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CHARACTERIZATION OF NANOCOMPOSITE CASEIN-CHITOSAN WITH ADDITION TiO_2 TOWARD PHYSICAL STABILITY, EMULSIFYING ACTIVITY INDEX, AND MICROSTRUCTURE

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ABSTRACT

The purpose of this research was to determine the characteristic of physical properties and emulsifying activity index with different concentrations (0%, 1%, 3%, and 5% (v/v)) on the viscosity, solubility, turbidity, emulsifying activity index, and microstructure. The experiment was designed by Completely Randomized Design (CRD). The data were analyzed by Analysis of Variance (ANOVA) and continued by the Least Significant Difference (LSD) test. The addition of TiO_2 with different concentrations gave a highly significant difference characteristic on syneresis, viscosity, solubility, turbidity, and emulsifying activity index ($P<0.01$) and the addition of TiO2 with different concentrations show the different effect on protein casein and chitosan aggregate. The surface microstructure of nanocomposite casein-chitosan addition of TiO_2 for low-level concentration TiO2. It can be presumed presence of TiO_2 as a photocatalyst indicated increasing the syneresis, viscosity, solubility, turbidity, and emulsifying activity index.

Keywords: Casein-chitosan; TiO2; solubility; turbidity; and packaging

INTRODUCTION

Currently, there is a lot of consumer demand for fresh ready-to-eat food products. This is both a big challenge and an opportunity for the food industry. The food industry to improve quality assurance and food safety requires the development of food processing technology to prevent contamination of food. There are still many technologies that require high energy and use expensive equipment, so that their commercial applications are limited. Therefore, it requires a solution so that the food products needed by consumers can meet the criteria, of course, at competitive costs. The packaging is an important thing that is needed in a food product. In addition to protecting the product from environmental contamination, it can also provide an attractive appearance. To be used as food packaging (food grade), packaging requirements are required, namely without the effect of packaging contamination on the packaged product, so that it is safe for human health (Linssen *et al.*, 2003). Nanotechnology for the development of food packaging that can guarantee food safety and product quality has attracted attention and has a major influence on the food packaging industry. The application of nanotechnology in polymer compounds opens new opportunities for improvement not only in the properties of the polymer concerned but also for their more efficient manufacturing costs (Haldorai *et al.*, 2014).

Egg coating technology is a method for extending the shelf life of eggs (Liang *et al.*, 2018). Chitosan is the most studied natural polysaccharide polymer. Due to its good film formation, gas barrier, and unique antibacterial properties, it has various sources, so it has been widely studied in egg film preservation (Liu *et al.*, 2009; Tezotto-Uliana *et al.*, 2014). Food packaging that is commonly used today is plastic because it is very affordable in terms of price and its flexibility, non-corrosive, not easily broken, and can be combined with other packaging materials. But consider that plastic can endanger health and cause environmental pollution.

Plastics in hot food can cause the breakdown of plastic polymers into their monomers, which can migrate into the food they are packed with. Plastics are widely used to wrap fresh to processed food. It is necessary to develop packaging materials that are safe and environmentally friendly (biodegradable) and can maintain the quality of foodstuffs that are being stored. Based on research Kustiningsih et al (2019), to synthesis TiO_2 nanoparticles were added with 0 g; 0.1 g; 0.2 g; 0.5 g; and 1 g). The purpose of this research is to find the right way to determine the characteristic of physical properties and emulsifying activity index with different concentrations (0%, 1%, 3%, and 5% (v/v)) on the viscosity, solubility, turbidity, emulsifying activity index, and microstructure. The urgency of this research is to produce superior packaging materials that are useful in the food industry because by developing packaging materials that are safe against disease-causing contamination and are also environmentally friendly. Casein-chitosan nanocomposite-based packaging material, which has the potential as food safety for livestock products.

MATERIALS AND METHODS

As for the addition of TiO_2 to the casein-chitosan nanocomposite solution, the physical analysis was performed on the

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parameters of syneresis, viscosity, solubility, turbidity, emulsifying activity index, and microstructure. Research implementation of stock preparation of casein-chitosan nanocomposite solution can be done by dissolving 10 g of chitosan powder in 100 ml of HCL. The stock of chitosan solution can be added to the TiO_2 solution according to the treatment. The stock of chitosan solution can be added to the TiO_2 solution according to the treatment, as follows. The research was performed experimentally using a completely randomized design (CRD) with 4 treatments and 3 replications to obtain 12 experimental units. Treatment with different concentrations in nanocomposite casein and chitosan (NCC) nanocomposites:

NCC 0 = NCC without TiO_2
NCC 1 = TiO_2 as much as 1% of NCC (v/v)
NCC 2 = TiO_2 as much as 3% of the NCC (v/v)
NCC 3 = TiO_2 as much as 5% of the NCC (v/v)

Here are the test variables:

Viscosity (Ismawanti *et al*., 2020)

Measurements were made at room temperature then the spindle is immersed solution for 1 min to adjust the temperature balance between the solution and the stems. Three viscometers of each information are read, and the average of these readings is recorded.

Solubility (Ahmad *et al*., 2012)

The sample (foem film) was weighed (W2) to determine dry material that is not soluble in water. Solubility was calculated using where W1 = weight of the sample and W2 = the sample was weighed, the following formula:

$$Solubility\ (\%) = \frac{W1 - W2}{W1}\ X\ 100\ \%$$

Turbidity (Huppetz *et al*., 2007)

The determination of the turbidity value was measured using a spectrophotometer, then determines the absorbance of 600 nm at a cuvette length of 10 mm. Turbidity (τ = Turbidity, Tr = Transmission A = Absorbance) the following formula:

$$Tr = 1/10^{A} \qquad \tau = -\ln(Tr)$$

Emulsion activity index (Zheng *et al*., 2014; Lam, 2014)

Inserted into a spectrophotometer with a wavelength of 500 nm Calculate the emulsion activity index using the formula: EAI (m2 / g) = (2 x 2.303 x A x DF) / IxC Where: A = A500 DF = dilute factor (100) I = length of cuvette (m) X = volume of oil fraction C = concentration of sample

Microstructure (Setiani *et al*., 2013)

Morphological analysis using SEM (Scanning Electron Microscopy) JEOL JSM-6360LA. Sample is attached to the set holder with double adhesive, then coated with gold metal in a vacuum. After that, the sample is inserted into its place in SEM, then topographical image observed and magnified 5000 times.

Data analysis

Data were analyzed using analysis of variance (ANOVA), and the LSD test was performed to determine a significant difference between the treatment (Yitnosumarto, 1991).

RESULTS AND DISCUSSION

Viscosity

Data and analysis of the various solubility levels are listed in Table 1. The results of the analysis of variance showed that the NCC with the addition of TiO2 levels to the casein-chitosan solution gave a very significant difference (P <0.01) to the viscosity.

The higher the level of TiO_2 addition, the increase in viscosity of the casein-chitosan solution. This is by the opinion of Setianto *et al*.. (2013) that the greater the number of solids, the greater the viscosity contained in the liquid; therefore, viscosity can also be used as an index of the number of solids present in the liquid. However, Hamid *et al.* (2019) said that the concentration of TiO_2 increases so increasing the viscosity also. In addition, Namburu (2007), that nanoparticle material will increase viscosity and resistance molecules of fluid. According to Manab (2007), the strength of the gel only comes from the amount and strength of the bond between casein-casein. The bond strength is easily damaged, so that it can affect the binding capacity of water, the level of syneresis, texture, and viscosity.

Solubility

The data and analysis of variance are listed in Table 1. The results of the analysis of variance showed that the addition of the TiO_2 level to the casein-chitosan solution had a significant difference (P <0.01) in insolubility. Casein has a low ability to dissolve, so that it affects turbidity, microstructure and causes the formation of sedimentation, so it needs to be modified to improve its functional properties. Nano TiO_2 was prepared need fully washed precipitate to control temperature of calcination against agglomeration. According to Shkol'nikov (2016) that the dissolution TiO_2 modifications on thermodynamics cause of molecular–ionic solubility, TiO2 structure and dispersity. The enhancement of the functional properties of milk protein can be carried out through chemical, enzyme, and physical modification.

Increasing the physico-chemical properties of the protein, especially milk protein such as casein, can be done chemically by adding minerals. Minerals are known to increase microstructure, sedimentation, solubility, and turbidity. These minerals can interact with protein 5 through electrostatic interactions. The minerals addition can increase protein solubility because the salt in minerals weakens the interaction between protein groups with different loads (Karlsson *et.al.*, 2005).

Turbidity

The data and analysis of variance are listed in Table 1. The results of the analysis of variance showed that the addition of the TiO_2 level to the casein-chitosan solution had a significant difference (P <0.01) in turbidity. This is presumably because the addition of TiO_2 resulted in an interaction between the casein-chitosan solution so that the turbidity value increased. Increased turbidity affects the value of turbidity because casein micelles can maintain their structure in an adverse environment or condition called intermicellar stability. Intermicellar stability refers to the stability of casein micelles against aggregation (Huppertz *et al*., 2007).

Increasing of TiO_2 concentration has a high affects turbidity because TiO_2 absorbs UV radiation effectively for small particles which have a high specific surface area so effectively in absorption (Beetsma, 2017).

Emulsion activity index

The data and analysis of variance are listed in Table 1. The results of the analysis of variance showed that the NCC with the addition of TiO_2 addition with different percentages had a very significant difference (P <0.01).

That is because the ability of casein as an emulsifier is capable to stabilize the mixed solution. This is by İbanoğlu and Karataş (2001), who explains that emulsifier molecules are adsorbed at the interface because they orient themselves with the hydrophilic part of water and the hydrophobic part of the oil. This emulsifying activity will be related to the ability of the protein to cover the oil-water surface (Sabolovic, Brncic, and Lelas., 2013).

Microstructure

Figure 5 presents the surface visualization results of NCC dried treatment using SEM. The surface of the film appears to have an uneven texture, while the chitosan surface has a homogeneous texture. The surface of the NCC2 composite film is more homogeneous, smooth, and without pores compared to other films. The surface of the alginate film looks the roughest and less homogeneous.

The cross-sectional morphology of the composite film is shown in other Figs. The cross-section of the composite film made from mixing the casein solution of chitosan + TiO_2 with a concentration of 1% and 10% were seen not homogeneous and porous (like cracks) and formed two layers. The low complexity and roughness of the casein chitosan + TiO_2 casein film were caused by the phenomenon of phase separation (agglomeration) molecules (Meng *et al*., 2010; Norajit *et al.,* 2010). Moreover, addition of TiO_2 in the chitosan made the distribution of granules chitosan matrix increases (Kustiningsih *et al.*, 2019) and the performance matrix polymers were homogeneous dispersion (Visurraga *et al.,* 2016). Microstructure properties of composite edible film which added with modified casein could affect elasticity value, so made film unbroken and potencial for packaging (Apriliyani, *et al*., 2020).

Table 1. Data of viscosity, solubility, turbidity, and emulsifying activity index

Treatment	Viscosity (mPas) 18° C	Solubility (%)	Turbidity (1/cm)	Emulsion activity index (%)
NCC0	$131.67^a \pm 7.63$	$98.56^e \pm 0.01$	$0.66^a \pm 0.02$	$67.45^a \pm 2.34$
NCC1	$208.33^b \pm 20.82$	$98.02^d \pm 0.06$	$0.84^b \pm 0.05$	$90.99^b \pm 6.39$
NCC2	$278.33^c \pm 15.27$	$97.59^c \pm 0.18$	$0.99^c \pm 0.04$	$112.49^c \pm 4.69$
NCC3	$335.33^d \pm 20.02$	$96.16^b \pm 0.23$	$1.13^d \pm 0.05$	$129.89^d \pm 6.14$

Note: alpha 0.01 (very significant) on viscosity, solubility, turbidity, and emulsifying activity index

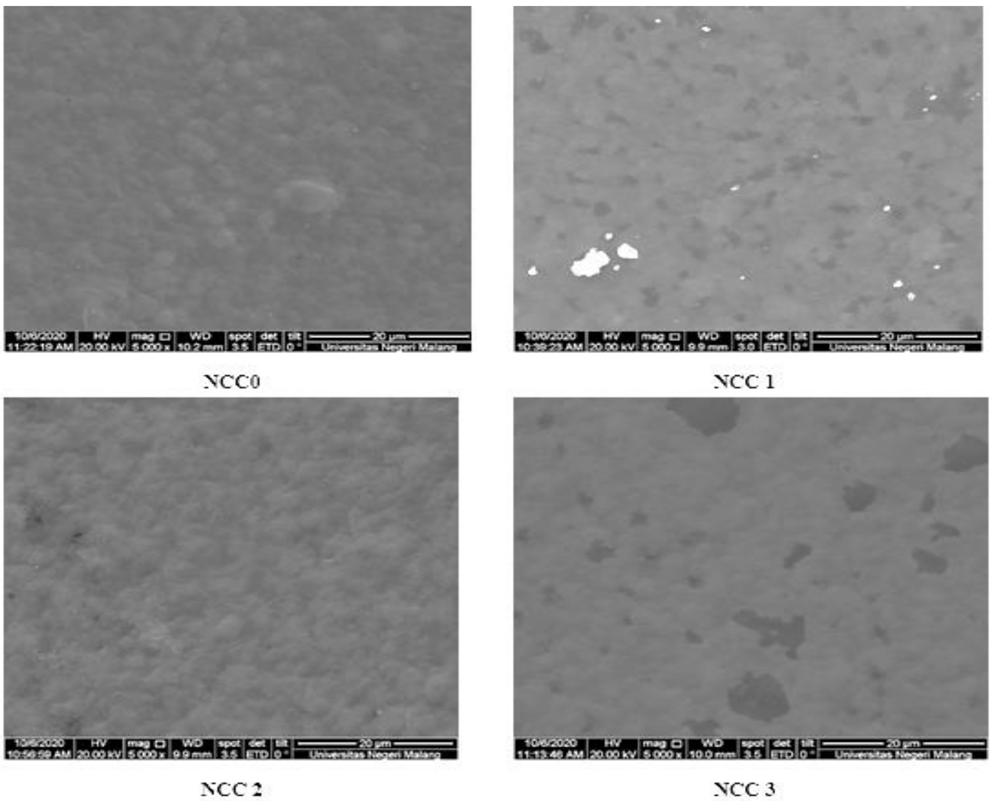

Figure 1. Microstructure of Nanocomposite casein-chitosan with addition of TiO2

CONCLUSION

In the conclusion of this study, added TiO_2 with different concentrations (0%, 1%, 3%, and 5% (v/v)) would increase the viscosity, turbidity, emulsifying activity index and will decrease on solubility. The surface of the NCC2 composite film is more homogeneous, smooth, and without pores compared to other films.

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REFERENCES

Abdul Hamid, K., Azmi, W. H., Nabil, M. F., & Mamat, R. (2019). Viscosity determination of titanium dioxide in water and ethylene glycol mixture based nanofluid. *Indian Journal of Pure and Applied Physics*, *57*(7), 461–465.

Ahmad, M., Benjakul, S., Prodpran, T., & Agustini, T. W. (2012). Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. *Food Hydrocolloids*, *28*(1), 189–199. https://doi.org/10.1016/j.foodhyd.2011.12.003

Apriliyani, M. W., Andriani, R. D., Rahayu, P. P., Purwadi, P., & Manab, A. (2020). Mechanical, chemical and microstructure properties of composite edible film added with modified casein. *Jurnal Ilmu Dan Teknologi Hasil Ternak*, *15*(3), 162–171. https://doi.org/10.21776/ub.jitek.2020.015.03.4

Beetsma, J. (2017). *Transparent inorganic pigments: titanium dioxide and iron oxide*. https://knowledge.ulprospector.com/6818/pc-transparent-inorganic-pigments-titanium-dioxide-iron-oxide/

Díaz-Visurraga, J., Meléndrez, M. F., García, A., Paulraj, M., & Cárdenas, G. (2010). Semitransparent chitosan-TiO 2 nanotubes composite film for food package applications. *Journal of Applied Polymer Science*, *116*, 3503–3515. https://doi.org/10.1002/app.31881

Haldorai, Y., & Shim, J.-J. (2014). Novel chitosan-TiO 2 nanohybrid: Preparation, characterization, antibacterial, and photocatalytic properties. *Polymer Composites*, *35*(2), 327–333. https://doi.org/10.1002/pc.22665

Huppertz, T., & de Kruif, C. G. (2007). High pressure-induced solubilisation of micellar calcium phosphate from cross-linked casein micelles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *295*(1–3), 264–268. https://doi.org/10.1016/j.colsurfa.2006.09.010

İbanoğlu, E., & Karataş, Ş. (2001). High pressure effect on foaming behaviour of whey protein isolate. *Journal of Food Engineering*, *47*(1), 31–36. https://doi.org/10.1016/S0260-8774(00)00096-0

Ismawanti, R. D., Putri, W. D. R., Murtini, E. S., & Purwoto, H. (2020). Edible film made of corn starch-carrageenan-rice bran: the characteristic of formula's viscosity, water content, and water vapor transmission rate. *Industria: Jurnal Teknologi Dan Manajemen Agroindustri*, *9*(3), 173–183. https://doi.org/10.21776/ub.industria.2020.009.03.2

Karlsson, A. O., Ipsen, R., Schrader, K., & Ardö, Y. (2005). Relationship between physical properties of casein micelles and rheology of skim milk concentrate. *Journal of Dairy Science*, *88*(11), 3784–3797. https://doi.org/10.3168/jds.S0022-0302(05)73064-2

Kustiningsih, I., Ridwan, A., Abriyani, D., Syairazy, M., Kurniawan, T., & Barleany, D. R. (2019). Development of chitosan-TiO2 nanocomposite for packaging film and its ability to inactive staphylococcus aureus. *Oriental Journal of Chemistry*, *35*(3), 1132–

1137. https://doi.org/10.13005/ojc/350329

Lam, R. H. L. (2014). *Tailoring of whey protein isolate stabilized oil-water interfaces for improved emulsification*.

Liang, X., Yang, L., Ye, L., Luo, W., Zhao, Y., & Wang, Z. (2018). A brief talk on egg coating preservation technology. *Jiangxi Journal of Animal Husbandry and Veterinary Medicine*, *5*, 4–6.

Linssen, J. P., van Willige, R. W., & Dekker, M. (2003). *Packaging-flavour Interactions*. Woodhead Publishing Limited.

Liu, X. De, Jang, A., Kim, D. H., Lee, B. D., Lee, M., & Jo, C. (2009). Effect of combination of chitosan coating and irradiation on physicochemical and functional properties of chicken egg during room-temperature storage. *Radiation Physics and Chemistry*, *78*(7–8), 589–591. https://doi.org/10.1016/j.radphyschem.2009.03.015

Manab, A. (2008). Kajian sifat fisik yogurt selama penyimpanan pada suhu 4^{o}C. *Jurnal Ilmu Dan Teknologi Hasil Ternak*, *3*(1), 52–58.

Meng, X., Tian, F., Yang, J., He, C.-N., Xing, N., & Li, F. (2010). Chitosan and alginate polyelectrolyte complex membranes and their properties for wound dressing application. *Journal of Materials Science: Materials in Medicine*, *21*(5), 1751–1759. https://doi.org/10.1007/s10856-010-3996-6

Namburu, P. K., Kulkarni, D. P., Misra, D., & Das, D. K. (2007). Viscosity of copper oxide nanoparticles dispersed in ethylene glycol and water mixture. *Experimental Thermal and Fluid Science*, *32*(2), 397–402. https://doi.org/10.1016/j.expthermflusci.2007.05.001

Norajit, K., & Ryu, G. H. (2011). Preparation and properties of antibacterial alginate films incorporating extruded white ginseng extract. *Journal of Food Processing and Preservation*, *35*(4), 387–393. https://doi.org/10.1111/j.1745-4549.2010.00479.x

Sabolović, M. B., Brnčić, S. R., & Lelas, V. (2013). Emulsifying properties of tribomechanically treated whey proteins. *Mljekarstvo*, *63*(2), 64–71.

Setiani, W., Sudiarti, T., & Rahmidar, L. (2013). Preparasi dan karakterisasi edible film dari poliblend pati sukun-kitosan. *Jurnal Kimia VALENSI*, *3*(2), 100–109. https://doi.org/10.15408/jkv.v3i2.506

Setianto, Y., Pramono, Y. B., & Mulyani, S. (2013). Nilai pH, viskositas, dan tekstur yogurt drink dengan penambahan ekstrak salak pondoh (Salacca zalacca). *Jurnal Aplikasi Teknologi Pangan*, *3*(3), 110–113.

Shkol'nikov, E. V. (2016). Thermodynamics of the dissolution of amorphous and polymorphic TiO2 modifications in acid and alkaline media. *Russian Journal of Physical Chemistry A*, *90*(3), 567–571. https://doi.org/10.1134/S0036024416030286

Taterka, H., & Castillo, M. (2015). The effect of whey protein denaturation on light backscatter and particle size of the casein micelle as a function of pH and heat-treatment temperature. *International Dairy Journal*, *48*, 53–59. https://doi.org/10.1016/j.idairyj.2015.01.017

Tezotto-Uliana, J. V., Fargoni, G. P., Geerdink, G. M., & Kluge, R. A. (2014). Chitosan applications pre- or postharvest prolong raspberry shelf-life quality. *Postharvest Biology and Technology*, *91*, 72–77. https://doi.org/10.1016/j.postharvbio.2013.12.023

Yitnosumarto, S. (1991). *Percobaan - Perancangan Analisis dan Interpretasinya*. Gramedia Pustaka Utam.

Zheng, M., Jia, Z. B., & Jiang, J. X. (2014). Emulsifying and foaming properties of soy protein isolates with covalent modification by (-)-Epigallocatechin-3-Gallate. *Advance Journal of Food Science and Technology*, *6*(2), 238–240. https://doi.org/10.19026/ajfst.6.17