addition of casein promotes hydrogen bonding, but decreased when casein percentage was stepped up. Figure 8 shows the highest intensity in the range 3600-2800 cm^{-1} was obtained in the 20% casein films followed by the 80% and 40% casein films and the lowest was for 60% casein films. Intensity was greater for the 80% casein films at pH 7 than at pH 4. Figure 9 shows in the range 3600-2800 cm^{-1} the highest intensity was obtained from the 60% casein films followed with those of 80%, 40% and the lowest was for 20% casein films. At pH 9, highest carbohydrate-protein interaction with hydrogen bond formation was obtained from the 60% casein film. The result was different with pH 4 and pH 7. Higher pH possibly made protein more soluble and the ability to form hydrogen bonds with carbohydrates increased.

The intensity of absorption band was highest at 80% casein even higher than 100% casein, in the area 1800-1200 cm^{-1} and followed with 60%, 40% and 20% casein. Probably, 20% cassava starch addition in the casein films matrix developed macromolecule interaction at amide I and amide II sites of the protein molecule. According to Kong and Yu (2007), the most sensitive region for protein secondary structure is the amide I band in the area 1600-1690 cm^{-1} . But the interaction decreased as casein ratio decreased. The same result was obtained at pH 4, 7 and 9 (Fig. 7 to 9).

Figure 7 to 9 show that in the region 1200-1000 cm^{-1} , the highest intensity of the blend was obtained from 20% casein films and decreased when casein percentage was stepped up. The intensity of the 20% casein films was higher than the 100% cassava films. Probably, 20% casein addition in the cassava film matrix developed macromolecule interactions on the C-

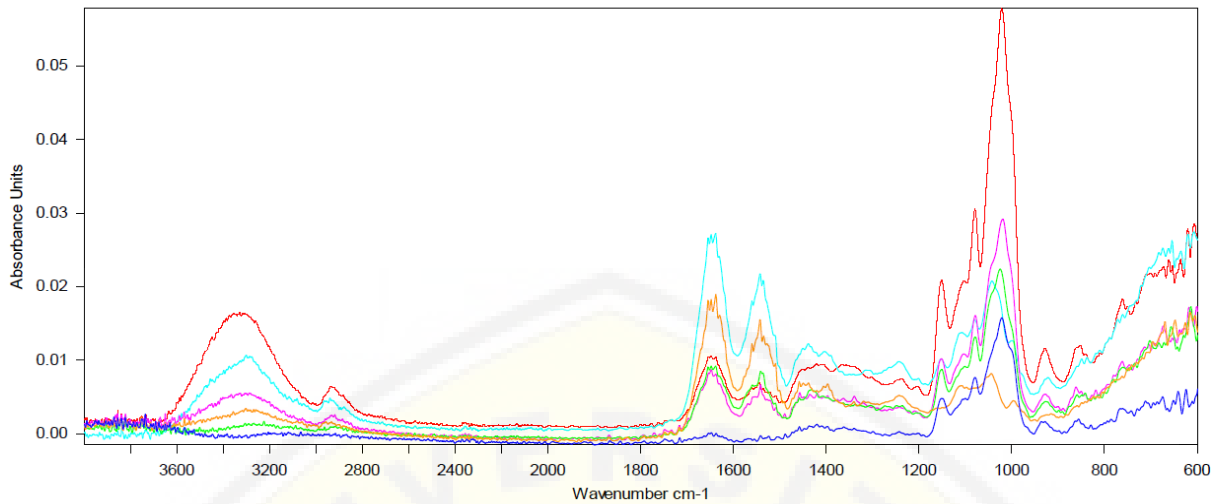


Fig. 8: Spectral comparison of edible films from cassava starch-casein blend at pH 7 with different level of casein ratio (Note: orange = casein 100%; toscablue = casein 80%; green = casein 60%; magenta = casein 40; red = casein 20; blue = cassava starch 100%)

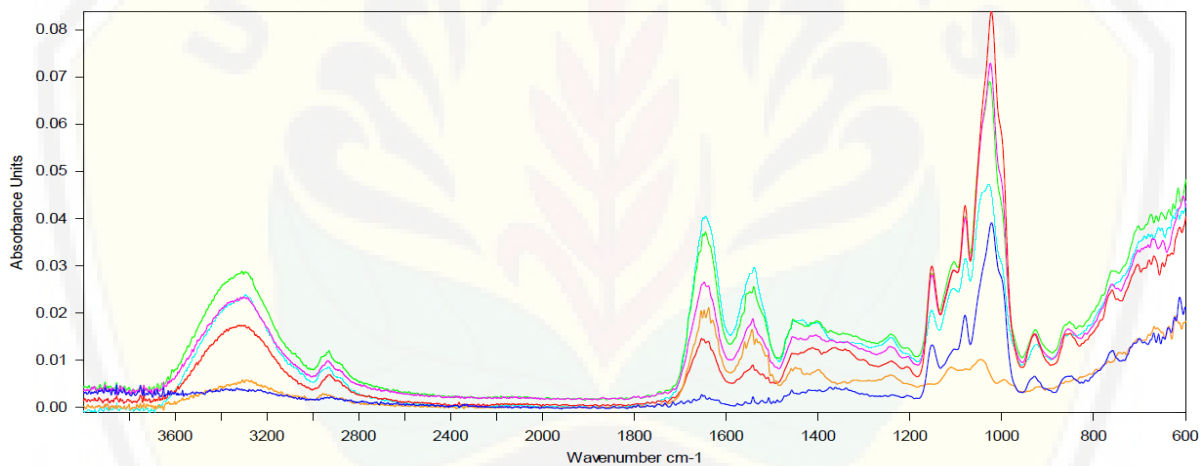


Fig. 9: Spectral comparison of edible films from cassava starch-casein blend at pH 9 with different level of casein ratio (Note: orange = casein 100%; toscablue = casein 80%; green = casein 60%; magenta = casein 40; red = casein 20; blue = cassava starch 100%)

OH stretch site. The same result was obtained at pH 4, 7 and 9.

Figure 7 to 9 show increased in band intensity for the cassava starch-casein edible films when pH level was stepped up. The enhancement can be seen in the region 3600-2800 cm⁻¹, 1800-1200 cm⁻¹ and 1200-1000 cm⁻¹. Presumably increasing protein solubility promotes hydrogen bond formation, interactions at amide I and II and interaction at the C-OH site of carbohydrate molecules.

CONCLUSION

FTIR spectra can be used to study cassava starch-casein interaction in the edible film matrix with a variation in pH and casein percentages. Carbohydrate-carbohydrate interaction promoted formation of

hydrogen bonding at C-OH stretch site, the highest interaction was obtained at pH 9. Formation of hydrogen bonds in protein-protein interaction can be observed in the secondary structure of protein. The highest interaction was obtained at pH 9. Interaction of carbohydrate-protein promoted the formation of hydrogen bonds at the C-OH stretch site of the carbohydrate molecules and secondary structure of proteins. The highest hydrogen bond formation was obtained from 20% casein in pH 4 and pH 7 solutions and 60% casein when the pH value was 9. Hydrogen bonding increased with solubility as pH values were stepped up. Highest interaction between cassava starch and casein molecules occurred at 3600-2800 cm⁻¹ in the 60% casein films at pH 9, in 1700-1500 cm⁻¹ it was the 80% casein films at pH 9 and at 1200-1000 cm⁻¹ it was the 20% casein films at pH 9. Presumably optimum

interaction of carbohydrate-protein occurred at pH 9. The complex interaction between carbohydrate-carbohydrate, protein-protein and carbohydrate-protein affected the %E, TS and S. Increasing the casein ratio decreased %E and S but improved TS. With increasing pH %E increased but saw a decrease in TS and S ($p < 0.05$).

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