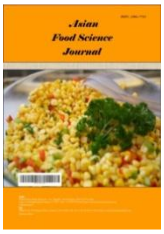


*Asian Food Science Journal*

*21(3): 58-65, 2022; Article no.AFSJ.85555*
*ISSN: 2581-7752*

# Fortification of Nano Whey Protein-Meniran Phyllanthine (*Phyllanthus niruri* L.) Encapsulated Casein Hydrolysates on Physicochemical Properties of Skimmed Milk Powder

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author WFS wrote the draft of manuscript and performed the statistical analysis. Authors AM and MES designed the study, managed the analysis study and the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/AFSJ/2022/v21i330418

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85555

***Original Research Article***

***Received 02 February 2022***
***Accepted 31 March 2022***
***Published 02 April 2022***

## ABSTRACT

Skimmed milk powder is one of dairy products that have processing through heating process and impact decreasing nutritional contents, thus would required fortification. Fortification skimmed milk powder with the addition of encapsulation is carried out using meniran phyllanthine and whey protein as core material and casein hydrolysates as wall material. The research was conducted to obtain skimmed milk powder fortified nano whey protein-meniran phyllanthine encapsulated casein hydrolysates with better physicochemical properties. The research material used was skimmed milk powder with different concentrations of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, designed by 5 treatments and 3 replications consisting of T0: 0% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T1: 2% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T2: 4% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T3: 6% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T4: 8% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates. The variables tested were solubility, turbidity, sedimentation, water activity

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(aw) and hygroscopicity. The research method was a laboratory experiment analyzed by Analysis of Variance (ANOVA) using Duncan's Multiple Range Test (DMRT) if there were differences. The results showed a highly significant difference ($P<0.01$) on water activity (aw). However, there was no significant difference ($P>0.05$) on skimmed milk powder's solubility, turbidity, sedimentation, and hygroscopicity. The data of physicochemical properties in this study were solubility ranged from 57.983-75.295%, turbidity 0.459-0.518, sedimentation 0.036-0.043, water activity 0.434-0.467, and hygroscopicity 4.601-5.528%. In conclusion, fortified nano whey protein-meniran phyllanthine encapsulated casein hydrolysates in skimmed milk powder can be used as a product with better physicochemical properties.

*Keywords: Casein hydrolysates; encapsulated; fortified; nano whey protein-meniran phyllanthine; skimmed milk powder.*

## 1. INTRODUCTION

Skimmed Milk Powder, which is also called Nonfat Dry Milk (NDM) is 52.15% lactose, 38.71% protein (31.18% casein, 7.53% whey protein), 1.08% fat, and 8.06% ash. Skimmed milk powder must contain no less 95% milk solids and not exceed 4% moisture [1]. Skimmed milk powder is one of the dairy products through a heating process by hot air to produce low moisture content. Lower moisture content will prevent microorganism growth and extend the shelf life of skimmed milk powder. Skimmed milk powder is usually processed using spray drying, which produces from a liquid material into dried powder using hot air [2]. The principle of the spray drying method was atomization, which sprayed liquid into fine droplets with hot air and became dried particles. However, the heating process during manufacture skimmed milk powder reduced water content and other nutritional contents such as protein and vitamins, which would require fortification. The fortification was an effort to enrich nutrients to improve nutritional quality and human health benefits [3].

Meniran is an herbal plant from Indonesia that has health benefits with its bioactive compounds. Bioactive compounds of meniran consist of lignans (phyllanthine, hypophillanthine, nirantin, linetetratin), flavonoids (quercetin and rutin), phenolics, alkaloid, saponin and tannin. The quality of meniran is based on a single marker compound from the lignan group [4,5]. Phyllanthine bioactive can be obtained by the extraction method. Microwave-assisted extraction is one of the extraction methods often used to extract phytochemicals in the plant using water solvent and microwave energy [6]. Microwave energy will be converted into heat energy, plant tissue and cell walls will cause lysis and extracted the phyllanthine bioactive. The application of meniran phyllanthine bioactive to one of the dairy by-products, such as whey protein, can be used as a functional food and is easy to absorb.

Whey protein is obtained from cheese making and casein production by-products, which still have high protein content. Whey protein mainly by β-lactoglobulin (β-Lg, 50%), α-lactalbumin (α-La, 20%), bovine serum albumin (BSA, 10%), immunoglobulins (10%) and peptones protease (<10%) [7]. Whey protein was rich in essential amino acids and complex structures consisting of primary, secondary, tertiary and quaternary structures [8], so if interacted with meniran bioactive can change the structure and functional properties of the protein. Amino acids from whey protein are composed of one hydrogen atom when heated at 65-70ºC, which can interact with hydroxyl group from meniran through covalent and non-covalent binding (hydrophobic interactions, van der Walls and hydrogen bonds) [9]. One of the disadvantages of whey protein is easily denatured when heated up to 70ºC, so an encapsulation process is needed.

Encapsulation is alternate protection by adding active substances into particles to coat and protect core material from high pressure and temperature during the heating process and reduce product stickiness [10]. This research used casein hydrolysates as encapsulant composed of αs1, αs2, β- dan κ-casein fragments [11]. The properties of casein hydrolysates that do not react to other food constituents were a concern if applied as a functional food or supplement [12]. Based on the description above, the analysis of solubility, turbidity, sedimentation, water activity (aw) and hygroscopicity of fortification nano whey protein-meniran phyllanthine encapsulated casein hydrolysates was carried out to evaluate the quality of skimmed milk powder based on their physicochemical properties.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The materials used in the research were skimmed milk powder, nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, SDS 0.1%, $NaN_3$, $Na_2SO_4$ and aquades. The equipment used was aw meter (Rotronic® Instruments), microwave (Sharp), spray dryer (Buchi), glass funnel, beaker glass, measure glass, centrifugator, centrifuge tube, analytical scale, oven 105ºC (WT-Binder), desiccator, hotplate and magnetic stirrer (SBS-06), mini hand mixer, UV-Vis spectrophotometer (721-Faithful), micropipette (HumaPette) and filter paper (Whatman No.1 and 4).

### 2.2 Methods

The research method was a laboratory experiment with 5 treatments and 4 replications. Fortification skimmed milk powder with different concentrations of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates consisting of T0: 0% (control), T1: 2% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T2: 4% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T3: 6% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates, T4: 8% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates.

#### 2.2.1 Meniran phyllanthine extraction

Extraction of meniran phyllanthine based on [13]. Meniran powder as much as 3 g diluted in 100 mL aquades and macerated for 24 hours. Meniran solution was extracted with microwave at 70ºC for 10 minutes (one-minute radiation, two minutes off and so on until ten minutes). After that, The extract of meniran phyllanthine was filtered using filter paper (Whatman No.4).

#### 2.2.2 Interaction whey protein and meniran phyllanthine

The interaction according to [9], 5 g of whey protein were heated using hotplate stirrer at 65-70ºC for 40 minutes and phyllanthine meniran was gently added into whey protein solution until it reached 100 mL volume.

#### 2.2.3 Encapsulation nano whey protein-meniran phyllanthine with casein hydrolysates

Four grams of casein hydrolysates were added into 100 mL nano whey protein-meniran phyllanthine solution and homogenized. The solution was allowed to stand at 4ºC for 24 hours. After that, spraying was carried out using a spray dryer (Buchi) until all of it became powder.

#### 2.2.4 Fortification nano whey protein-meniran phyllanthine encapsulated casein hydrolysates in skimmed milk powder

Fortification of skimmed milk powder can be designed by following different concentrations of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates consisting of T0 (0%), T1 (2%), T2 (4%), T3 (6%) and T4 (8%).

#### 2.2.5 Solubility

Solubility was analyzed according to [14] with slight modification. Filter paper (Whatman No.1) and centrifuge tube were oven at 105ºC for 1 hour, and then removed to desiccator for 15 minutes. The filter paper (A) and centrifugation tube (B) were weighed. A sample of as much as 6 mL was taken and stirred for 30 minutes, centrifuged later for 10 minutes at 400 rpm to separate supernatant and precipitate. The supernatant was filtered using filter paper (Whatman No.1). After that, filter paper with solid sample and centrifuge tube containing precipitate were placed into the oven for 24 hours and desiccated for 15 minutes. The filter paper with solid sample (A') and centrifugation tube with precipitate (B') was weighed and analyzed using the formula :

$$\text{Solubility (\%)} = \frac{A' - A}{(A' - A) + (B' - B)} \quad (1)$$

Description :
A = Filter paper
B = Centrifugation tube
A' = Filter paper + Solid sample
B' = Centrifuge tube + Precipitate

#### 2.2.6 Turbidity

Turbidity was determined using [15,16] method. Sample was taken as much as 3 mL and allowed stand for 2 hours. The analysis was carried out using UV-Vis spectrophotometer with a wavelength of 600 nm. The result was observed and recorded.

### 2.2.7 Sedimentation

Sedimentation was described by [17]. Filter paper (Whatman No.1) and centrifuge tube were oven at 105ºC for 1 hour, and then removed to desiccator for 15 minutes. The filter paper (A) was weighed. Sample as much as 5 mL was taken and filtered using filter paper (Whatman No.1). The filter paper with solid sample was weighed and analyzed using formula:

$$\text{Sedimentation} = \frac{\text{B}-\text{A (gram)}}{\text{mL sample}} \tag{2}$$

Description :
A = Filter paper
B = Centrifugation tube

### 2.2.8 Water Activity (aw)

The measurement water activity based on [18] research using aw meter (Rotronic® Instruments) which has been calibrated with $BaCl_2$ / $Mg(NO_3)_2$ / NaCl / KCl. Set the sample in sample container and waited until aw meter rang. The water activity value shown in digital display, then recorded and analyzed.

### 2.2.9 Hygroscopicity

Hygroscopicity analysis was carried out [19] method. The sample was weighed about 1 gram and placed in petri dish, then analyzed the water content's sample. Put the analyzed sample into desiccator which containing saturated solution of $Na_2SO_4$ and allowed stand for 7 days. Hygroscopicity percentage calculated using formula:

$$\text{Hygroscopicity (\%)} = \frac{\frac{\text{x}}{\text{ax}}-\text{Wi}}{1-\frac{\text{x}}{\text{ax}}} \text{ x } 100\% \tag{3}$$

Description :
X = The increase in weight of sample
Ax = The amount sample
Wi = Water content of sample before exposure humid environment (%)

## 2.3 Data Analysis

The data of solubility, turbidity, sedimentation, water activity (aw), and hygroscopicity were analyzed by analysis of variance (ANOVA) was carried out using the Microsoft Excel Software. The level of significance was determined at P<0.05.

## 3. RESULTS AND DISCUSSION

## 3.1 Solubility

The research showed that fortification nano whey protein-meniran phyllanthine encapsulated casein hydrolysates had no significant differences (P>0.05) on the solubility of skimmed milk powder. The lowest solubility was obtained on T3 (6% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates) at 57.983% and the highest on T2 (4% nano whey protein-meniran phyllanthine encapsulated casein hydrolysates) at 75.295%. The difference of water-binding ability on the hydrophilic side from whey protein and casein causes an increase or decrease in solubility. Solubility is based on protein's hydrophilic and hydrophobic sides [20].

Solubility percentage reduction on T1 was 68.480% to 61.854% on T2. It happened because of the non-covalent interaction of phenolic compounds from meniran and amino acids from whey protein and casein. Non-polar interaction on polyphenol with protein increases the surface hydrophobicity of protein molecules by reducing solubility properties [21]. Casein hydrolysates utilization as wall material that processed using spray drying method is one of the encapsulation materials with heat tolerance, however heating process up to 120ºC casein gradually becomes insoluble and form precipitate [22]. Insoluble particles cause turbidity and, if continuous, will produce precipitate at the bottom solutions or form sediment.

## 3.2 Turbidity

Turbidity with different concentrations of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates in Table 1 did not significantly affect (P>0.05). Therefore, the product's material composition was an indication of determining the turbidity value of skimmed milk powder. T1 without nano whey protein-meniran phyllanthine encapsulated casein hydrolysates has a turbidity value of 0.482. Bioactive meniran, which interacts with milk protein (whey protein and casein), provides partial conformational change to protein structure which can change the physicochemical properties of the protein. The processing is one of the causes of turbidity, the higher temperature during the heating process impact lower water content. The lower water content indicated skimmed milk powder products were more hygroscopic. Thus, the particles combine to form larger and insoluble particles causing turbidity. However, in research by [23], increasing particle size resulted in reduced turbidity value. Therefore, the particle size only can not be used indicator of turbidity.

Skimmed milk powder fortified nano whey protein-meniran phyllanthine encapsulated casein hydrolysates on T1-T2 decrease turbidity with an average 0.518-0.459. Whey proteins are proteins that have been isolated and filtered to be easily absorbed by the body. Casein hydrolysates have been involved in hydrolysis, which partly changes protein to amino acids. This amino acids group is more soluble in water and did not give solution turbidity. The addition of phenolic compounds from meniran is not involved in turbidity, but it will be oxidized to protein. The excessive polyphenol concentration causes the protein not to provide cross-linking sites, so the addition phenolic compounds must match [24].

### 3.3 Sedimentation

Sedimentation is physical property to identify the quality of skimmed milk powder. The decreased solubility of skimmed milk powder presents poor solubility, which results in high sedimentation. The different concentrations of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates had no significant differences ($P>0.05$) on sedimentation of skimmed milk powder, shown in Table 1. Skimmed milk powder without nano whey protein-meniran phyllanthine encapsulated casein hydrolysates had the lowest sedimentation value at 0.036 because milk powder contains fat, water, lactose, protein and mineral that have to affect milk sedimentation properties [25–27].

The differences concentration of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates in skimmed milk powder resulted in sedimentation value on T1 (2%), T2 (4%), T3 (6%) and T4 (8%) consecutive at 0.041, 0.042, 0.043, and 0.043. The encapsulation process with casein hydrolysates protected core materials (whey protein and meniran phyllanthine) during spray drying. Casein hydrolysates are assumed can stabilize protein by reducing the tendency of protein particles to combine or form sediment. The fortification nano whey protein-meniran phyllanthine encapsulated casein hydrolysates did not significantly different of skimmed milk powder sedimentation value. However, there have been increased sedimentation trends.

### 3.4 Water Activity (aw)

The result of fortification nano whey protein-meniran phyllanthine encapsulated casein hydrolysates with different concentrations showed a highly significant difference ($P<0.01$) on skimmed milk powder water activity (Table 1). The highest to lowest average value of water activity was T3 ($0.467^{b} \pm 0.001$), T2 ($0.463^{b} \pm 0.0006$), T4 ($0.462^{b} \pm 0.0071$), T1 ($0.460^{b} \pm 0.0032$), T0 ($0.434^{a} \pm 0.001$). It can be due to covalent bonds from carboxyl groups of amino acids reacting with other amino acid groups by releasing water molecules through hydrogen bridges. Chemically, an amino acid has one carboxyl group and one hydrogen atom, so it will play a role in determining water activity value.

The water activity values in this study were 0.434-0.467, which can not be used for microorganism growth. Microorganism growth in food products identified according to free water content inside. The more free water in food products means the higher water activity. Water activity value to the growth of microorganisms such as bacteria: 0.90; yeast: 0.80-0.90; mold: 0.60-0.70 [28].

Water activity showed a tendency of increasing value on T0 to T3. Processing with drying method using spray dryer aims to produced low water activity product. According to [29,30] research, the spray drying method is a drying method by hot air to reduce significant water content. The higher temperature and longer time processing impact the lower water activity. The reduction of water activity (aw) on T4 is thought to be due to the addition of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates was too much. It is known that the functional properties of protein are as a water binder.

**Table 1. Physicochemical analysis of fortification skimmed milk powder with nano whey protein-meniran phyllanthine encapsulated casein hydrolysates**

| Treatment | Analysis | | | | |
|---|---|---|---|---|---|
| | Solubility (%) | Turbidity | Sedimentation | Water Activity (aw) | Hygroscopicity (%) |
| T0 | 68.480±13.835 | 0.482±0.0029 | 0.036±0.0049 | $0.434^a$±0.001 | 4.933±0.275 |
| T1 | 61.854±9.864 | 0.518±0.0684 | 0.041±0.0079 | $0.460^b$±0.0032 | 4.896±0.407 |
| T2 | 75.295±17.554 | 0.489±0.0171 | 0.042±0.0037 | $0.463^b$±0.0006 | 4.601±0.669 |
| T3 | 57.983±8.247 | 0.478±0.0064 | 0.043±0.0039 | $0.467^b$±0.001 | 5.376±0.412 |
| T4 | 59.179±6.411 | 0.459±0.0078 | 0.043±0.0053 | $0.462^b$±0.0071 | 5.528±0.145 |

### 3.5 Hygroscopicity

The fortification of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates had no effect (P>0.05) in hygroscopicity of skimmed milk powder with values of T0 (4.933%), T1 (4.896%), T2 (4.601%), T3 (5.376%) and T4 ( 5.528%). The protein content of skimmed milk powder is related to their water content. The higher protein content means drier products [31]. The production of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates was carried out through a spray drying process by the rapid evaporation into dried powder. The lower water content will indicate that the product is more hygroscopic. Increasing the hygroscopicity of skimmed milk powder is related to free water content (water activity/aw), which triggers the growth of microorganisms and then impacts the physicochemical properties.

The increased value of hygroscopicity is evidenced by more addition of nano whey protein-meniran phyllanthine encapsulated casein hydrolysates on T2 (4%), T3 (6%) and T4 (8%), resulting in 4,601%, 5,376% and 5,528%. However, some characteristics of protein encapsulants (especially casein hydrolysates) in the food industry must be limited because of their hygroscopic, hydrophobic, reactive and common allergy triggers [32]. Encapsulation using protein (whey and casein) fortified in skimmed milk powder impact to hygroscopicity reduction, showed on T2 (2%) and T2 (4%) at 4,896% and 4,601%. This may be caused by interaction between polyphenols from meniran with protein and then the change of protein structure and functional properties during encapsulation using the spray drying method. The core material in this study was whey protein and meniran that cause an interaction through hydrophobic bonds. The hydrophobic side of polyphenols binds whey protein, so if casein hydrolysates were added, these hydrophobic bonds were weakened eventually, which reduces hygroscopic of skimmed milk powder. Casein has low hygroscopic properties, therefore unable to absorb moisture from the air again. [33] reported that encapsulation of *curcumin* in casein micelle powder produced by spray-drying impacted its hygroscopicity, which their less hydrophobic bonds because curcumin bind casein.

## 4. CONCLUSION

The encapsulation using casein hydrolysates as wall material has been succesfully to protect whey protein-meniran phyllanthine in core material. Fortification nano whey protein-meniran phyllanthine encapsulated casein hydrolysates 2% (T1) can improve physicochemical properties of skimmed milk powder. Based on this research, which resulted solubility at 61.854%, turbidity 0.518, sedimentation 0.041, water activity (aw) 0.460, and hygroscopicity 4.896%.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## ACKNOWLEDGEMENTS

The acknowledgment of this research was supported by Penelitian Penerimaan Negara Bukan Pajak (PNBP) 2020, Universitas Brawijaya.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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