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Review Article

Enzymes Involving in Chocolate Processing

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ABSTRACT

Indonesia is one of the largest cocoa-producing countries in the world, but the productivity of Indonesian cocoa is relatively low compared to other cocoa-producing countries. Many cocoa beans are exported in raw form (raw cocoa beans) so they do not have high economic value. In addition, cocoa beans also can not be consumed directly and must be processed first. The processing of cocoa beans includes curing stage, breaking the fruit, fermentation, soaking and washing, drying, sorting, and storage to producing cacao almonds. The fermenting process of cocoa beans involves the role of the polyphenol oxidase to produce flavor precursor compounds. Furthermore, to improve the quality of the resulting chocolate, protease and carboxypeptidase can be used in the processing of cacao almonds to enhance flavor precursor compounds forming to produce chocolate with stronger flavor characteristics. Processed chocolates can be developed into confectionery with a soft or liquid filler by utilizing invertase to obtain a solid filler during processing that may turn into liquid during storage.

Keywords : cocoa, chocolate, enzyme, processing.

1. Introduction

Indonesia is one of the largest cocoa producing countries in the world, but the productivity is still lower compared to other cocoa producing countries. Compared with the processed chocolate products, the original exports of cocoa is more, so the economic value is small. In addition to cocoa beans, other types of exports include cocoa shells, cocoa paste, cocoa butter, cocoa powder, the rest are chunky chocolate products and other processed products. According to Central Bureau Statistics of Indonesia issued by Director General of Plantations, in 2015 Indonesia's cocoa exports amounted to 350,730 tonnes valued at US$ 1,316,867,000. The number increased by 333,679 tons over the previous year.

Cocoa has astringent bitterness by the content of tannin and polyphenols (Paembong, 2012). Chocolate, cocoa beans raw materials cannot be consumed directly as raw materials, because of its composition and taste must be addressed. An important phase of cocoa processing is fermentation that reduces bitterness and fermentation. In addition, fermentation can also improve the mouthfeel and cocoa, with a flat chocolate aroma, and lower levels of impurities and acidity (Kim *et al.*, 2010). The fermentation process involves altering the enzymatic action of cocoa's chemical compounds that initially provide undesirable properties associated with the desired characteristics of compounds such as typical chocolate flavor compounds.

In addition to the processing of cocoa beans, the enzymes used in the chocolate processing can also be cocoa processed in advanced processes, and the chocolate processed into candies. Each stage of processing involves different enzymes and compounds. Therefore, it is necessary to do research on the role of enzymes that play a role in the mechanism of chocolate as well as the functioning of these enzymes.

2. Chocolate

Chocolate is one of the most widely known foods, both as confectionery and as a flavor of other foods. In general, chocolate has a sweet taste due to the addition of sugar, but naturally, chocolate has a bitter taste. Commercially, chocolate can be in the form of a solid, liquid, pasta or powder. The widespread popularity of chocolate has made chocolate a part of many traditions and cultures, especially in Europe, for example on Easter and Valentine's Day.

According to its history, chocolate was originally introduced into the modern civilization from the Mayan in Mexican by Spanish colonists and became popular among the aristocrats of the 17th century. In 1828, Conrad Marriott London, a Dutch chemist, developed the Dutch method of separating fat from the cocoa beans process, which was subsequently called cocoa butter. Subsequently, in 1847, the British company Fletcher and Son (now part of Cadbury and Billion International) will produce the first chocolate bar in full by mixing cocoa butter with cacao and sugar. Thirty years later, the collaboration between Swiss innovator Nestle and Swiss chocolate Daniel Pete produced the first milk chocolate mixed with powdered milk. Rudolf Swiss lotus, a Swiss inventor, further develops chocolate refining techniques that can enhance the flavor and texture of chocolate in 1879. Although it may seem simpler, chocolate requires considerably more complex processing involving a variety of complex chemical reactions (Tannenbaum, 2004).

3. Cacao

According to its etymology, the name cocoa or cocoa is derived from the term "Kakava" in the Mixe-Zoquean language group and is highly regarded as "God's food." At the same time, the Aztec first discovered chocolate drink called “*cacahuatl*”, meaning cocoa water. Thus, in 1735, Linneas gave the scientific name *Theobroma cacao*. (Tannenbaum, 2004). The name chocolate itself is considered to be chocolate by the name of Aztec tribal civilization found by Spanish explorers.

The main component of chocolate processing is cocoa beans. The main habitat for cocoa plants is in the tropical forest. Cocoa consists of five parts, namely leaves, stems, lateral roots, roots and cocoa fruit (Prihastanti *et al.,* 2015). Cocoa is a group of *cauliflori*s, a plant that grows flowers from a stem or branch. Cocoa plant classification is shown in Table 1.

Cocoa was first found in Central and South America and then spread to all areas along the equator 10-20 degrees. Cocoa plants have large stems and branches, and small flowers produce large pieces of fruit that can reach 1 kilogram in size. Each cocoa fruit has 30 to 40 seeds surrounded by the flesh of the fruit. It is believed that humans are first attracted to cocoa due to the flesh of the fruit is sweet (Tannenbaum, 2004).

Cocoa plants include three varieties, namely Criolo, Forastero and Trinitario (hybrids). Criolo is also known as fine cocoa or noble cocoa. At present, the use of Criolo processed is few, but chocolate varieties dominate the market until the mid-eighteenth Century, also known as the form of cocoa, cacao ordinary or bulk cocoa is the largest breed, the two types processing and planting of small chocolate handles. Criolo and Forastero hybrids produce Trinitario. Repairs in cocoa plantations are a must-do and are trimming weak, weeding tree branches to protect, fertilize, water and pest insecticide-eradicating pesticides (Secretary General of Department of Industry, 2007).

Table 1. Classification of cacao plants

Classification	Scientific name
Division	Spermatophyta
Subdivision	Angiospermae
Class	Dicotyledonae
Subclass	Dialypetalae
Order	Malvales
Family	Sterculiaceae
Genus	*Theobroma*
Species	*Theobroma cacao* L.

Source: Jurniati(2013)

The young cocoa greens whitey, when they mature yellowish and young cacao fruit is red, while fully mature is orange (Wahyudi, 2008). The cocoa fruit consists of four parts, namely skin, placenta, pulp, and seeds. A mature cocoa can have 30-40 seeds covered with white paste or mucus (Jurniati, 2013). According to NSAI (2008), cocoa beans are divided into five groups based on the weight of the seed, and the water content of AA, A, B, C and S in cocoa beans is an important property of storage in affecting its durability. If the moisture content, they are highly susceptible to fungal or insect damage. However, if the water content is too low, cocoa beans can easily become brittle or broken.

Pests, which usually attacking cocoa beans is *C. cramerella,* cause wrinkling, small, dark, and hard-to-separate from the flesh of fruit (Azim *et al.,* 2016). In addition, *Moniliophtora perniciosa* can also lead to the broom disease, which reduces the quality of cacao beans and causes huge losses in the cocoa industry in Brazil. Pest attacks on cocoa plants may affect the quality and quantity of cocoa beans and add additional production costs to separate good cocoa and bad cocoa beans. The damage can also be caused by larvae.

4. Cacao Processing

One of the most common cocoa processed products is chocolate. Cocoa processing will determine the final quality of the product, both of the form of taste and aroma, as well as the reduction of bitterness and taste. Processed cocoa is a seven-phase chocolate product that cures, crushed fruit, fermented, soaked, washed, dried, sorted and stored (Secretary General of Department of Industry, 2007).

Curation of cocoa fruit is designed to make the maturity of fruit is more uniform and promote seed release from the fruit. Curing continues for 5-7 days and is done in the shade. During the curing process, cocoa beans produce a unique brown flavor on the final product by known important germination processes (Jurniati, 2013). Breaking the fruit is to remove the cocoa beans from the fruit. After removal, cocoa beans are fermented for several days. Fermentation is performed to obtain seed color characteristics, increase flavor and taste, and improve seed pieces (Sari, 2016). However, the chemical processes that occur during cocoa fermentation remain unclear and remain a research topic.

There are two ways of fermentation, fermentation and bamboo basket fermentation. Cocoa beans are placed into a bamboo box or basket and covered with banana leaves (Secretary General of Department of Industry, 2007). Incomplete or too short fermentations result in grainy and gray grain texture, while too long will result in moldy and fragile seeds, reducing the quality of the beans. During fermentation, proteins and peptides react with polyphenols and produce brown color (Diansari *et al.,* 2016).

The next step is to soak and wash the seeds to stop the fermentation process. The cocoa beans are washed to remove residual fermentation mucus that inhibits the drying process. Seed drying can be accomplished by drying or heating until the water content reaches 7-8%. During the drying process, water, volatile compounds, and low-boiling acidic compounds drastically decreased, producing cacao beans with poor bitter and astringent taste (Secretary General of Department of Industry, 2007). After that, cocoa beans are sorted according to their quality and stored.

There are cocoa beans which are processed without fermentation. Cocoa beans can only be washed and dried using sunlight. However, the resulting cocoa beans have one drawback, such as the unique brown aroma is not strong, the seeds feel bitter (Schwan and Wheals, 2004). Once dry, cocoa beans are sometimes also roasted to produce products with better flavor characteristics. The roasting process led mainly to Maillard's reaction to produce color and flavor compounds

5. Enzyme Utilization on Chocolate Processing

The main ingredient in making chocolate is cacao almonds or dried fermented cacao beans. Although chocolate has a distinctive flavor, cacao beans have a chocolate-like flavor that is separated from the freshly-ripe pods and has a very bitter and astringent taste. In order to obtain the desired flavor, cocoa beans must undergo a curing process that is divided into fermentation and drying processes. This process leads to the formation of flavor precursors. At the same time, the commercial value of cocoa is dependent on the characteristics of cocoa butter and chocolate, making the correct handling of cocoa beans important for determining the quality and economic value of the chocolate product (Oliveira *et al.,* 2011).

The main problem encountered in the chocolate industry is the low quality of cocoa almonds which reduces the formation of fermentation compounds and flavoring compounds. Cocoa almonds, the initial treatment for fermentation and drying, are usually carried out directly on the plantation to prevent spoilage of cocoa beans for distribution to the industry. However, this treatment was done in direct plantations not under controlled conditions, resulting in many changes in cocoa almonds that were sufficient to allow enzymatic reactions. In the absence of enzymatic reactions, the cocoa almonds obtained with low quality. Enzymatic reactions play important roles in protein hydrolysis in cocoa almonds to produce flavoring precursor compounds are not formed during the fermentation process. Therefore, in the production of a standardized chocolate mass, the addition enzyme can be used in the treatment of cocoa (Gray, 2011).

The development of food processing technology has made the development of chocolate products an important part of the candy industry. Also sold separately, chocolate is also commonly marketed with other products such as crackers, or with stuffing. Variation adding in chocolate processing products can increase the economic value of chocolate. This goal can be achieved through the use of enzymes produced in chocolate products that have special characteristics that increase consumer buying interest (Chandrasekaran *et al.,* 2016).

5.1. Polyphenol oxidase

Polyphenol oxidase (1,2-benzenediol: oxidoreductase oxygen; EC 1.10.3.1), or often referred as phenolase, catechol oxidase, monophenol oxidase, cresolase, or catecholase, was first discovered in 1856 by Schoenbein on fungi as aerobic oxidation reaction catalyst of certain compounds in plants (Putra *et al.,* 2010; Singh *et al.,* 2015). Polyphenol oxidase (PPO) can be found in many plant tissues, as well as some fungi especially those producing brown silk), and in some higher animals, including insects and humans. In those higher plants, this enzyme is used to protect plants from insects and microorganisms, and when they are injured, allowing plants to form melanin-scar tissue that protects from drought and other attacks. Meanwhile, in insects, PPO participates in the sclerotization in the exoskeleton (chitin hardening) and melanin formation. In humans, PPOs are involved in the pigmentation of the skin, hair, and eyes (Whitaker, 1995).

PPO is stable at a temperature of 25-65°C and has an optimum activity at a pH in the range of 5.0-7.0 at a temperature of 25-35°C and a pH of neutral (in the range of 6.0-7.0) and can be extracted from various materials, some of them are plums (Ioniță *et al.,* 2017), eggplant (Harish *et al.,* 2017), avocado (George and Christophersen, 2016) and apple (Liu *et al.,* 2015). The boiling point of the PPO phenolic compound, 181.7°C, renders the compound resistant to high temperatures, but 300°C (Wiranata *et al.,* 2016) will be damaged if the temperature is too high. PPO activity can be inhibited by using various compounds including tropolone (Valero *et al.,* 1991), dithiothreitol, sodium metabisulfite (Wuyts *et al.,* 2006), and potassium pyrosulfite (Signore *et al.,* 1997). However, the maximal inhibitory activity is shown by ascorbic acid, L-cysteine, quercetin (Ioniță *et al.*, 2017; Bravo and Osorio, 2016). In food, many of the roles of PPO in enzymatic browning reactions are neither desirable because of the desire in apples and potatoes, nor desired as in black tea and chocolate (Quesnel and Jugmohunsingh, 1970). Browning occurs by the PPO oxidation reaction occurring through the amino acid tyrosine to form an o-quinone monophenolic compound ring followed by the polymerization of the pigment o-quinone compound that produces the polyphenolic compound of black, brown or red (Mayer, 2006).

In chocolate production, PPO is used to form the flavor compound precursor from the oxidation stage of the fermentation process and continues to the dry stage. Some flavor changes occur when bitterness and sourness are reduced due to the interaction between polymerized polyphenols and proteins. During the drying process, the infiltration of oxygen into the cocoa bean maximizes the oxidation of the compound epicatechin and the pro-cyanidin, producing melanin and melanoprotein confer typical of brown of chocolate (Hammer, 2013). The study by Lima *et al.* (2001) and de Brito *et al.* (2002) also pointed out that the action of polyphenol oxidase reduces the polyphenols, tannins and the bitter and astringent features that affect cocoa beans.

In general, PPO activity can be carried out at a moisture content of 40 to 10% at 40-60°C (Lopez, 1986). Optimizing PPO activity in cocoa beans will require rehydration (water back-up), as well as pH, temperature conditions, and time is optimal. Therefore, PPO from previous cocoa beans should be isolated and characterized by its optimal conditions (Kim *et al.*, 2010). Measurement of PPO activity can be accomplished by reducing the concentration of phenol or catechol with quinone formation by increasing reaction time (Murniati *et al.*, 2014).

5.2. Invertase

Invertase (β-fructofuranosidase, EC 3.2.1.26) or also commonly known as saccharase, glucosidase and invertase are enzymes that catalyze the hydrolysis of sucrose. Thus, the result of the hydrolysis reaction of sucrose in the form of a mixture of fructose and glucose is referred to as inverted syrup. Invertase breaks down sucrose by cleaving O-C bonds in the fructose portion (Schiweck *et al.*, 2012).

Invertase works optimally at 60°C and pH 4.5. For industrial purposes, invertase is usually extracted from yeast, but it is also naturally produced by honeybees that produce nectar from honey. In the human body, invertase acts as an immune enhancer, as an antioxidant, as an antiseptic, and as a treatment for some cases of patients with bone and stomach cancer. In the meantime, in plants, sucrase plays a role in regulating osmosis, metabolism, supporting growth and fighting viral attacks. Invertase activity can be inhibited by fructose in the form of Hg^{2+}, Ag^{+}, Ca^{2+}, Cu^{2+} ions and furanose (Kulshrestha *et al.*, 2013).

In the confectionery industry, invertase is used to make chocolates with filling that dominate the market. A variety of padding variations and their favor and quality allow chocolate with padding to become an expensive product. The chocolate stuffing can be milk, fruit, beans, etc. But the most popular stuffing is the filling cream with a unique texture and taste. In order to produce such confectionery products, the chocolate filling should be solid enough for handling and processing, but softened and creamy when consumed.

The technology currently used to achieve this goal in the confectionery industry is the use of invertase. Invertase can convert the sucrose content of the filling into glucose and fructose. This change allows the filling form to be changed from solid to liquid during storage during processing. The very high fructose solubility properties can help prevent the occurrence of sugar crystals in the fillings so the fillings will continue to have a soft and creamy texture. Using invertase can also extend the shelf life by maintaining the required product consistency over time. The conversion of sucrose to glucose and fructose also results in a decrease in Aw value and minimizes the possibility of microbial contamination and growth which can lead to product damage (Chandrasekaran *et al.*, 2016).

5.3. Protease and carboxypeptidase

The study by Oliveira *et al.* (2011) showed the better quality of the chocolate produced by protease and carboxypeptidase (flavor protease) used in the processing of cocoa almonds. This enzyme utilization is accomplished as a means of overcoming the low-quality problems of cocoa almonds processed early in plantations. The results show that application of Flavourzyme for the treatment of cocoa albumen produces a flavorful precursor compound with a better flavor due to a sufficient amount of flavoring resulting from the hydrolysis reaction by Flavourzyme. This research has also shown that the potential of almonds processed for cocoa is considered to be of low quality, to produce a quality of 50% better with sensory quality chocolate.

Carboxypeptidase (EC 3.4.16-3.4.18) is responsible for breaking the peptide bond of the carboxyl terminus of a compound of a protein or peptide. Karboksipeptidase, also known as beta-glucanase, works at optimum pH in the range of 5.5 and 45-50°C of temperature. For commercial purposes, carboxypeptidases are commonly obtained from *Aspergillus oryzae* cultures. In addition to microorganisms, carboxypeptidases are also naturally found in various plant seeds and play a role in the seed germination. At the same time, it assists in digestion, protein modification (eg, in the synthesis of insulin), and regulate biological processes including production of growth hormone, clotting, wound healing, reproduction, and various other treatments in humans (Whitehurst's and van Oort's 2009). Carboxypeptidase is also useful in the medical field for the treatment of apoptosis and adipogenesis.

Flavourzyme itself is a combination of several enzymes produced by Novozymes, an international biotechnology company headquartered in Denmark. Commercially, the Flavourzyme produced by *Aspergillus oryzae* results in the extraction of peptides and the hydrolysis of proteins is marketed, both in the industry and in research. Several enzymes constitute the estimated flavor protease component, but studies by Merz *et al.* (2015) showed that eight major components of Flavourzyme are two aminopeptidases, two types of dipeptidyl peptidases, tripeptidases, The alpha-amylase ATCC 42149 / RIB 40 of the *Aspergillus* strains is a mold of koji. The optimal conditions for each enzyme are different so that the processing conditions and the best quality of chocolate-flavored cocoa almonds have yet to be further studied to produce the product.

6. Conclusion

There are many enzymes used in chocolate processing, PPO, invertase, and a combination of protease and carboxypeptidase. During cocoa bean fermentation, PPO functions during the formation of flavor-precursor compounds, producing cocoa almonds. The fermented and dried cocoa beans (cocoa almonds) can then be treated by adding Flavourzyme and carboxypeptidase to improve the quality of the chocolate produced by disrupting the peptides in cocoa almonds. Once processed into ready-to-eat chocolate, the chocolate fillings in the form of liquid cream can be given additional invertase to produce fillings that are soft and liquid in character.

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