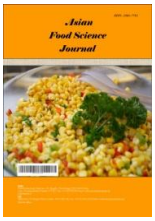

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Interactions between Cocoa Husk Catechin and Casein Micelles and their Impact on Physico-chemical Properties

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Authors' contributions

This work was carried out in collaboration among all the authors. Authors did the research in the laboratory, analysis and describe about interaction using UV-Vis detection, chemical structure using Fourier Transform Infra-Red, microscopy analysis by Scanning Electron Microscopy, particle size by Zetasizer Nano Series Software Version 7.01, Malvern Instrument and electrophoretic analysis, wrote the draft of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this research was to investigate the influence of physicochemical properties of interaction casein with different concentration catechin sources of the cocoa husk.
Study design: Casein was added catechin with various concentration treatments (0 (control), 20, 40 and 60 (µg/ml)).
Place and Duration of Study: This study was conducted between April until August 2020 at the Faculty of Animal Science, Universitas Brawijaya.
Methodology: Casein was added catechin with various concentration treatments (0, 20, 40 and 60 (µg/ml)). An analysis is carried out to determine interaction using UV-Vis detection, chemical structure using Fourier Transform Infra-Red, microscopy analysis by Scanning Electron Microscopy, particle size by Zetasizer Nano Series Software Version 7.01, Malvern Instrument and electrophoretic analysis.

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Results: The interaction between casein and catechin was investigated by the method of UV-Vis detection and chemical structure analysis by Fourier Transform Infra-Red showed that it was indicated that catechin could detected in casein. It is similar with recent research. The interaction of casein and catechin showed to decrease the particle size and showed on microscopy analysis. Protein profile showed to increase the molecular weight with the addition of catechin, it can be indicated that presence interaction both on casein with catechin.
Conclusion: Casein interacted with catechins 60 µg/ml was able to increase casein stability, maintain nano size, casein components were still detected in the protein profile, the resulting microstructure looked compact and functional groups of bioactive compounds were still detected using Fourier Transform Infra-Red.

Keywords: Microscopy analysis; milk protein; nano; particle size.

1. INTRODUCTION

Milk protein is used as an additive because of its properties as a gelling, foaming and emulsifier, especially casein. Milk protein consists of casein and whey protein with a composition of 80% and 20% of total milk protein. Casein consists of several fractions such as α casein, β casein and kappa casein [1]. Casein is a natural vehicle for essential micronutrient carriers to be added to a product. The application of catechins in dairy products can increase the bioavailability of catechins through increased absorption in the digestive tract [2].

In a study that explains that casein micelles bind hydrophobic molecules such as curcumin and vitamin D, so that an interaction between casein micelles and these compounds is formed [3]. Single molecule β-casein has a gyration radius of 4.6 nm, while casein micelles consisting of several casein molecules have a gyration radius of between 7.3-13.5 nm. The phenolic -OH group of catechins plays a role in the interaction between catechin molecules and casein to form casein-curcumin complex nanoparticles [4,5].

Nano technology is defined as the design, production, and application of structures, tools and systems that control the size and shape of a material in the nanometer scale (10-9 nm) and are used to improve texture, taste, nutritional content, be able to detect pathogenic microbes, food packaging, and functional food alternatives [6]. One way of making nanoparticles using polymers is through the spray-drying method or dispersed into an organic solvent containing a polymer and then sprayed in a hot air stream. The solvent will immediately evaporate and dry nanoparticles can be obtained [7].

The benefits of nano technology in functional food can respond to the need for nutrients in the body efficiently and bioactive nutrients are absorbed easily by the body. One of the applications of nanotechnology in the food sector is the addition of nano capsules in food ingredients that are easily dissolved, so that they do not cause distortion of the flavor and color of food ingredients. Efforts to create products of this size take into account the materials used and the processes for forming their interactions. One of the ingredients that is often used is milk protein.

2. MATERIALS AND METHODS

2.1 Process of Casein Interaction with Bioactive Compounds from Cocoa Husk

Casein was dissolved in aqueous solution by adding 2,5 gr casein in 100 ml phosphate buffer (pH 6,8). Casein was homogenized by Ultra-Turrax at the speed of 7,600 rpm for 10 min heated and added catechin with various concentration treatments (0, 20, 40 and 60 (µg/ml)) with temperature of 30-55°C. after that homogenized by hand mixer for 1 min. Casein solution can be stored in a refrigerator at 4°C. Catechin prepared using microwave-assisted extraction [8]. Furthermore, an analysis is carried out to determine interaction using UV-Vis detection, chemical structure using FTIR, microscopy analysis by SEM, particle size by Zetasizer Nano Series Software Version 7.01, Malvern Instrument and electrophoretic analysis.

2.2 Spectrophotometry Determination of the Casein-catechin Interaction

Spectrophotometry determination to know the interaction of protein with a slight modification [9]. 0,1 ml emulsion of casein with catechin (different concentration as treatment) were prepared. After 10 min the solid with adsorbed casein was

separated by centrifugation of a suspension at 12,000 rpm (twice, 10 min). The supernatant absorbance was measured at 280 nm by UV–Vis spectrophotometry. Simultaneously, a reagent blank without catechin was performed according to the same procedure. The absorption spectra of the suspension were measured between 210 and 350 nm by spectrophotometry.

2.3 Particle Size Distribution

The particle size of casein was measured by a static laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Montreal, QC, Canada), using deionized water as the dispersion medium (refractive index is 1.465).

2.4 Electrophoretic Analysis

Inter-protein crosslinking was evaluated by polyacrylamide gel electrophoresis in sodium dedocyl sulfate (SDS–PAGE) using precast gradient gels (4–15%). Gels were calibrated by the broad range (BRM) SDS calibration standard that contains nine proteins ranging in size from 6.5 to 200 kDa. Samples (approximately 0.5 mg) of casein dissolved in sample buffer (10 mMTris–HCl at pH 8.0 containing 1 mM EDTA, 25 mg/ml SDS, 50 ll/ml b-mercaptoethanol and 0.1 ll/ml bromphenol blue)(Merck) were heated at 40^{o}C for 4 h. Coomassie blue was used to stain the gels [10].

2.5 Scanning Electron Microscopy (SEM) Analysis

A scanning electron microscope (SEM-model SU8010, Hitachi High-Technologies Canada, Inc. Toronto, ON, Canada) was used to characterize the surface morphology of casein control and casein with catechin treatment. The sample was placed on object glass then coated with a layer of gold powder with a coating time of ± 30 seconds. The sample was observed using SEM with a voltage of 15 kV and magnification up to 5000 x.

2.6 Determination of Chemical Structure – Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model, Digilab, Mississauga, ON, Canada). One milligram of casein and casein with catechin treatment dried in a vacuum desiccator was ground and mixed thoroughly with 200 mg of oven-dried KBr powder (Merck, DAC, USP). The powder was placed in a die and compressed into a transparent disk. The sample was observed with the spectral range of 4000–600 cm^{-1} with a nominal resolution of 2 cm^{-1} and 100 scans [11].

3. RESULTS AND DISCUSSION

3.1 Spectrophotometry Determination of the Casein-Catechin Interaction

Interaction of casein with catechin could be observed using UV-Vis spectra at wavelengths between 210-350 nm and determined the maximum wavelength at each treatment. All of the treatment produced the same maximum waves at a wavelength of 278 nm. Image from that sample showed in Fig. 1. The maximum wavelength is not significantly different from the literatur study [9], that analysis of protein interactions using wavelengths between 210-350 nm with λ max of 280 nm.

The sample absorbs light because the compound/molecule in the sample contains a functional group called a chromophore which can absorb UV radiation. Chromophores tend to have unsaturated bonds or contain functional groups with double bonds. When a molecule absorbs light, the energy will cause excitation of electrons so that the electrons move to higher energy levels. Molecule can only absorb energy if the energy is equivalent to the energy difference of the two energy levels.

Based on the results of the analysis using UV Vis spectrophotometers, Increasing the amount of catechins added can increase the absorbance value of the casein-catechin complex. Casein without the addition of catechins has an absorbance value of 0.132 while the treatment of P1 with the addition of catechins in the amount of 20 µg/ml produces an absorbance value of 0.143. The higher the concentration added will increase the value of the absorbance produced. Addition of catechins causes more chromophore groups, so absorbing UV radiation is increased.This is due to the formation of a complex of casein nano solutions of catechins which are influenced by the presence of covalent bonds, van der walls interaction and hydrogen bonds. Interaction betweenphenolic compound with amino chains or sulfhydryl groups of polypeptides forming covalent bonds of C-N or C-S with phenol rings.

The higher absorbance value produced by the casein-catechin solution showed that more

catechins are trapped in the casein molecule. The interaction between casein and catechin was dominated by Van der walls interactions which can stabilize casein because it prevents coagulation of casein granules with other casein. The interaction formed between casein and catechins is classified as a weak bond so that it has the advantage that the role of catechins as antioxidants in the fight against free radicals still functions. This is supported by the statement that encapsulation of bioactive compound with casein was done by formed interaction both [12]. The hydrophobic casein is bound to curcumin. The interaction between the two can cause increased casein stability. The increased stability shows that the keto-enol group contained in curcumin is protected. Casein stability is affected by temperature and pH. The interaction of casein with phenol compounds is influenced by many factors, one of which is the level of proline in casein [13]. Based on proline levels, it is suspected that β-casein has a higher affinity for phenol compounds compared to α-casein [14]. The proline group has a strong affinity for the hydroxyl group (-OH) in catechins [15]. Casein has a tendency to interact with other proteins and some ligands, based on the hydrophobic character of casein micelles.

Casein bind polyphenols to through hydrophilic and hydrophobic interactions [14]. Polyphenol-protein interactions are dominated by non-covalent interactions, namely hydrophobic interactions that are stabilized by hydrogen bonds [16]. The structure and molecular weight of polyphenols have an important role in protein-polyphenol interactions, polyphenols with high molecular weight can be more strongly binding to proteins [17].

Based on the discussion above, it can be concluded that the absorbance value analysis using UV-Vis spectrophotometer with the higher addition of catechin concentrations results in higher absorbance values indicating the interaction of nano-casein-catechins. The higher absorbance value indicates that the stability of the casein is higher. The results of this analysis can be concluded that the treatment of adding catechins in the amount of 60 µg/ml produces the highest value of casein stability compared to other treatments.

3.2 Particle Size Distribution

The particle size analysis aimed to determine the particle size in casein-catechin complex and their distribution from a representative sample. Particle testing at this stage uses Delsa Nano. The principle of the working system of this tool uses laser diffraction. Particles will pass through a beam of laser light, then the light is scattered by these particles and collected over a range of direct facing angles. The distribution of the scattered intensity will be analyzed by the computer as a result of the particle size distribution.

Each treatment of different catechin concentrations produced particle sizes between 159.43-213.6 nm (Table 1). This is in accordance with several studies which explain that particles are said to be nano if they have a size of 50-1000 nm, and casein micelles have a diameter between 150-300 nm [18,19]. These results still reach the size that is in accordance with the study, so it can be concluded that the particle size in nano-casein-catechins is as expected, which is nano size.

The average particle size of nano-casein-catechins gets smaller as more concentrations of catechins are added. This happens because the interaction between OH phenolic from catechins that interact with casein [4,5]. Casein interacted with other compounds tends to produce smaller particles. Casein interacted with phenolic has a diameter of 78.1 nm, while casein interacted with dextran has a diameter of 75.3 nm [12]. Casein that is interacted with other compounds will stabilize the surface which has larger particles. The addition of dextran has the benefit of making it more stable, because of the bonds between proteins and polysaccharides. There is a conjugation between the hydrophilic dextran and casein to form bonds that are more compact and produce smaller particles of casein micelles. The process of interaction to form nano-sized particles requires the right role of temperature and pH.

Casein is rich in proline amino acids which have an open structure so that they can interact with other compounds. Bioactive compounds can interact with proline proteins through non-covalent interactions including hydrogen bonds and hydrophobic bonds. Polyphenols can be loaded in colloidal particles because of the high proline. Polyphenols have excellent solubility making it easier to make nanoparticles. The interactions formed between casein and polyphenols can cause changes in structure, stability, solubility and functional properties [20,21,22].

Non-covalent bonds including hydrogen bonds, hydrophobic interactions, and van der Waals interactions play a role in the formation of reversible interactions between proteins and polyphenol compounds. This reversible interaction was achieved by forming covalent bonds under certain conditions through enzymatic processes, heating or o-quinone formation that interacts with nucleophilic proteins such as -NH2 and -SH. Hydrogen bonds are formed by electronegative atoms (acceptor atoms) with hydrogen atoms covalently bonded to other atoms, mainly protein groups, namely amino and hydroxyl, and positive hydrogen and hydroxyl atoms from polyphenol molecules. Hydrophobic interactions are formed through the formation of bonds between aromatic amino acids from proteins and aromatic rings of polyphenols which have nonpolar properties. Non-covalent protein-polyphenol interactions are more common than covalent bonds [12,22].

It can be concluded that the results of particle size analysis in all treatments produce particle sizes that are still categorized in the range of nano particles. The higher concentration that is added produces a smaller size. The results of the analysis can be concluded that the treatment of adding catechins in the amount of 60 µg/ml produces the smallest particle size compared to other treatments.

3.3 Electrophoretic Analysis

Casein-catechins were analyzed protein profiles using SDS PAGE electrophoresis in order to determine the molecular weight of the protein. The working principle of electrophoresis is to separate charged biomolecules based on the rate of migration of biomolecules in the electric field. The results of the analysis of protein profiles can be seen in Fig. 2.

Based on the curve obtained by the equation y = -1.5187x + 2.302 with R2 = 0.9595. X is Rf and Y is the log molecular weight. Molecular weights of nano-casein-catechin samples can be obtained from these linear formulas. Based on the electrogram analysis results of electrophoresis using SDS PAGE, it can be seen that the bands that appear in samples P0, P1 and P2 have the same molecular weights. The addition of catechins with concentrations of 20 and 40 µg / ml did not change molecular weight (MW), but the P3 sample with the addition of 60 µg / ml produced 2 different bands that did not appear before in treatments P0, P1 and P2.

The molecular weights in samples P0, P1, and P2 were 116.09 kDa, 98.55 kDa, 79.21 kDa, 57.08 kDa, 28.06 kDa and 23.82 kDa. The molecular weights of 23.82 kDa, 28.06 kD and 79.21 kDa in nano-casein-catechins are suspected that the casein in nano-casein-catechin is β casein, α casein and lactoferrin. MW β casein was 24.1 kDa and α casein was 27.3 kDa [1]. MW in αS1 casein is 22.1-23.7 kDa, αS2 casein is 25.2-25.4 kDa, β casein is 23.9-24.1 kDa, k casein of 19 kDa [22] and MW of lactoferrin is 78 kDa MW [23].

Sample P3 produced two different bands when compared to other samples Fig. 2. The molecular weights in P3 samples are 116.09 kDa, 98.55 kDa, 79.21 kDa, 57.08 kDa, 38.94 kDa. 33.06 kDa, 28.06 kDa and 23.82 kDa. The molecular weight is 23.82 kDa, 28.06 kDa in nano-casein-catechins it is assumed that the casein that appears is β casein and α casein. . MW β casein was 24.1 kDa and α casein was 27.3 kDa [1]. MW in αS1 casein is 22.1-23.7 kDa, αS2 casein is 25.2-25.4 kDa, β casein is 23.9-24.1 kDa, k casein of 19 kDa [22]. Bands that differ from the others are MW 33.06 kDa, and 38.94 kDa. MW of lactoferrin is 78 kDa MW [23].

All samples based on the data above are thought to contain β casein and α casein with molecular weights of 23.82 kDa and 28.06 kD, respectively. All samples that have been tested have molecular weights with protein bands around the molecular weights of casein and α casein. Based on the electrogram it can be concluded that the casein protein bands in treatments P0 through P2 do not have MW differences. There were two different bands in the P3 sample with the highest concentration of extract addition namely MW 33.06 kDa, and 38.94 kDa. Changes in the intensity of the band staining refer to the relative abundance of protein fractions, migration of the band can be caused by the treatment of the percentage of catechin added.

The results showed that the addition of catechins caused the formation of high molecular complexes (33.06 kDa, and 38.94 kDa) in the treatment of addition of 60 µg/ml. Casein contains amino acid proline which has an open structure so that it can interact with other compounds [22,24]. Interactions are formed due to hydrogen bonds and hydrophobic interactions. Interaction occurs between phenolic acids with amino side chains or with sulfhydryl groups of polypeptides forming covalent bonds of C-N or C-S with phenol rings. The complex formation of casein and catechin results in changes in the

structure, stability, solubility and functional properties of casein [20,21,25].

3.4 Scanning Electron Microscopy (SEM) Analysis

Microstructure analysis using SEM-model SU8010, Hitachi High-Technologies Canada specifications with 5000x magnification can be seen in Fig. 3. The purpose of the analysis was to determine the casein microstructure without addition and casein that had been interacted with cocoa husk extract. The analysis was carried out using a dry sample so that the initial preparation was made to make a casein-catechin solution into powder. The condition of nano-casein-catechin powders has hygroscopic properties so that the images generated from microstructure analysis tend to stick together.

Microstructure analysis results obtained an average size for P0 of ± 3.95 µm, P1 of ± 1.44 µm, P2 of ± 1.36 µm, P3 of ± 1.16 µm. Based on these data it can be seen that the higher the concentration of added catechins will reduce the particle size, this is because the more catechins added it is suspected that more bonds are formed. The microstructure at the time of SEM observation compared with the analysis of particle in the form of solution using PSA has a different size value. That is because SEM analysis uses powder samples. The powder has hygroscopic properties, resulting in a larger size than when the sample was in solution. The hygroscopic nature indicates that the sample has the ability to absorb water molecules well so that the solubility is high. Although the resulting values are different due to the different sample shapes, the results obtained are still in line with the results of particle analysis using PSA, namely the addition of cocoa husk extract at 60 µg resulting in smaller particle sizes compared to other treatments.

Samples P0 through P3 showed that the resulting microstructure is increasingly compact, characterized by the formation of intensive bonds. It is showed in Fig. 3. This is due to the interaction between casein and catechins, causing changes in the microstructure. The interaction of the two forms a complex structure. The complex structure enhances the functional properties of casein, so it produced the stable polymers that on heating [26]. The stability of heat is caused by the interaction of casein with OH phenolic from cocoa peel extract. Supported by several studies that explain that the nanoparticles formed in casein are caused by OH groups that bind amino acids [4,5].

Samples P0 to P3 show that the resulting microstructure is progressively smaller, tighter, by ordering intensive bonds. This is due to the interaction between casein and catechins, causing changes to their microstructure. Interaction in forming complex structures. The complex structure increases the functional properties of casein to produce a heat-stable polymer [26]. The heat stability was caused by the interaction of casein with phenolic OH from the extract of the cocoa shell. Supported by several studies that explain that the nanoparticles formed in casein are caused by the OH group that binds to the amino [4,5].

3.5 Determination of Chemical Structure–fourier Transform Infrared Spectroscopy (FTIR)

Observation of functional groups on nano-casein-catechins aims to determine the functional groups that refer to the phenolic compounds that are still present in the emulsion, besides ensuring that the bioactive components in the cocoa shell extract are still and not lost during the processing process. Functional groups that refer to polyphenol compounds are observed by testing using an infrared spectrophotometer, then analyzing the images obtained.

IR spectroscopic analysis techniques aim to determine functional groups to identify functional groups that refer to certain compounds, determine molecular structures, analyze purity and study the reactions that are taking place. Used to analyze organic and inorganic compounds [27]. The results of nano-casein-catechin analysis using FTIR for more details are presented in Fig. 4. The results of the nano-casein-catechin FTIR analysis are tabulated in Table 2.

Based on (Table 2) it can be seen that in all treatments the concentration of cocoa shell extract that was added still contained a group of bioactive components of cocoa shell. It can be seen that in all treatments P0, P1, P2 and P3, spectra appear in the absorption region containing catechin groups ranging from 500-1900 cm^{-1}. This is in accordance with several studies that explain the absorption area which refers to bioactive components between 500-1900 cm^{-1}. Clusters of numbers can undergo changes caused by several factors including the

time and temperature used during the process [28,29].

In the addition of catechins P1, P2 and P3, there was an absorption between 2970.38 cm^{-1} which refers to the O-H bond of the phenol number. This is in accordance with the treatment that the addition of bioactive compounds is only in that treatment. There are several absorption areas that appear in the P3 treatment which differentiates it from other treatments. This treatment produces more spectra. There are several absorption areas different, one of the most important is in the absorption area of 1986.68 cm-1 which is thought to be the C = C Strecth group which is an aromatic number. The more catechin concentrations are added, it can reduce the possibility of bioactive compounds being lost during the heating process. This is proven by the presence of these groups which are only what in the P3 treatment.

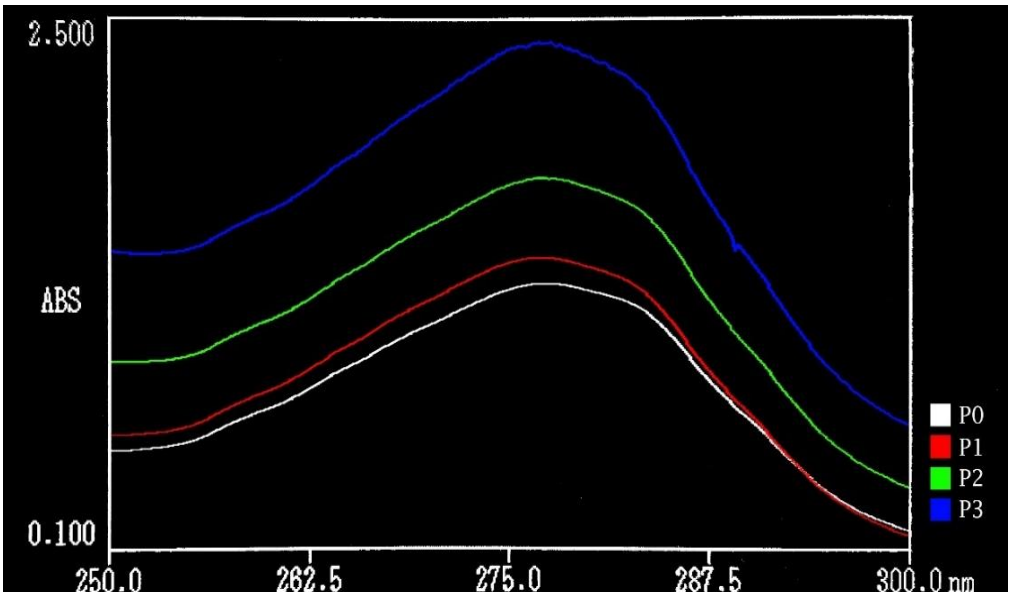

Fig. 1. Curve spectra of casein-catechin using spectrophotometer

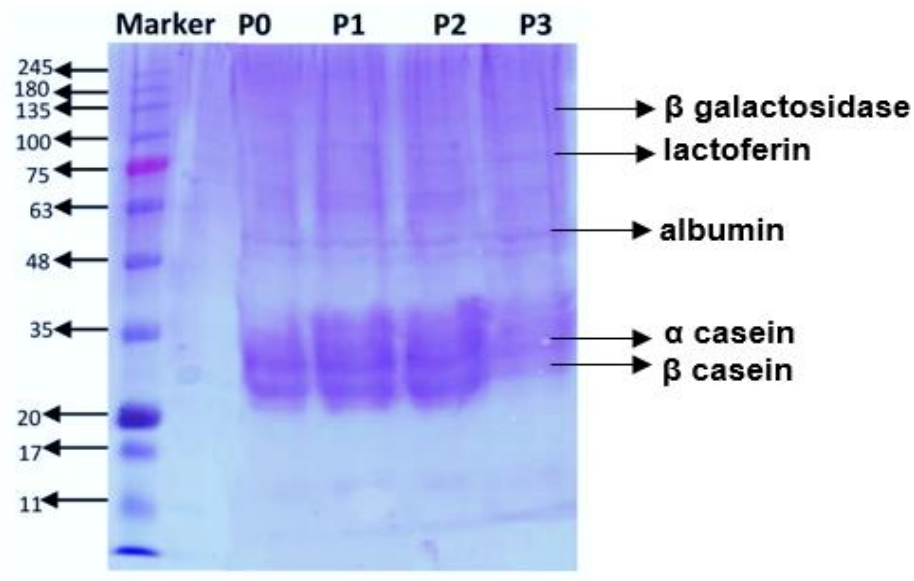

Fig. 2. Electrogram of Nano-casein-catechin Protein Profile

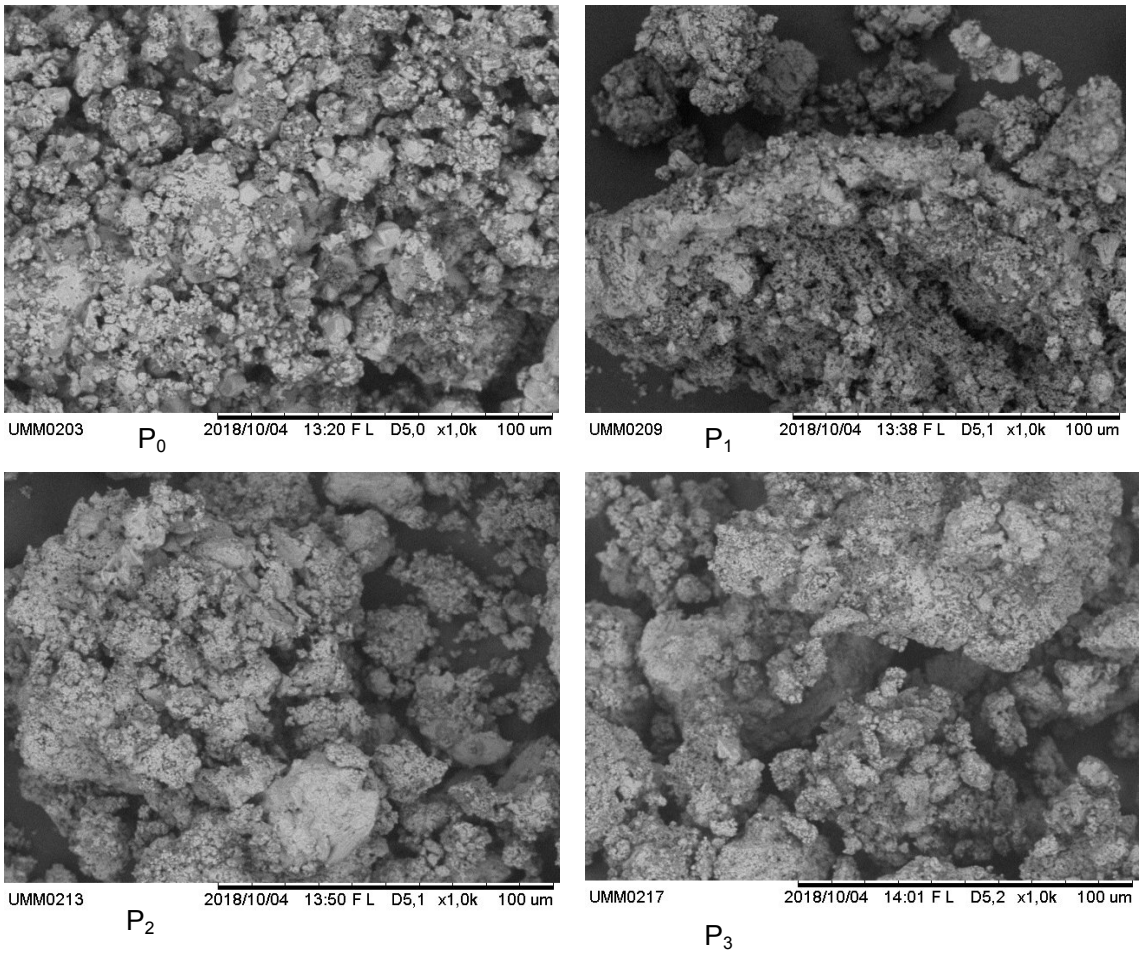

Fig. 3. The nano-casein-catechin microstructure

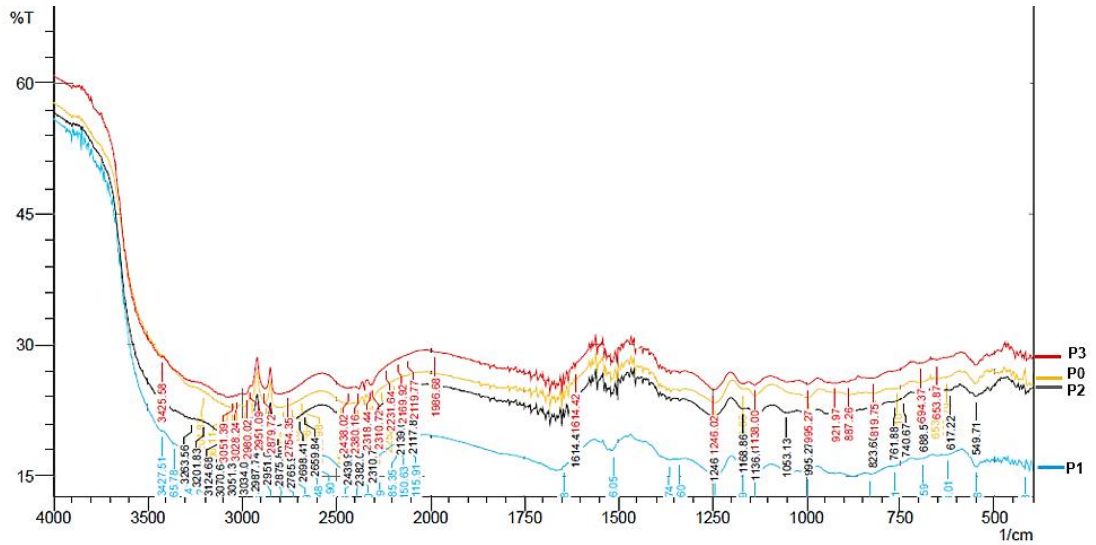

Fig. 4. Casein FTIR spectra with the addition of different catechins

Casein ftir spectra with the addition of different catechins separate each treatments.

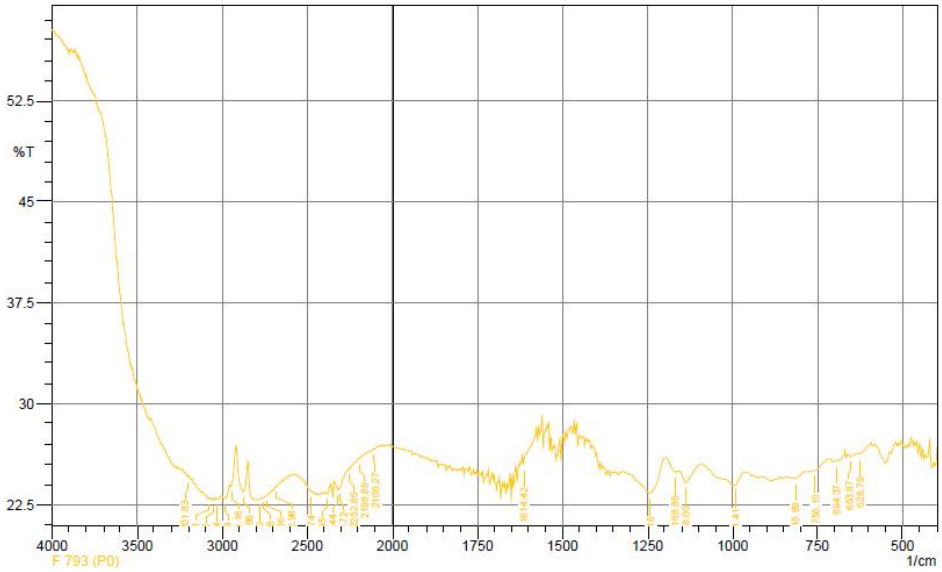

Fig. A. Casein ftir spectra with 0 µg/ml

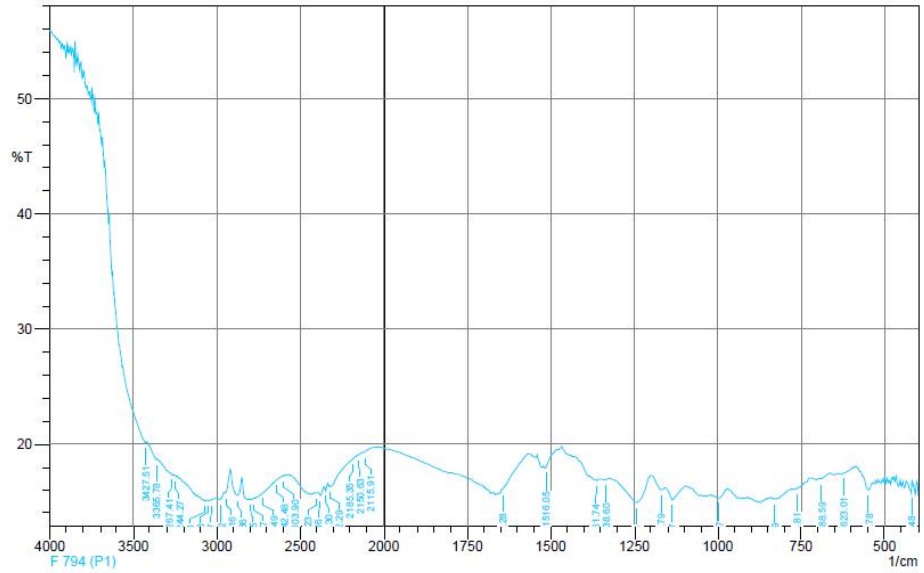

Fig. B. Casein ftir spectra with 20 µg/ml

Table 1. Particle size of nano-casein-catechin

Treatment	Average of particle size (nm)
0 µg/ml	213,6
20 µg/ml	167,40
40 µg/ml	162.57
60 µg/ml	159,43

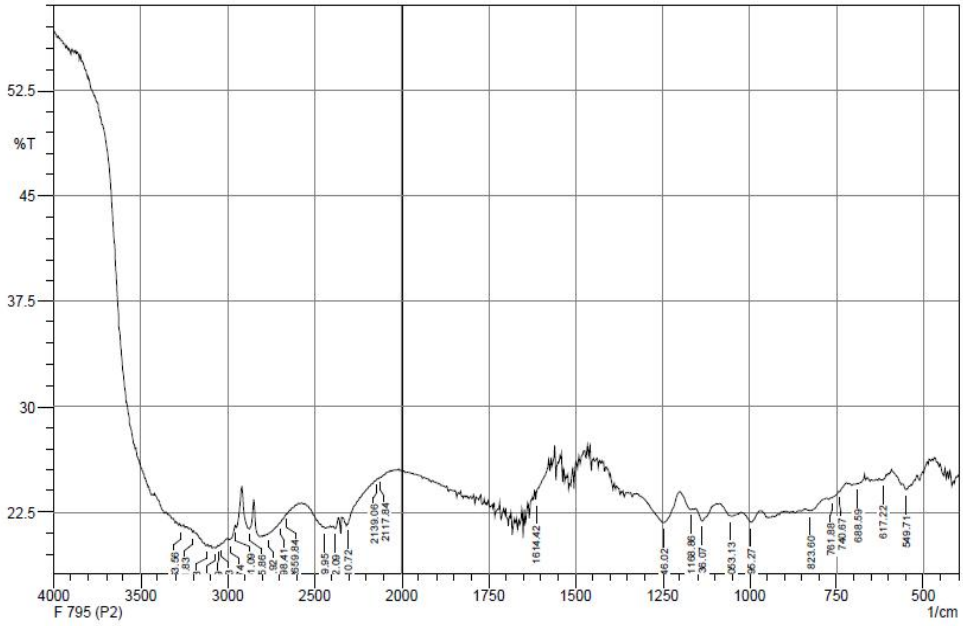

Fig. C. Casein ftir spectra with 40 µg/ml

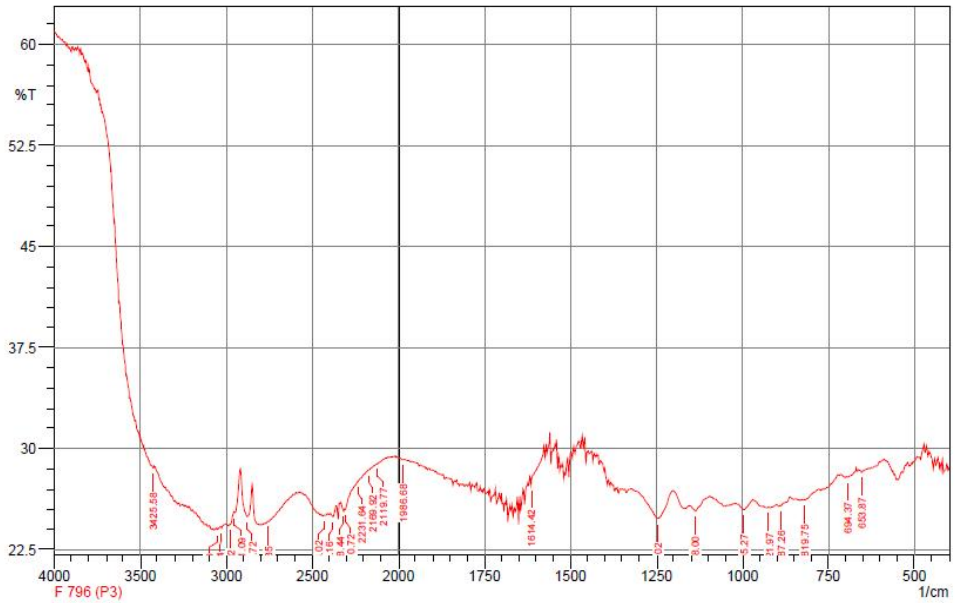

Fig. D. Casein ftir spectra with 60 µg/ml

Based on the qualitative results of functional group testing using FTIR, it was concluded that all treatment concentrations of the addition of different cocoa shell extracts were confirmed to contain catechins by looking at the functional groups that appeared. The more concentration of extract added to this treatment will produce more spectra. This is presumably because the heating treatment can reduce the content of bioactive compounds in the nano-casein-catechin emulsion. It can be concluded that the best treatment based on functional group parameters was obtained in the P3 treatment, namely the addition of the catechin extract of 60 µg/ml.

Table 2. Observations of the nano-casein-catechin functional groups using FTIR

Kakao	P_0	P_1	P_2	P_3	Functional Groups	Chemical compound
	756.1	763.81	740.67	819.75	N-H	Amina
952.84				921.97	C-H	Alkana
	991.41	995.27	995.27	995.27	C-H	Alkana
1037.7					C-O stretch	Alkohol, ester, asam karboksilat
1228.88					C-N Strech	Aliphatic amines
	1242.16	1242.16	1246.02	1246.02	N-H	amida III
1456.26					C-C Strech (in ring)	Aromatik
1712.79	1614.42	1645.28	1614.42	1614.42	C=O Strech	Karbonil
1766.8				1986.68	C=C Strecth	Aromatik
2472.74	2472.74	2405.23	2439.95	2438.02	N-H	Amida A
	2756.28	2775.57	2765.92	2754.35	C-H	Regangan
2883.58	2875.86	2875.86	2875.86	2879.72	C-H Strech	Alkana
2931.8						
	2949.16	2949.16	2951.09	2951.09	C-H Strech	Alkana
2970.38		2980.02	2987.74	2980.02	O-H. OH-Bonding	Fenol, alcohol
		3030.17-3070.68	3034.03	3028.24	N-H	Amida
3344.57-3352.28	3201.83	3244.27-3427.51	3201.83-3263.56	3425.58	N-H	Amida

4. CONCLUSION

Based on the results, casein with different concentration of catechins indicate that casein interacted with catechins 60 µg/ml was able to increase casein stability, maintain nano size, casein components were still detected in the protein profile, the resulting microstructure looked compact and functional groups of bioactive compounds were still detected in FTIR.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Minard R. Isolation of casein, lactose and albumin from milk. Thesis. Penn State University; 2000.
2. Livney YD, Dalgleish DG. Casein micelles for nanoencapsulation of hydrophobic compounds. U.S. Patent US2009311329 (A1); 2009.
3. Livney YD. Milk proteins as vehicles for bioactives. Current Opinion in Colloid and Interface Science. 2010;15:73-83.
4. Pool H, Quintanar D, Figueroa JD, Marinho Mano C, Bechara JEH, Godínez LA, et al. Antioxidant effects of quercetin and catechin encapsulated into plga nanoparticles. Journal of Nanomaterials. 2012;1-12.
5. Mehranfar F, Bordbar AK, Fani N, Keyhanfar M. Binding analysis for interaction of diacetylcurcumin with β-casein nanoparticles by using fluorescence spectroscopy and molecular docking calculations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2013;115:629-635.
6. Afroz QM, Swaminathan K, Karthikeyan P, Ahmed KP, Sudhir U, Kumar MU. Application of nanotechnology in food and dairy processing: An overview. The Pakistan Journal of Food Science and Technologies. 2012;22(1):23-31.
7. Riwayati I. Pemanfaatan teknologi nano di dalam industri pengolahan bahan makanan. Momentum. 2007;3(2):1-4.
8. Rahayu PP, Rosyidi D, Purwadi Thohari I. Characteristics of catechin extracted from cocoa husks using microwave-assisted extraction (MAE). Biodiversitas. 2019;20: 3626-3631.
9. Farhadian S, Shareghi B, Salavati-Niasarib M, Amooaghaeia R. Spectroscopic Studies on the Interaction of Nano-TiO2 with Lysozyme. Journal of Nanostructure. 2012; 1:95-103.
10. Hernàndez-Balada E, Taylor MM, Phillips JG, Marmer WN, Brown EM. Properties of biopolymers produced by transglutaminase treatment of whey protein isolate and gelatin. Bioresource Technology. 2009; 100(14):3638-3643.
11. Maoela MS, Arotiba OA, Baker PGL, Mbusela WT, Jahed N, Songa EA, et al. Electroanalytical determination of catechin flavonoid in ethyl acetate extracts of medicinal plants. J Electrochem. 2009;4: 1497-1510.
12. Wu Y, Wang X. Binding, stability, and antioxidant activity of curcumin with self-assembled casein–dextran conjugate micelles. International Journal of Food Properties. 2017;20(12):3295-3307.
13. Bohin MC, Vincken JP, Westphal AH, Tripp AM, Dekker P, Harry TWM van der Hijden, H Gruppen. Interaction of flavan-3-ol derivatives and different caseins is determined by more than proline content and number of proline repeats. Food Chem. 2014;158:408-416.
14. Hasni I, Bourassa P, Hamdani S, Samson G, Carpentier R, Tajmir-Riahi HA. Interaction of milk a- and b-caseins with tea polyphenols. Food Chemistry. 2011; 126:630-639.
15. Arts MJ, Haenen GR, Wilms LC, Beetstra SA, Heijnen CG, Voss HP, et al. Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. J Agric Food Chem. 2002;50(5): 1184-1187.
16. Yuksel Z, Avci E, Erdem YK. Characterization of binding interactions between green tea flavanoids and milk proteins. Food Chemistry. 2010;121: 450-456.
17. Frazier RA, Deaville ER, Green RJ, Stringano E, Willoughby I, Plant J, Mueller-Harvey I. Interactions of tea tannins and condensed tannins with proteins. Journal of Pharmaceutical and Biomedical Analysis. 2010;51(2):490-495.
18. DeFrates K, Markiewicz T, Gallo P, Rack A, Weyhmiller A, Jarmusik B, et al. Protein polymer-based nanoparticles: Fabrication and medical applications. International Journal of Molecular Sciences. 2018;19(6): 1717.

19. Nagi HPS, Kair J, Dar BN, Sharma S. Effect of storage period and packaging on the shelf life of cereal bran incorporated biscuits. American Journal of Food Technology. 2012;7(5):301-310.
20. Mootse H, Pisponen A, Pajumägi S, Polikarpus A, Tatar V, Sats A, et al. Investigation of casein micelle particle size distribution in raw milk of Estonian Holstein dairy cows. Agronomy Research. 2014; 2(3):153-158.
21. Yildirim-Elikoglu S, Erdem YK. Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. Food Reviews International. 2017;34(7):665-697.
22. Wang B, Timilsena YP, Blanch E, Adhikari B. Lactoferrin: Structure, function, denaturation and digestion. Critical Reviews in Food Science and Nutrition. 2017;1-17.
23. Sęczyk L, Świeca M, Kapusta I, Gawlik-Dziki U. Protein-phenolic interactions as a factor affecting the physicochemical properties of white bean proteins. Molecules. 2019;24(3):408.
24. O Connell JE, Fox PF. HeatInduced coagulation of milk. In P. F. Fox and P. L. H. McSweeney (Eds.). Advanced Dairy Chemistry (3rd ed.). New York, NY: Kluwer Academic/ Plenum Publishers. 2001;879-945.
25. Somanathan N, Subramanian V, Mandal AB. Thermal stability of modified caseins. Thermocimica Acta. 1997;302:47-52.
26. Ramos-Tejada MM, Dura´n JDG, Ontiveros-Ortega A, Espinosa-Jimenez M, Perea-Carpio R, Chibowski E. Investigation of alumina/(+)-catechin system properties. Part I: A study of the system by FTIR-UV–Vis Spectroscopy. J Colloids and Surfaces B Biointerfaces. 2002;24:297-308.
27. Chen YM, Wang MK, Huang PM. Catechin transformation as influenced by aluminum. J Agric Food Chem. 2006;54:212-218.
28. Senthilkumar SR, Sivakumar T, Arulmozhi KT, Mythili N. FT-IR analysis and correlation studies on the antioxidant activity, total phenolics and total flavonoids of Indian commercial teas (*Camellia sinensis* L.) - A novel approach Int Res J Biol Sci. 2017;6:1-7.
29. Chen YM, Wang MK, Huang PM. Catechin transformation as influenced by aluminum. J. Agric. Food Chem. 2006;54: 212-218.

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