Study on the use of various concentration of acetic acid and different precipitation duration on casein characteristics

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Abstract. Casein can be precipitated from skim milk using acetic acid. The effectiveness of the casein formed during precipitation was determined by precipitation duration. The purpose of this study was to determine the characteristics of casein (water content, the amount of isolated casein and/or yield and the amount of dissolved protein) with various concentrations of acetic acid and precipitation duration. This study used a completely randomized design (CRD) factorial pattern with three replications. Factor A was the various concentration of acetic acid (6%, 9%, and 12%) and factor B was the different precipitation duration (30 minutes, 60 minutes, and 90 minutes). The parameters measured were casein yield, water content and dissolved protein. The results showed that the concentration of acetic acid and the precipitation duration significantly affected the characteristics of casein yield, water content and dissolved protein. The casein yield values ranged from 20.16 to 26.49%, the water content ranged from 71.55 to 76.63% and dissolved protein ranged from 1.17 to 1.98%. The use of 6% acetic acid and the 60-minute precipitation duration produced casein with low water content and a less amount of dissolved protein. The casein yield produced was in line with the casein water content.

1. Introduction
Casein is the main protein of milk and contains 80% of total milk protein. Casein is very useful for human life, both as an ingredient in the manufacture of food and non-food products. Food products such as ingredients for cheese, bread, biscuits, baby food, ice cream, and other edible products. Non-food products such as glue, plastic, paint, and others [1,2]. Casein can be precipitated from milk by the addition of acid [3]. Types of acids that can be used as a precipitate include lemon juice, citric acid, tartaric acid, and acetic acid. Acetic acid is the most common precipitate used for the separation of milk proteins [4,5].

Casein characteristics are also determined by the precipitation duration. Precipitation is a process of separating the bonds of calcium with phosphate contained in milk. The precipitation process by the addition of acetic acid and the right precipitation duration is important to produce good quality casein [6,7]. In the precipitation process, a transition from natural to denatured forms occurs. The gel will be formed after a portion of the protein is denatured. This study aimed to determine the characteristic of casein (water content, yield content, and the dissolved protein) with various concentrations of acetic acid and precipitation duration.
2. Materials and methods

2.1. Skim preparation.
The skim was separated using cream separator and pasteurized at 85°C for 5 minutes. The skim then cooled at 5°C for 24 hours while removing the fat. The skim was separated from the clotted fat after cooling.

2.2. Skim-based casein preparation
As much as 1 N acetic acids with the concentration of 3%, 6%, 9% and 12% (v/w) were added to the skim and precipitated for 30, 60, and 90 minutes. The curd then filtered to obtain whey. The whey was calculated in volume to determine the washing materials.

2.3. Washing of casein curd.
Washing was done three times with sterile distilled water. At the first and the second time, the curd produced was washed with sterile distilled water. The volume of the distilled water was as much as the volume of whey obtained from curd filtration. At the third time, the casein curd was weighed and distilled water was added with the ratio of 1:1. The pH of casein then measured and neutralized to pH of 6–7 by 1N NaOH and left for five minutes.

2.4. Yield value.
The yield was the ratio between the weight of casein formed after washing compared with the weight of the skim to produce the casein. The yield value was calculated using the modified formula by [8]:

\[
\text{yield value} = \frac{\text{casein weight (g)}}{\text{skim weight (g)}} \times 100\%
\]

2.5. Water content
Water content was determined by counting the weight loss of the heated sample. The AOAC [9] formula used:

\[
\text{water content} = \frac{(B-C) \text{ (g)}}{(B-A) \text{ (g)}} \times 100\%
\]

A = dry weight of dish (g)
B = dry weight of the dish and initial sample (g)
C = dry weight of the dish and dried sample (g)

2.6. Dissolved protein
Dissolved protein percentage was determined by the Lowry method [10].

\[
\text{Dissolved protein (\%)} = \left(\frac{A \text{ sample} - A \text{ control}}{A \text{ sample}}\right) \times 100\%
\]

A sample = sample absorbance
A control = control absorbance

2.7. Data Analysis
The study used an analysis of variance with Completely Randomized Design (CRD) factorial pattern and three replications. If there was a significant effect of the treatment a further Multiple Range Duncan tests were carried out [11].
3. Results and discussion

3.1. Casein yield value.
A yield is a proportion of the weight of casein produced and the weight of skim used. The casein yield values using the various concentration of acetic acid and different precipitation duration ranged from 20.16 to 26.49% (table 1). The analysis of variance showed that various concentration of acetic acid and different precipitation duration significantly affected (p<0.05) the casein yield. However, the interaction between the concentration of acetic acid and different precipitation duration did not significantly affect the casein yield. The casein yield is also affected by water content. The higher water content will produce higher casein yield concentration.

Table 1. The casein yield value (mean±SD) using a various concentration of acetic acid and different precipitation duration.

| Acetic acid (%) | Precipitation duration (minutes) | Average   |
|-----------------|----------------------------------|-----------|
|                 |                                  | 30        | 60        | 90        |
| 6               |                                  | 22.71±0.00| 20.16±1.56| 23.29±1.47| 22.05±1.79A |
| 9               |                                  | 23.19±0.87| 20.79±0.58| 26.25±1.01| 23.41±2.47B |
| 12              |                                  | 23.82±2.17| 22.85±1.87| 26.49±2.41| 24.39±2.48C |
| Average         |                                  | 23.24±1.26| 21.27±1.75| 25.34±2.15 |

A,B,C Superscript with different notation letter on the same row or column showed a significant difference (p<0.05)

As the concentration of acetic acid increases, the yield of casein increases. Acetic acid will hydrolyze protein bonds into simpler peptide bonds. This change causes more simple proteins to dissolve in water and more casein yields are produced. Further test results showed that high concentrations of acetic acid also resulted in a high average casein yield value. This was caused by the hydrolysis of protein-peptide bonds during acid addition. Triyono [12] suggested that the longer the protein reacts with acids or bases the more peptide bonds are hydrolyzed and caused damage in the primary structure of the protein. Further, [13] suggested that excessive acid can cause denaturation of protein structures.

Further test on different precipitation duration showed that the average value of casein yields precipitated for 90 minutes was higher than 30 minutes and 60 minutes (table 1). The addition of acetic acid caused a slower reaction of protein termination resulting in an increase in the casein yield. The casein yield value decreased at 60 minutes due to the protein matrix termination reaction so that more water came out. However, casein yield value increased at 90 minutes due to the re-absorption of water content in the protein matrix. Milewski [14] suggested that the addition of high acid concentrations resulted in coagulation and rapid protein aggregation. The rate of protein coagulation will affect the amount of protein which forms the matrix and the ability of protein matrix to bind other components, especially water, hence will affect the casein yield value.

3.2. Casein water content.
Water content is a very important factor in determining the quality of food. The water content in food could change with food processing. The casein water content using the various concentration of acetic acid and different precipitation duration ranged from 71.55 to 76.63 % (table 2). There is no provision for wet casein water content yet. However, the codex stan 290-1995 mentioned provision for dry casein maximum water content is 12%.

The analysis of variance showed that the use of various concentrations of acetic acid significantly affected (p<0.05) the casein water content. While the different precipitation durations and interactions between both factors did not significantly affect the casein water content. High water content in casein caused higher dissolved protein levels and higher casein yield. The further tests showed that the highest water content was obtained by adding acetic acid at a concentration of 9% and 90-minutes...
precipitation, while the lowest water content was obtained by addition of acetic acid at a concentration of 6% and 90-minutes precipitation. The higher acid concentration produced higher water content.

Table 2. The casein water content (mean±SD) using a various concentration of acetic acid and different precipitation duration.

| Acetic acid (%) | Precipitation duration (minutes) | Average |
|----------------|---------------------------------|---------|
|                | 30                              | 60      | 90      |
| 6              | 71.64±0.03                      | 72.16±0.32 | 71.55±5.56 | 71.78±4.22\textsuperscript{A} |
| 9              | 72.34±0.01                      | 76.14±2.00 | 77.60±1.13 | 75.36±2.62\textsuperscript{B} |
| 12             | 74.56±1.47                      | 76.42±0.42 | 76.63±2.02 | 75.87±1.60\textsuperscript{B} |
| Average        | 72.84±1.51                      | 74.91±3.91 | 75.26±4.12 |

\textsuperscript{A,B} Superscript with different notation letter on the same column showed a significant difference (p<0.05)

The presence of H\textsuperscript{+} ions caused the released of peptide bonds. In acid condition, H\textsuperscript{+} will react with the COO\textsuperscript{-} group to form COOH while the rest will bind to the amino group NH\textsubscript{2} to form NH\textsubscript{3}\textsuperscript{+}. Further, the excessive acid given to an isoelectric peptide solution will increase the charged group which form an affinity to water and solubility in water. Milewski [14] suggested that the high water content due to acetic acid concentration will affect the rate of protein coagulation and aggregation. In high concentration, the protein coagulation and aggregation will occur faster. The rate of protein coagulation will affect the amount of protein that forms the matrix and the ability of protein matrix to bind other components, especially water, which in turn will affect the color and yield value produced.

3.3. Dissolved protein.
Milk protein consists of dissolved protein and non-dissolved protein. Dissolved protein is a protein that is soluble in whey, while non-dissolved protein is also called casein. At casein separation, the dissolved proteins could still be included. In addition, the dissolved proteins are also formed due to excessive denaturation during the casein separation process. The percentage of dissolved protein content using the various concentration of acetic acid and different precipitation duration ranged from 1.17 to 1.98% (table 3). The casein dissolved protein related to the casein yield produced.

Table 3. The percentage of casein dissolved protein (mean±SD) using a various concentration of acetic acid and different precipitation duration.

| Acetic acid (%) | Precipitation duration (minutes) | Average |
|----------------|---------------------------------|---------|
|                | 30                              | 60      | 90      |
| 6              | 1.59±0.42                       | 1.17±0.13 | 1.53±0.18 | 1.43±0.31 |
| 9              | 1.73±0.40                       | 1.37±0.11 | 1.44±0.45 | 1.52±0.35 |
| 12             | 1.37±0.27                       | 1.39±0.10 | 1.98±0.10 | 1.58±0.34 |
| Average        | 1.57±0.37\textsuperscript{AB}   | 1.30±0.14\textsuperscript{A} | 1.66±0.35\textsuperscript{B} |

\textsuperscript{A,B} Superscript with different notation letter on the same row showed a significant difference (p<0.05)

The analysis of variance showed that various concentration of acetic acid and different precipitation duration and the interaction between did not have a significant effect on casein dissolved protein. The highest dissolved protein was obtained by addition of acetic acid at a concentration of 12% and 90-minutes precipitation, while the lowest was obtained by addition of acetic acid at a concentration of 9% and 60-minutes precipitation. The high content of casein dissolved protein due to the concentration of acid affected the pH value of casein, causing the higher the proteolysis and in turn, produced more dissolved protein. Winarno [15] suggested that protein solubility will increase after excessive acid treatment. Acidification can decompose complex protein to simpler protein such as components of peptides and amino acids [16].
4. Conclusion
The use of 6% acetic acid and the 60-minutes precipitation duration produced casein with low water content and a less amount of dissolved protein. The casein yield produced was in line with the casein water content.

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