1. INTRODUCTION

1.1. Research Background

Milk is one of the major beverage consumed by human from children to elderly. It is because milk contains numerous nutritions such as calcium, selenium, magnesium, vitamin B5, vitamin B12, and riboflavin that give significant contribution to human body and health (FAO, 2013). Leonil *et al*., (2000) reported that enzymatic hydrolysis could enhance the functional properties of protein in milk. Protein usually used for building up body tissues, structure, and muscles such as collagen protein that stronger teeth and bones (Ngili, 2013). Protein in the milk will break down and could be absorbed by the human body as amino acids with the help of enzymatic hydrolysis. High digestibility of the protein resulting in higher amount of amino acids that could be adsorb and readily use for the body, these determine the quality of the protein. If the protein doesn't degrade much into smaller form, the protein adsorb by the body will be only in small amount and the rest will be discarded through feces (Anggraini & Yunianta, 2015).

Peptides derived from casein in the milk protein have been found to exert other functionality, such as antioxidant activites (Kumar *et al*, 2016). Antioxidant activity functionality is to prevent free radical agent that cause cardiovascular and inflammatory. This causes food industries also try to enhance antioxidant value in food by using synthetic antioxidant in food, which leads a lot of studies try to find source of antioxidant from natural sources. Generally, relationship between degree of hydrolysis and antioxidative activity for different whey protein hydrolyzates were not found. These results indicated that the antioxidant activity of the hydrolyzates was inherent to the characteristic amino acid sequences of peptides derived, depending on the protease specificity (Korhonen & Pihlanto, 2006).

Papain enzyme is difficult to obtain in small amount in Indonesia, which will cause very high cost for the research. Bromelain enzyme also rarely used as a proteolytic enzyme to hydrolyze milk, causing there is still limited information about it. The researcher also

wants to find enzyme that is effective for consumer acceptance's taste. Therefore in this study, bromelain enzyme was chosen as the proteolytic enzyme for hydrolysing milk to apply for debittering methods.

Bromelain enzyme could be used in the enzymatic hydrolysis because it's one of the proteolytic enzyme that have function to break down protein. But the side effect of using bromelain enzyme is the milk taste would turn out bitter and not suitable for consumer's acceptance. The signature taste of milk need to be maintained for the consumer's acceptance by using several debittering methods. Debittering methods of casein hydrolyzates can be obtained from application of masking and spray drying. Spray drying is based on microencapsulation and atomization to disperse the liquid or slurry into controlled drop size spray dry powder. Masking is based on cocoa powder addition to mask the bitter taste of hydrolyzate skimmed milk (Roy, 1992).

1.2. Objectives

The aims of this study are to determine the best debittering methods for bitter taste of cow's milk hydrolyzed with bromelain enzyme between masking with the addition of cocoa powder and spray drying treatment, to determine the degree of hydrolysis, pH, antioxidant activity, and sensory analysis between the methods.

1.3. Literature Review

1.3.1. Protein

Proteins are polymers made up of 19 different alpha amino acids and one amino acid linked via peptide bonds. Protein has several important roles in biological and food systems, such as biocatalysts (enzyme). Protein functions as catalyst, carrier, and storage of other molecules such as oxygen, mechanically support the body immune system (immunity), a nerve motion transmitter, and controlling growth and development. Role and activity of protein in biological process are enzymatic catalyst, that almost all of the chemical reaction in biological system is catalysted by macromolecules called as enzyme (Katili, 2009).

The functional properties of proteins in foods are related to their structural and other physicochemical characteristics. The understanding of physical, chemical, and functional properties of proteins and the changes these properties undergo during processing is essential if the performance of proteins in foods is to be improved and if underutilized proteins are to be increasingly used in processed food products. Combination factors such as pH, temperature, and environment, and interaction of other food components (lipids, carbohydrates) with proteins can cause unpredictable structural changes in proteins and thus affect their functional behaviors (Alain, 1997).

Solubilty of proteins is fundamentally related to its hydrophilicity or hydrophobicity balance. Thus, amino acid composition of a protein inherently affects its solubility characteristics. Protein solubilty in aqueous solutions is dependent on pH. At pH values above and below the isoelectric pH, proteins carry a net charge, electrostatic repulsion, and ionic hydration promote solubilization of the protein (Alain, 1997).

1.3.2. Enzymatic Hydrolysis

Enzymatic hydrolysis is a process where enzyme has role to create cleavage of bonds in molecules with addition of water. Hydrolyzate protein is a result from enzymatic hydrolysis process which makes proteins into amino acid and smaller peptide molecules by the presence activity of protease enzymes. After protein hydrolyzed, the reaction continued by hydrolyzing small peptide fractions. Resulting in various lengths mixture of amino acid and polypeptide that has ability as bioactive peptide and called as hydrolyzate protein (Saidi *et al.*, 2013; Whitehurst & van Oort, 2010). This protein hydrolyzate is easier to digest for the body, resulting in more of proteins adsorb by the body.

Protein structure is modified to improve solubility, emulsification, gelling, and foaming properties. Chemical modification is not something desirable for food applications because harsh conditions from removing residual reagents from the final product. Meanwhile, modification of protein structure with enzyme is one of the way to improve the functional and nutritional properties of proteins. Enzymes provide several advantages, such as fast reaction rates and high specificity.

Measurement of enzymatic hydrolysis activity is showed from degree of hydrolysis (DH). The degree of hydrolysis of enzyme-treated proteins determine the properties of relevance to food applications. It is important that degree of hydrolysis can be measured while the reaction is going on, to make it possible for stoping the reactions at a well-defined stage when the desired property of product has been obtained.

a. Enzyme

Enzyme is biomolecules in the form of proteins that function as catalysts in biological chemical reaction. Every cell biological process needs enzyme so that it can accelerate the metabolism rate. Enzyme stability is influenced by several factors such as substrate, temperature, acidity, cofactor, and inhibitor (Katili, 2009).

Protease is an enzyme that helps protein catabolism by hydrolysis of peptide bonds (proteolysis). Proteases are classified according to their source i.e animal, plant, microbial; their catalytic action (endo-peptidase or exo-peptidase) and active site. Proteolytic enzymes can be classified into two major groups as endo- and exopeptidases, based on its hydrolytic activity. Both can be used for the production of hydrolyzates. Endopeptidases hydrolyze the protein at the interior of the polypeptide chain; while exopeptidases hydrolyze either at the N-terminal or the C-terminal end of the protein, which are called aminopeptidase and carboxypeptidase, respectively (Whitehurst, 2010).

Bromelain enzyme is isolated from pineapple (*Ananas comosus L.*) and it has an ability to protein digestibility (Wuryanti, 2004). The mayor endopeptidases are stem bromelain and fruit bromelain. The minor endopeptidases are ananain and comosain. The optimum pH range from 6-7, and the optimum temperature range from 50-60ºC (Bala *et* al., 2012). Bromelain was also found having the highest enzyme activity at 55ºC with 4.05 U/ml activity, optimum pH was 7 with 3.05 U/ml, KM was 5.074 mg/ml, and Vmax was 0.666 mg/ml.second. KM stated complex dissociation constant of enzyme-substrate and Vmax showed that every millilitre of bromelain extract maximum produced 0.666 mg every minute. After 55ºC, the bromelain enzyme activity was found decreasing, mainly because enzyme is a type of protein and at high temperature protein will be denatured and enzyme will be damaged and having lower activity (Herdyastuti, 2006).

b. Bitter Taste

Several cause that affect the creation of bitterness are the average hydrophobicity of the hydrolyzed protein, the higher hydrophobicity is likely to result in high bitterness, DH also influences the concentration of soluble hydrophobic peptides and their chain length, the specifity of the enzymes, separation of the hydrolyzate to eliminate part of bitterness (Whitehurst & van Oort, 2010). The bitter taste also come from several amino acids such as methionine, tryptophan, lysine, isoleucine, valine, tyrosine, leucine, proline, and phenylalanine (Lemieux & Simard, 1992). Prevention of bitterness is achieved by selection of the type of proteases and

operating conditions of the hydrolysis process, which called with debittering process. Debittering can be done with several treatments, such as using peptidases to avoid the formation of bitter peptides, employing the plastein reaction to form larger non bitter peptides, selectively removing bitter peptides by chromatography, treating the hydrolyzate with activated charcoal as a hydrophobic absorbent for the hydrophobic peptides, masking the bitter taste (Uhlig, 1998).

Based on sensory analysis, chocolate has been considered to be an ideal substances, because of the flavourable taste. Cocoa is one of the food product that has a lot phenolic compound from *Theobroma cacao* plant and one of the source of flavanol compound that functions as natural antioxidant called flavonoid. The addition of cocoa powder is given more antioxidant values than it already is in the milk. Beside that, cocoa powder can cover the undesireable taste (bitter) from the hydrolysis process result. (Sudibyo, 2012).

Spray drying is a method of producing a dry powder from a liquid by rapidly drying with a hot gas. Spray drying is also one of the most used methods in microencapsulation, due to the wide availability of the equipment, low processing costs, possibility of using a large variety of carriers, and good final product stability. Microencapsulation and addition of hydrocolloids such as pectin and maltodextrin help spray drying to reduce the bitter taste (Uhlig, 1998). Favaro-Trindade *et al*., (2010) also reported that spray drying with the mixture of gelatine and Soy Protein Isolate (SPI) can cover or reduce the bitter taste on casein hydrolysate.

1.3.3. Milk

Major component of milk is water, which contributing on average of 68% of its weight. Milk is also the major source of protein, fat, and dietary energy contributing on average 8 g of protein per capita per day, 7.3 g of fat per capita per day and 134 kcal of energy per capita per day (FAOSTAT, 2017). The cattle milk compositions consist of 13% of

dry matter, 3.4-5.4% of fat, 3.5-4.0% of protein, 4.6% of lactose, and 5% of other nutrients (Park & Haenlein, 2006).

Milk also contains several antioxidant, such as enzymes and vitamins. Peptides derived from digestion of milk proteins are believed to have antioxidant activity (Korhonen & Pihlanto, 2006). Antioxidant activity can be measured with one of the antioxidant assay, which is 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. DPPH is a free radical that is acquired directly without preparation (ready to dissolve). DPPH can only be dissolved in organic media (especially alcoholic media), not in aqueous media, which was the reason ethanol 95% used. But this is actually an important limitation when interpreting the role of hydrophilic antioxidants (Arnao, 2001). DPPH is one of the few stable and commercially available organic nitrogen radicals. It is one of the most widely reported methods for the determination of antioxidant activity. The different for DPPH assay is the radicals did not have to be generated before the assay.