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Types of Acid and Drying Method Differently Affect the Chemical Profile of Sodium Caseinate

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HIGHLIGHTS

- Interaction of acids (hydrochloric acid and acetic acid), and drying methods (oven and freeze drying) significantly (p<0.01) affected chemical profile of sodium caseinate.
- The kinds of acid and drying methods altered the moisture, protein, and ash content of sodium caseinate.
- The combination of hydrochloric acid and freeze drying can produce good chemical characteristics of sodium caseinate.

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Acronyms and abbreviations
HCl=Hydrochloric acid

ABSTRACT

Background: Sodium caseinate is a rich source of protein and minerals originating from animals. Numerous food and non-food products are made from sodium caseinate. The present study investigated the chemical components (moisture, crude protein, ash, and soluble crude protein) of sodium caseinate prepared by different acids and drying techniques.

Methods: A completely randomized factorial design was used by different acids including hydrochloric acid (HCl) and acetic acid, and also drying methods including oven (50 °C for 48 h) and freeze drying (-40 °C for 48 h). In each experimental group, sodium caseinate was obtained for determination of moisture, crude protein, ash, and soluble crude protein. Data were statistically evaluated using an ANOVA in SPSS 18.0.

Results: The interaction of both acids and drying methods significantly (p<0.01) affected moisture, crud protein, and ash content. HCl treatment coupled with freeze drying was the best combination, resulting in an appreciably higher content of crude protein (52.90%), moisture (5.38%), and soluble protein (0.85%).

Conclusion: The kinds of acid and drying method altered the chemically profile of sodium caseinate. The combination of HCl and freeze drying could be the considered as the best approach, resulting in good chemical characteristics of sodium caseinate.

Introduction

Milk protein is an animal-based nutrient needed for human growth and development. It is divided into two, namely soluble proteins and non-soluble proteins. Casein is a dissolved protein that can be separated from milk (Anggraeni et al., 2010; Lestari et al., 2015; Sudibya and Purnomo, 2013). The variations in casein separation process leads to variety of casein products with different names and nutrient content. Casein processing is carried

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out with a series of important processes such as precipitation, washing, and grinding. Sodium caseinate is a kind of casein produced by the use of sodium hydroxide (NaOH) as a washing material. It is widely used as primary and additive ingredient for a myriad of food products, such as cheese, ice cream, infant foods, health products and enhancing the product quality, food wrapping and edible film (Khwaldia et al., 2004; Schou et al., 2005; Wagh et al., 2014). Sodium caseinate is rich in essential amino acids (primarily lysine) and minerals, and it can be used to enrich the nutritional features of the processed products (Sindayikengera and Xia, 2006).

Sodium caseinate can be prepared from skimmed milk through acid precipitation of casein. By changing pH to approximately 4.9, casein will precipitate together with calcium salts and phosphate associated with the protein. The chemical changes during processing can be affected by the type of acid used. Acetic acid and hydrochloric acid (HCl) are the two most acids used for casein processing, due to the difference in their acidification properties (Husnaeni et al., 2019; Mourad et al., 2014).

Drying is often done on foods that are rich in protein. This aims to extend the shelf life of food. After precipitation of casein, sodium caseinate is processed via dehydration to reduce moisture content. Variation in the drying temperature enables might modify its chemical characteristics (moisture, ash, crude protein, and soluble protein) of sodium caseinate. The use of varying temperatures in the dehydration process closely relates to the rate of either chemical or physical changes in the product, which contributes to the quality of the final products. Drying operations are commonly carried out using oven or freeze dryer. Freeze drying is a low-temperature dehydration process which involves freezing the product (Ciurzynska and Lenart, 2011). Different parameters in both drying methods inevitably may lead to differences in physical as well as chemical features of caseinate. Therefore, the present research investigated the chemical components of sodium caseinate prepared by different acids (HCl and acetic acid) and drying techniques (oven and freeze drying).

Materials and methods

Materials and instruments

Fresh milk was taken from Enrekang Regency, South Sulawesi (Indonesia). Chemicals included HCl, acetic acid, NaOH, H_2SO_4, H_3BO_3, HCl, and Trichloroacetic acid which were totally prepared from Sigma-Aldrich, Germany. The main instruments used included pasteurization, cream separator, oven (Ecocell SIS-B2V/EC111, D112457, Germany), autoclave (SX-500, Japan), UV-Vis spectrophotometer (Shimadzu UV-11800, Japan), and a freeze dryer (Christ, type Alpha -2 LD, Germany).

Sample preparation

The sample preparation procedure was according to Sindayikengera and Xia (2006), Husnaeni et al. (2019), and Sarode et al. (2016) with some modifications. Briefly, the cream in fresh milk was removed using a cream separator and the skimmed milk was pasteurized at 85 °C for 5 min. After storage in a refrigerator (5 °C for 24 h), the fat in the skimmed milk was aseptically removed. Casein was precipitated by adding 1 N HCl or 1 N acetic acid into the initial skimmed milk until the concentrations were 5% (w/v) and 10% (w/v), respectively. Subsequently, casein curd was collected after being separated from the whey and washed three times with distilled water using the same amount of removed whey (for the first and second washing). In the last washing process, casein curd was added to distilled water at a ratio of 1:1 (w/v) and then adjusted to pH 6-7 using NaOH, followed by dehydration using the oven (50 °C for 48 h) or freeze dryer (-40 °C for 48 h). The dried casein was vacuum-sealed aseptically and kept at 25-27 ^{o}C for further analysis.

Determination of crude protein

Crude protein was determined using the Kjeldahl method according to AOAC (2005). Each sample (0.3 g) was transferred into a 100 ml Kjeldahl flask, to which was added a 1 g mixture of selenium and 10 ml concentrated H_2SO_4. The sample was digested until a clear fraction was produced and transferred into a measuring flask and rinsed with distilled water. The sample was then mixed with 10 ml 2% H_3BO_3+4 drops of indicator reagent in an Erlenmeyer bottle. The mixture (5 ml) was then pipetted to a distillation apparatus and titrated using 0.01% HCl, and then 5 ml 30% NaOH+100 ml distilled water was added. Crude protein was calculated as follows:

$$\% \text{ Protein} = \frac{V1 \times \text{Normality HCl} \times 6.38 \times p \times 14}{\text{Sample weight}} \times 100\%$$

Determination of moisture content

A container was first dried in the oven at 105 °C for 30 min, cooled in a desiccator, and weighed. About 0.5 g of the sample was placed in the dried container (weighed as W1) and was dehydrated in an oven at 105 °C until achieving a constant weight, cooled in a desiccator for 15 min, and re-weighed (W2) (AOAC, 2005). The percentage moisture content was calculated as follows:

$$\text{Moisture content} = \frac{W1 - W2}{W1} \times 100\%$$

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Determination of ash content

The ash content was determined based on AOAC (2005). The casein sample from moisture content analysis was ignited to ash in a muffle furnace at 600 °C for about 3 h. With known weight of sample before and after the ashing process, the ash content was then determined using following formula:

$$\text{Ash content} = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \times 100\%$$

Determination of soluble protein

Each sample (1 g) was placed in a 100 ml measuring flask and homogenized for 10 min. A mixture of reagent A and B (25 and 0.5 ml, respectively) was made. A 10% trichloroacetic acid solution was then mixed with 5 ml of the sample and left to produce agglomeration, followed by filtration to collect the supernatant. The supernatant (0.1 ml) was mixed with 1 ml reagent and distilled water up to 10 ml, then left for 30 min to form a blue solution. The absorbance of the solution was then spectrophotometrically measured at 600 nm, and compared to a standard casein solution. The percentage of soluble protein was calculated as follows:

$$\text{Soluble protein (\%)} = \frac{\text{FP}\times\text{X}\times 100\%}{\text{mg/ml}}$$

Statistics analysis

The experiment was arranged according to completely randomized, 2X2 factorial design with 2 factors of different ratio of HCl and acetic acid as the first factor and drying method (oven and freeze drying) as the second factor. Each measurement was conducted in triplicate. Data were statistically evaluated using an ANOVA in SPSS 18.0. The significant difference between means was verified using the Duncan test.

Results

Moisture content

As shown in Table 1, the moisture content of sodium caseinate were significantly (p<0.01) influenced by the interaction of acid treatment and drying methods. The results of further tests showed that the moisture content of sodium caseinate with the use of HCl and freeze dryer was lower (5.38%), compared to the use of HCl with oven (5.64%), the use of acetic acid with freeze dryer (5.67%), and acetic acid with an oven (5.83%).

Content of crude protein

Crude protein content in sodium caseinate were significantly (p<0.01) related to the interaction of acid treatment and drying methods (Table 1). Further test results showed that the crude protein of sodium caseinate with the use of HCl and oven was lower (54.42%), compared with the use of HCl with a freeze dryer (52.90%), the use of acetic acid with a freeze dryer (51.42%), and acetic acid with an oven (51.97%).

Content of soluble protein

As shown in Table 1, soluble protein level of sodium caseinate were significantly (p<0.01) depended on various types of acids and drying methods. The soluble protein of casein using the oven method was higher (1.11%) compared to the freeze dryer method (0.82%). The soluble protein of sodium caseinate using HCl was higher than the use of acetic acid.

Ash content

There was significant (p<0.01) relationship between ash content of sodium caseinate and kinds of acid treatment and drying methods (Table 1). The ash content of sodium caseinate with the use of acetic acid and oven was higher (3.88%), compared with the use of acetic acid with a freeze dryer, and the use of HCl with a freeze dryer and HCl with oven.

Discussion

Moisture content indicates the availability of water present in the food matrix (Serin et al., 2018). Various types of food processing allow the reduction of water content in foodstuffs. In the present work, the acids were used to induce the release of casein from other milk components. Hotnida et al. (2017) and also Guo and Wang (2016) suggested that milk casein has an isoelectric point around pH 4.6, and when the temperature is above 8 ^{o}C, caseine will form an aggregate. The sodium caseinate was then collected after dehydration. The drying process aimed at reducing the water level, thus allowing the expansion of the product's shelf life.

We found that the water content of sodium caseinate using HCl and the freeze dryer method was lower than the use of HCl with oven, the use of acetic acid using the freeze dryer method and acetic acid by the oven method. HCl is a strong acid that can denature proteins. During denaturation, the protein will experience a decrease in biochemical activity and a decrease in solubility. The

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Table 1: Chemical composition of sodium caseinate prepared by various types of acids and drying methods

Parameters (%)	Type of acid	Drying method		Mean
		Freeze dryer	Oven	
Moisture	HCl	5.38±0.01	5.64±0.02	5.51±0.14
	CH_3COOH	5.67±0.02	5.83±0.03	5.75±0.09
Crude protein	HCl	52.90±0.02	54.42±0.02	53.66±0.84
	CH_3COOH	51.42±0.01	51.97±0.02	51.69±0.30
Soluble protein	HCl	0.85±0.03	1.15±0.02	1.00±0.17
	CH_3COOH	0.80±0.02	1.06±0.01	0.93±0.14
Ash	HCl	1.71±0.02	1.56±002	1.64±0.08
	CH_3COOH	1.94±0.01	3.88±0.02	2.91±1.06

folded protein structure will be made open. During denaturation, there is a breakdown of hydrogen bonds, hydrophobic interactions, salt bonds, and the opening of folds or pleases of protein molecules. Strong acidic HCl will be faster in opening/breaking the protein chain than acetic acid. This condition causes the water in the casein curd (free water and some bound water) to evaporate. Some researchers previously stated that HCl is capable of precipitating casein in milk much better compared to acetic acid which enhances water release (Glab and Boratynski, 2017; Sindayikengera and Xia, 2006). This is understandable since HCl as a strong acid which enables to complete ionization during protein hydrolysis.

Based on Table 1, the water content of sodium caseinate dried with a freeze dryer (5.53%) was lower than that of the oven (5.74%). This moisture content of studied groups was still lower than the maximum water level for edible caseinate i.e., 8.8%, according to the standard (Codex Alimentarius, 1995). This indicates that the freeze dryer method was able to release water in casein maximally compared to the oven method. Freeze drying has good air pressure for the release of moisture from the material. Hariyadi (2013) found that freeze drying had the best results in terms of dehydrated products' quality when compared to other dehydration techniques. The higher product's moisture content after oven-dried was mainly due to the used temperature in the process, i.e., 50 °C. Saenmuang et al. (2017) suggested that the ability of the material to release water from its surface is greater with the high drying air temperature. This low temperature was used to ensure that the protein is not destroyed by heat but it seems to be less effective in reducing water content. Temperature of 50 ^oC in the oven for drying food results in evaporation of water on the surface of the food. However, this temperature does not penetrate optimally to the deepest part of the dried food.

Casein is precipitated and separated from other skim components after being treated with acids (Sindayikengera and Xia, 2006); thus, the type of acid used determines the quality of sodium caseinate, as well as drying techniques. In the current study, crude protein in sodium caseinate was differently affected by the interaction of acid treatment and drying methods. It was also found that the content of crude protein was higher with the treatment of HCl and oven compared to acetic acid and freeze drying. This is clear that HCl, as a strong acid, performed better precipitation activity than acetic acid. Seo et al. (2013) revealed that HCl as a strong acid is completely ionized and hydrolyzes protein better than acetic acid.

In this work, crude protein content was higher when treated with oven process compared to freeze drying. Crude protein range was lower than the minimum standard of milk caseinate protein (dry matter), i.e., 88% (Codex Alimentarius, 1995). It might be associated with the high abundance of water trapped in the matrix as well as water chemically bound with the protein. The temperature (50 °C) in the oven treatment did not completely remove the water. Husnaeni et al. (2019) showed that water serves as an important component in foods, available in many forms, i.e., free water, adsorbed water and bound water. Among them, free water is most easily removed from food matrix. Whereas chemically bound water is difficult to remove and a certain amount of expenditure requires the study of certain methods.

The soluble protein in this research was sourced from the hydrolysis of complex proteins (casein) into simple proteins. Hydrolysis of proteins by acid catalysts opens up the potential of polar amino acids to be released and dissolves during rehydration. The soluble protein may also be derived from milk whey protein (α lactalbumin, β lactoglobulin, etc.). The whey protein component is soluble protein in milk. Whey protein found in casein is probably due to an imperfect washing process. We found that soluble protein was not affected by the interaction of acid treatment and drying method.

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Nonetheless, it was influenced by either acid type or drying method. In this case, it was found that total soluble protein in sodium caseinate dried with an oven was significantly higher than that dried with a freeze dryer. This finding demonstrates that temperature used in the oven causes denaturation. In this regard, Raikos (2010) indicated that milk protein is very dependent on heating conditions.

Casein has the ability to be slightly soluble in water. The nonpolar amino acid content is quite high at around 35-45% of the total amino acid residues so this condition makes it less soluble in water. It was suggested that the solubility of casein in water can be increased up to 20% by heat treatment (Elzoghby et al., 2011; Haque et al., 2008; Mocanu et al., 2012). The treatment can induce the hydrophobic and electrostatic interactions in the composition and structure of amino acids.

According to results of this investigation, denaturation was higher in the HCl treatment compared to acetic acid. HCl more strongly induced cleavage of protein chains, in which some of them were denaturized. It might occur due to the ionization of HCl. Glab and Boratynski (2017) stated that denaturation may occur due to some factors, i.e., heat, high pressure, and the presence of alcohol, alkaline, urea, acid, and other reagents. Pereira (2014) and Ye and Harte (2013) found that the internal structure of micelle of casein may change depends on pH. When pH is about 4.8, the charge of casein tends to neutral meanwhile in low pH, it has positive charge. Aggregation, calcium release, and micelle deposition occur when the pH decreases below the isoelectric point (4.6-4.8). Furthermore, Hariyadi (2013) asserted that freeze drying could better perform the drying process compared to other drying techniques.

Some of minerals like calcium affect the dissolution of protein in milk. Protein denaturation with acids or physical treatment (use of temperature in the drying method) can release mineral bonds and further leach is easily removed from sodium caseinate during the washing process. In our study, ash content was affected by the interaction of both factors. Acetic acid treatment, followed by either oven or freeze drying, showed higher ash content than HCl. This is understandable since acetic acid is a weak acid and therefore, it is not ionized perfectly during protein hydrolysis (McIntyre, 2017). Consequently, protein structure is not opened well, thereby avoiding associated organic salts from completely soluble during the washing process. Similar with this research, Sarode et al. (2016) previously reported the effect of various acid volumes on ash content of casein and found that a higher level of acid could attenuate ash content.

The current work showed that ash content in samples prepared with acetic acid was 1.94-3.88%, which was higher than those prepared with HCl, which ranged from 1.56-1.71%. The ash content was far away from good standards for caseinates according to the Codex Alimentarius (1995), which must be zero. Nevertheless, ash content in this work was still under maximum level prescribed by Sarode et al. (2016), i.e., acid casein (2.5%), rennet casein (7.5%), sodium caseinate (3.8%), and calcium caseinate (3.6%).

Conclusion

The kinds of acid and drying method altered the chemically profile of sodium caseinate. The combination of HCl and freeze drying could be the considered as the best approach, resulting in good chemical characteristics of sodium caseinate. Further detailed researches are needed in future to understand the duration of precipitation using acid.

Author contributions

F.M. and R.M. designed the study; S.S., S.B., and H.A. conducted the experimental work; M.T., S.S., and H.A. analyzed the data; F.M., S.B., M.T., and R.M. wrote the manuscript. All the authors read and approved the file manuscript.

Conflicts of interest

The authors have no conflict of interest.

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