CHARACTERIZATION OF NANOCOMPOSITE CASEIN-CHITOSAN WITH ADDITION TiO\textsubscript{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\textsubscript{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 TiO\textsubscript{2} with different concentrations show the different effect on protein casein and chitosan aggregate. The surface microstructure of nanocomposite casein-chitosan addition of TiO\textsubscript{2} for low-level concentration TiO\textsubscript{2}. It can be presumed presence of TiO\textsubscript{2} as a photocatalyst indicated increasing the syneresis, viscosity, solubility, turbidity, and emulsifying activity index.

Keywords: Casein-chitosan; TiO\textsubscript{2}; 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\textsubscript{2} solution according to the treatment. The stock of chitosan solution can be added to the TiO\textsubscript{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\textsubscript{2}
NCC 1 = TiO\textsubscript{2} as much as 1% of NCC (v/v)
NCC 2 = TiO\textsubscript{2} as much as 3% of the NCC (v/v)
NCC 3 = TiO\textsubscript{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:

\[
\text{Solubility} \text{ (\%)} = \frac{W1 - W2}{W1} \times 100 \%
\]

**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:

\[
Tr = 1/10^\tau \quad \tau = - \ln (Tr)
\]

**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:

\[
\text{EAI} (\text{m}^2/\text{g}) = \frac{2 \times 2.303 \times A \times DF}{I \times C}
\]

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 TiO2 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 TiO₂ 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 TiO₂ 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 TiO₂ was prepared need fully washed precipitate to control temperature of calcination against agglomeration. According to Shkol’nikov (2016) that the dissolution TiO₂ 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 TiO₂ level to the casein-chitosan solution had a significant difference (P <0.01) in turbidity. This is presumably because the addition of TiO₂ 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 TiO₂ concentration has a high affects turbidity because TiO₂ 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 TiO₂ 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) $18^\circ C$ | Solubility (%) | Turbidity (1/cm) | Emulsion activity index (%) |
|-----------|------------------------------|----------------|-----------------|-----------------------------|
| NCC0      | $131.67^a \pm 7.63$          | $98.56^c \pm 0.01$ | $0.66^a \pm 0.02$ | $67.45^a \pm 2.34$         |
| 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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