



Application of Casein Phosphopeptide Isolate in Cheese as Dental Caries Prevention

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Abstract

Dental caries is an infectious disease that results from bacterial interactions with teeth. One of the preventive effort against dental caries is consuming foods containing a bioactive compound called casein phosphopeptide (CPP). Cheese is one of the food products that contain CPP. This study aimed to measure the potential of CPP compounds obtained from cheese protein as a preventative for dental caries. This research was conducted from April to July 2019. The method used in this study was measuring the inhibition power on the growth of bacteria causing dental caries (*Streptococcus mutans*) and measure the process of teeth remineralization in vitro. The results showed that the yield of crude CPP isolates obtained from commercial parmesan cheese was 16.65 ± 2.97 %. CPP isolates inhibited the growth of *S. mutans* at concentrations of 10 %, 15 %, and 20 % with inhibitory zone values of 1.22 ± 0.04 cm, 1.30 ± 0.00 cm, and 1.36 ± 0.08 cm, respectively. The application of 0.1 g CPP isolates on tooth surface for 10 days was shown to increase tooth hardness by 28.36 %.

Introducton

The largest problem currently faced in the field of dental and oral health is dental caries. According to Basic Health Research (Riskesmas) in 2007-2013, the prevalence of dental caries was in Indonesia increased from 43.4% in 2007 to 53.2% in 2013. In the same year, WHO (World Health Organization) data showed that the largest increase occurred at the age of 12 years (13.7%) and 65 years (14.3%). The cause for such high prevalence of dental caries is the consumption of foods containing carbohydrates (especially sugar) and the lack of attention towards oral health problems. Caries is an infectious disease that results from the interaction of bacteria with the teeth. Bacteria use food scraps as a substrate for the fermentation process and produce acids that will damage tooth enamel and cause cavities (Mustika et al., 2014).

Streptococcus mutans and *Lactobacillus* are reported as the main cause of caries due to their activity in fermenting sucrose into lactic acid, resulting in enamel demineralization. Such bacteria can form plaques on teeth surface which is firmly attached to teeth surface and resistant to gargling. Plaque will form on the surface of teeth and dental fillings. Plaques develop best in areas that are difficult to clean, such as the gingival edge, the proximal surface, and fissures (Ramayanti & Purnakarya 2013). Efforts that can be made to prevent dental caries is brushing teeth properly. Tooth brushing, when done properly, can remove the remaining dirt that sticks to the teeth, therefore, preventing the growth of bacteria that cause dental caries. In addition, dental caries can also be prevented by applying materials that contains certain bioactive compounds, one of which is CPP. In the study of Kanako et al., (2005) and Maki et

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al., (2007) conducted on cattle teeth, the use of CPP paste was proven to prevent dental caries by preventing demineralization and increasing tooth remineralization.

CPP is found in relatively high amount in milk and its derivatives. Cheese is a milk-derived product made by coagulating milk casein. Milk clumping is caused by the addition of acid, chymosin enzyme found in rennet or other proteolytic enzymes produced by bacteria. The addition of acid is done to reduce the pH to 4.5-5.4 which is the isoelectric point of milk casein. The addition of rennet to milk is done when the desired acidity has been reached so that the chymosin enzyme can work optimally. The addition of rennet is intended to agglomerate milk to form the desired curd character (Budiman et al., 2017; Sari et al., 2014). The stages of cheese production consists of pasteurizing milk, clumping casein, cutting, cooking curd, whey separation, salting, pressing, and aging. During the curing process, microbial activity results in the breakdown of carbohydrates, proteins, and fats resulting in the specific taste, aroma, and texture of each cheese (Quarto et al. 2018). Protein degradation by bacterial proteolytic enzymes will break down milk casein molecules into various bioactive peptides, one of which is CPP. The presence of CPP compounds in cheese causes cheese to have potential in prevention of dental caries. CPP is reported to prevent dental caries through two mechanisms, namely preventing demineralization of tooth enamel and inhibit the attachment of caries-causing bacteria to teeth surface (Merrit et al., 2006; Sugito et al., 2008)

Cheese consumption in Indonesia has increased from 8000 tons per year (2002) to around 19,000 tons per year (2013) (Rakhman, 2010). Today many food and beverage products in Indonesia are made from cheese and is consumed by many. The West Java Animal Husbandry Department survey results stated that 52% of the people are fond of cheese. This shows that cheese has the potential to be used as an alternative solution to prevent dental caries; therefore, study on CPP compounds in cheese is needed to measure the potential of cheese as a prevention of dental caries. Siregar (2011), Farooq et al., (2013), and Divyapriya GK et

al., (2016), reported that CPP compounds in dairy products have an effect on preventing dental caries, but there has been no further research into the potential of CPP compounds in cheese. Therefore, this study was conducted with the aim of measuring the potential of CPP compounds obtained from cheese protein as a prevention of dental caries in vitro.

Methods

This research was conducted from April to July 2019. The materials used in this study include commercial parmesan cheese obtained from supermarkets, frozen dried culture of *Streptococcus mutans* bacteria (BCC 2760) obtained from the Bogor Veterinary Research Center, and molars obtained from Dental and Mouth Clinic. The study was conducted in three stages, namely CPP isolation, *S. mutans* inhibitory test, and dental remineralization test.

CPP isolation was carried out according to the Aranachalam and Raja (2010) method with modifications. A 6% cheese suspension was made by dissolving the cheese in distilled water and stirred using a magnetic stirrer. The sample solution's pH was increased using 2 N NaOH until pH 7 then centrifuged at 4000 rpm for 10 minutes. 1 N HCl was added to the supernatant to obtain a pH of 4.6 and then centrifuged at 4000 rpm for 10 minutes to remove proteins and peptides that do not have a phosphate group. The supernatant from centrifugation was taken and 2N NaOH solution was added until the pH of the solution reached 7.0. 7% calcium chloride was added to the sample supernatant allowed to stand for 10 minutes at room temperature. Afterwards, 50% ethanol solution (V / V) was added to the sample and centrifuged at 4500 rpm for 15 minutes. The precipitate obtained was a CPP compound.

The inhibition testing of *S. mutans* was carried out according to the method of Mahmudah & Atun (2017) with modifications. The CPP sample was dissolved in a pH 4.6 buffer solution at various concentrations (10%, 15%, 20%). Paper blanks were soaked in each sample solution for 10 minutes then placed in a blood agar medium containing *S. mutans* culture and incubated at 37°C for 48 hours. The inhibition zone formed was measured using a horizontal and vertical ruler. The average

of the measurements were calculated and the inhibition zone diameters expressed in centimeters (cm).

Tooth remineralization test was carried out according to the method of Wiryani et al., (2016) with modifications. Tooth samples (molars) were brushed and cleaned by soaking them in disinfectant solutions. The tooth roots were separated from the crowns, and the crowns were cut vertically in mesiodistal plane. Tooth samples were immersed in carbonated solution for 3 days for demineralization. The remineralization process was carried out by applying 0.1 g of CPP to the teeth surface every 12 hours for 10 days. CPP-treated teeth were immersed in artificial saliva (a mixture of 0.65 g of potassium chloride, 0.058 g of magnesium chloride, 0.165 g of calcium chloride, 0.804 g of potassium hydrogen phosphate, 0.365 g of potassium dihydrogen phosphate, 2 g of carboxymethyl cellulose, and 1 liter of water). The positive control used was commercial toothpaste while the negative control used was teeth without CPP treatment. Tooth hardness testing was done before and after the remineralization process using the Vickers Hardness Tester (Future-tech FM-810) with a 200 gf test load for 10 seconds at two indentation points. Hardness value was calculated according to a predetermined formula and stated in Hardness Vickers (HV) units.

Result and Discussion

CPP isolation results from commercial parmesan cheese obtained a yield of $16.65 \pm 2.97\%$. The isolates obtained were white and paste-shaped rough isolates as shown in Figure

1. CPP is a group of peptides derived from casein, which is the largest constituent protein component in milk. The maximum solubility of CPP is at pH 4.6 and it does not dissolve well at pH 7 (Pinto et al., 2012; Arunachalam et al., 2010). These properties distinguish CPP from proteins or other groups of peptides found in cheese.

Generally, CPP is produced by enzymatic hydrolysis of casein (Pinto et al., 2012). In the cheese manufacturing process, CPP is formed in large amount at curing stage. During curing, cheese protein will undergo hydrolysis and produce bioactive peptides with small molecular weights. The curing time of the cheese will determine the amount of CPP contained in the cheese. The longer the duration of curing, the larger the amount of CPP (Korhonen, 2009).

According to curing duration, cheese is divided into three types, namely soft cheese, medium cheese, and hard cheese. Hard cheese has a longer curing time and therefore highest CPP content. Parmesan cheese is one type of hard cheese with relatively high CPP content. Pinto et al., (2012) stated that there are 23 types of CPP in parmesan cheese with molecular weights between 0.8–4 kDa.

The inhibitory test result of CPP isolates and fluorinated toothpaste against *S. mutans* bacteria is presented in Figure 1. The test results showed that CPP isolates were able to inhibit the growth of *S. mutans*. CPP isolates produce inhibition zones greater than fluorinated toothpaste at the same concentration with values of 1.22 ± 0.04 cm and 1.16 ± 0.07 cm, respectively. Fluoride toothpaste inhibits the growth of *S. mutans* by inhibiting the action

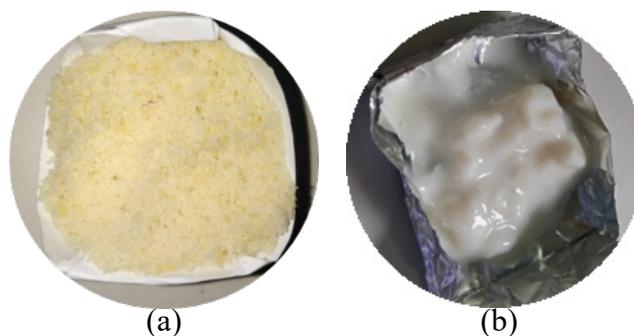


Figure 1.(a) Commercial Parmesan Cheese, (b) Caseine Phosphopeptide Isolate

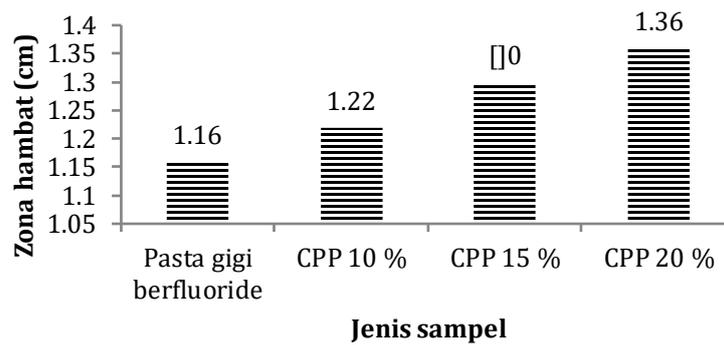


FIGURE 2. Inhibitory Zone Measurement of *Streptococcus mutans*

Table 1. Increase in Tooth Hardness After Treatment

Treatment	Hardness (HV)	
	Before remineralization	After remineralization
No treatment (control)	325.3±17.1	331.6±36.8
Fluoridated Toothpaste treatment	309.6±4.5	338.4±13.4
CPP treatment	307.1±0.3	394.2±43.7

Source: Primary Data, 2019

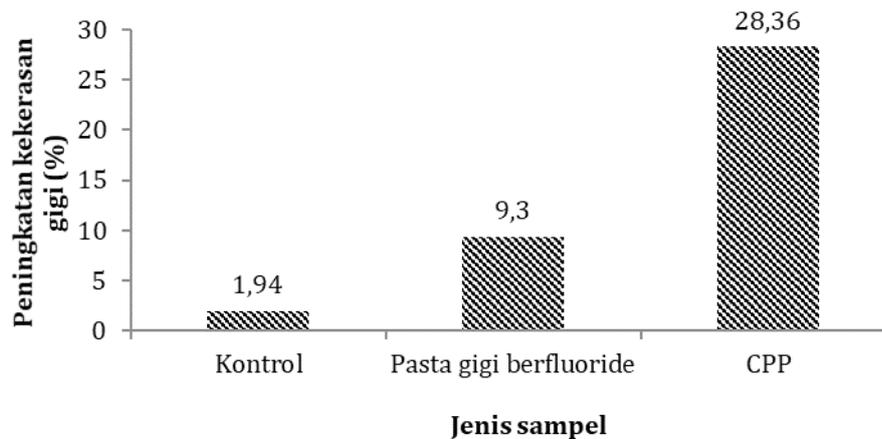


Figure 3. Percentage of Increase in Tooth Hardness

of enolase enzyme and the active proton-transport ATPase enzyme, whereas the enzyme works in the process of glycolysis. Inhibition of the two enzymes causes disruption of bacterial metabolic activity (Sinaredi et al., 2014).

The higher the concentration of CPP isolates used, the greater the inhibition zone produced. CPP isolates with concentrations of 15% and 20% produced inhibitory zones of 1.30 ± 0.00 cm and 1.36 ± 0.08 cm, respectively. According to Zhao et al., (2016), CPP can

act as both bactericidal and bacteriostatic against cariogenic bacteria especially at high free calcium concentrations. The mechanism of CPP in inhibiting *S. mutans* growth starts with electrostatic interactions between CPP molecules and anionic molecules in the cell membrane of *S. mutans*. This interaction causes disruption of *S. mutans* growth (Pinto, 2009). CPP also plays a role in reducing *S. mutans* adhesion by inhibiting their adhesion to the tooth surface (Siregar, 2011).

The result of tooth remineralization test is presented in Table 1. The result of dental hardness test showed that untreated dental samples tend to have the same hardness before and after the remineralization process, whereas teeth treated with CPP isolates and fluoridated toothpaste showed increased tooth hardness. The use of CPP isolates increased tooth hardness three times higher than fluoride toothpaste, with an increase in the percentage of hardness 28.36% and 9.30%, respectively (Figure 3). This shows that CPP isolates was more effective in increasing teeth remineralization compared to fluoride toothpaste. This is because CPP plays a role in preventing demineralization and increasing tooth remineralization. Prevention of tooth demineralization occurred through stabilization of Amorph Calcium Phosphate (ACP) to form multiphosphoryl sequences. CPP binds to ACP and prevents calcium and phosphorus ions dissolution. CPP concentrates ACP on tooth surfaces and in biofilms, so as to maintain tooth mineral saturation and prevent tooth demineralization (Thakkar et al., 2017; Zhao et al., 2016). CPP aids remineralization of teeth by localizing ACP from the teeth and increasing the concentration of calcium phosphate, which will turn into free calcium and phosphate ions reservoir. These conditions serve to prepare tooth enamel in receiving calcium and phosphate ions to enhance remineralization process of the teeth (Siregar, 2011; Walsh, 2005).

Conclusions

Casein phosphopeptide obtained from commercial parmesan cheese has a yield of $16.65 \pm 2.97\%$. CPP isolates obtained was shown to inhibit the growth of one of the main bacteria that cause dental caries, namely *S. mutans*, and enhance teeth remineralization. The result showed that CPP isolates were effective in preventing dental caries.

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