

Effect of Solvent Temperature in Effervescent Granule Denture Cleanser with Cacao Pod (*Theobroma cacao* L) 6,5% toward the Growth of *Streptococcus mutans* and *Candida albicans*

Mohammad Dharma Utama¹, Chusnul Chotimah², Harun Achmad³, Nur Fadhillah Arifin⁴,
Andi Winaya Furqani²

¹Departement of Prosthodontic, Faculty of Dentistry, Hasanuddin University

²Departement of Prosthodontic Faculty of Dentistry, Moslem University of Indonesia

³Departement of Pedodontics, Faculty of Dentistry, Hasanuddin University

Departement of dental Material Faculty of Dentistry, Moslem University of Indonesia

Corresponding author: Harun Achmad, email: harunachmader@gmail.com

ABSTRACT

Introduction and Objectives: Generally, the materials and/or ingredients that are often used in the denture cleanser products are available in several dosage forms, including effervescent tablets. The basic materials and/or ingredients of denture cleanser are not only in the form of chemicals, but can also use herbals. The use of herbs for dentures cleanser can be obtained from the skin of cocoa fruit or cocoa pod (*Theobroma cacao* L). To determine the effect of the solvent temperature of effervescent granules denture cleanser of cocoa pod (*Theobroma cacao* L) 6.5% in inhibiting the growth of *Streptococcus mutans* and *Candida albicans*. **Materials and Methods:** The research method used was True Experimental through the Pre-Post Test with Control Group research design, the total samples used was 24 acrylic resin plates that immersed in *Streptococcus mutans* and *Candida albicans* suspensions, then immersed in granules effervescent of cocoa pod with the solvent temperature of 5- 10°C, 20-25°C and 25-31°C then the colony count was performed with a colony counter. **Results:** ANOVA test that was carried out has obtained result of p value <0.05, it means that there was a significant influence between the solvent temperature of 5-10°C, 20-25°C and 25-31°C of effervescent granules denture cleanser of cocoa pod (*Theobroma cacao* L) toward the growth of *Streptococcus mutans* and *Candida albicans*. **Conclusion:** The solvent temperature 20-25°C of effervescent granules denture cleanser of cocoa pod (*Theobroma cacao* L) 6.5% can inhibit the growth of *Streptococcus mutans* and *Candida albicans*

Keywords: Effervescent granules; Cocoa pod, *Streptococcus mutans*, *Candida albicans*, Solvent temperature

Introduction

Loss of one or several teeth that experienced by a person requires rehabilitation measures, among others, by making artificial teeth or denture.⁽¹⁾ The prevalence of denture users in Indonesian population aged 12 years and over is 4.5%. The type of treatment in the form of removable and fixed denture installation ranges from 0.6% -10.8%, the highest in the age of 65 years and above.⁽²⁾ In dentures can be found several types of bacteria such as *Candida albicans* 65.5%, and *Streptococcus mutans* 53.3%.⁽³⁾ *Streptococcus* sp. is the first bacterium that attaches to the base of the denture and forms a colony. *Streptococcus mutans* can produce Extracellular Polysaccharides (PSE) that is not possessed by other bacteria. The substrate can open access for other microorganisms in attaching the base of the denture. *Candida albicans* can penetrate acrylic resin and grow on the surface of denture so that it can infect soft tissue. *Candida albicans* can release endotoxins which damage the oral mucosa and cause denture stomatitis, to prevent denture stomatitis, it is necessary to maintain oral hygiene and dentures.⁽⁴⁾

Denture cleaning can be done in two ways namely mechanically and chemically. Mechanical cleaning is done by using a toothbrush and ultrasonic, while chemically cleaning by immersing dentures in disinfectants, alkaline peroxide, chlorhexidine, alkaline hypochlorite, sodium hypochlorite, enzymes and herbs.⁽³⁾ Materials that are often used in denture cleanser products are available in several dosage forms, including effervescent tablets. Denture cleanser materials in the form of effervescent tablets on the market are effervescent tablets which containing sodium Perborate.⁽⁵⁾ Other dosage forms that can be used as denture cleansers are effervescent granule preparations:

Effervescent granules are the result of a combination of acid and base compounds which when added to water (H₂O) will react to release carbon dioxide (CO₂), so this effect will produce froth on the preparation, the speed of reaction also depends on the temperature of the water.^(6,7) The temperature of the solvent has a role in the process of decomposition of chemical reactions from effervescent granules, the effect of the reaction produced in the form of CO₂. Mechanical cleansers can release *Streptococcus mutans* and *Candida albicans* colonies on acrylic resin plates.⁽⁸⁾

The basic materials of denture cleanser are not only in the form of chemicals, but can also use herbal. The government has launched the use of drugs that come from nature (herbal). The use of herbs for dentures cleanser can be obtained from the cocoa pod (*Theobroma cacao* l). Cocoa pod is the biggest waste produced by farmers and cocoa cultivators. This cocoa pod can only be used as fodder. Cocoa pods contain many bioactive components that can be used as dental therapeutic agents. Cocoa pod extract concentration of 0.25% can inhibit the growth of *Streptococcus mutans*, while a concentration of 6.25% can inhibit the growth of *Candida albicans*.^(9, 10,11)

The bioactive components of cocoa pod, namely flavonoids, saponins, catechins, might prevent the initiation of pellicle attachment and glucan formation by *Streptococcus mutans*, so that *Candida albicans* attachment does not occur.⁽⁹⁾ Effervescent granules which have the advantage and bioactive components of the cocoa pod (*Theobroma cacao* l) and the role of solvent temperature which plays an important role in the antibacterial and antifungal activities, this is the background of researchers in observing the effect of solvent temperature granular effervescent denture cleanser with cocoa pod (*Theobroma cacao* l) in inhibiting the growth of *Streptococcus mutans* and *Candida albicans*.

Material and Methods

This research has obtained ethical approval under number 294 / A.1 / KEPK-UMI / XII / 2019. The study was conducted at the Microbiology Laboratory of the Faculty of Pharmacy, Moslem University of Indonesia, which was conducted in November 2019 to February 2020. The materials used were 6.5% effervescent granules of cocoa pod, distilled aquades, *Candida albicans* mushroom suspension, *Streptococcus mutans* bacterial suspension, nutrient broth, potato dextrose broth, nutrient agar, potato dextrose agar, sterile saliva and phosphate buffer saline. The tools used are petri dishes, test tubes, tweezers, measuring cups, syringe tuberculin, thermometers, incubators, autoclaves and colony counters.

The research method used was True Experimental research design through Pre-Post test with Control Group. The total samples in this study was 24 with a simple random sampling method, acrylic resin plate samples were divided into 12 groups, consisting of 6 treatment groups (3 treatment groups for *Streptococcus mutans* and 3 treatment groups for *Candida albicans*) and 6 groups control (3 control groups against *Streptococcus mutans* and 3 control groups against *Candida albicans*). Acrylic resin plates were sterilized with 121°C autoclave for 15 minutes, soaked in sterile saliva for 1 hour then rinsed with phosphate buffer saline, acrylic resin plates were put into *Streptococcus mutans* and *Candida albicans* suspensions and incubated for 24 hours at 37°C for acrylic resin plates inserted into *Streptococcus mutans* suspension and for 48 hours at 25°C for acrylic resin plates which inserted into *Candida albicans* suspension, acrylic resin plates inserted into nutrient broth and 10 ml potato dextrose broth were vibrated for 30 seconds to release *Streptococcus mutans* and *Candida albicans* attached to nutrient broth and 10 ml potato dextrose broth were vibrated for 30 seconds to release *Streptococcus mutans* and

candida albicans attached to the sample. 0.1 ml of Streptococcus mutans suspension and candida albicans (from nutrient broth and potato dextrose broth 10 ml) were taken using 1 cc tuberculin syringe, dripped on nutrient agar and potato dextrose agar and then incubated for 24 hours and 48 hours at 37°C and 25°C. The calculation of Streptococcus mutans and Candida albicans colonies by using colony counter (Pre-test).

Acrylic resin plates were incubated for 24 hours to let the Streptococcus mutans and Candidaalbicans colonies to reattach to the surface of the acrylic resin plates then the acrylic resin plates were immersed in 6.5% effervescent granules of cocoa pod with solvent temperatures of 5- 10°C, 20- 25°C and 25-31°C and then put into 10 ml nutrient broth and potato dextrose broth, vibrated for 30 seconds to release Streptococcus mutans and Candida albicans that attached to the sample 0.1 ml of Streptococcus mutans suspension and candida albicans (from nutrient broth and potato dextrose broth 10 ml) were taken using 1 cc tuberculin syringe, dripped on nutrient agar and potato dextrose agar and then incubated for 24 hours and 48 hours at 37°C and 25°C. The calculation of Streptococcus mutans and Candida albicans colonies using a colony counter (Post-test). Data processing using SPSS version 25 with the ANOVA test.

Result

Table 1 ANOVA test result shows the effect of solvent temperature in effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) in inhibiting the growth of *Streptococcus mutans*

Source	Number of Squares	df	Average Squares	F	P- Value
Corrected Model	1448665.000a	5	289733.000	175.844	.000
Intercept	1119963.000	1	1119963.000	679.727	.000
Temperature	99276.500	2	49638.250	30.126	.001
Sample	1245496.333	1	1245496.333	755.915	.000
temperature * Sample	103892.167	2	51946.083	31.527	.001
Error	9886.000	6	1647.667		
Total	2578514.000	12			
Corrected Total	1458551.000	11			

a. R Squared = .993 (Adjusted R Squared = .988)

*Corrected model sig < 0, 05

Table 1 shows the results of the anova test on the effect of the solvent temperature of the effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) in inhibiting the growth of *Streptococcus mutans*, overall the model is significant because a p- value of 0,000 is less than 0.05. Table 1 also shows that temperature has a significant effect on the test model carried out, because it has a p-value of 0.001 which is smaller than than 0.05 so that it is concluded that there is a significant effect of solvent temperature of 5-10°C, 20-25°C, 25-31°C effervescent granules denture cleanser of cocoa pod extract (*Theobroma cacao* l) toward the growth of *Streptococcus mutans*, next, to determine the temperature of the solvent that most inhibits the growth of streptococcus mutans, advanced testing was done by using the Tukey Test. The test results are shown in table2.

Table 2 Results of advanced different tests of the average decrease in the number of *Streptococcus mutans* (CFU / mL) colonies at each temperature

(I)Temperature	(J)Temperature	Mean	P-value	95% Confidence Interval	
		Difference (I-J)		Lower Bound	Upper Bound
5-10 ⁰ C	20-25 ⁰ C	-213.2500*	.001	-301.3171	-125.1829
	25-31 ⁰ C	-50.7500	.258	-138.8171	37.3171
20-25 ⁰ C	5-10 ⁰ C	213.2500*	.001	125.1829	301.3171
	25-31 ⁰ C	162.5000*	.003	74.4329	250.5671
25-31 ⁰ C	5-10 ⁰ C	50.7500	.258	-37.3171	138.8171
	20-25 ⁰ C	-162.5000*	.003	-250.5671	-74.4329

*Post Hoc Test: Tukey's HSD; $p < 0, 05$: significant

Table 2 shows the results of further testing can be seen a comparison between the overall temperature observed, the ratio between the temperature of 20-25⁰C with 5-10⁰C has an average difference in the decrease number of bacterial colonies by 213.25, it shows that a decrease in the number of bacterial colonies at a temperature of 5-10⁰C is less than the temperature of 20-25⁰C, so there is a significant difference between the temperature of 20-25⁰C with 5-10⁰C because it has a p- value of 0.001. The temperature between 20-25⁰C with 25-31⁰C has an average difference in the decrease number of bacterial colonies by 162.50, this shows that the decrease number of bacterial colonies at 25-31⁰C is less than the temperature of 20-25⁰C, so there is a significant difference between temperatures 20-25⁰C with 5-10⁰C because it has a p-value of 0.00

Table 3 ANOVA test results on the effect of the temperature of solvent effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) in inhibiting the growth of *Candida albicans*

Source	Number of Squares	Df	Average Squares	F	P- Value
Corrected Model	4671850.750a	5	934370.150	505.452	.000
Intercept	4251870.750	1	4251870.750	2300.070	.000
Temperature	130202.000	2	65101.000	35.217	.000
Sample	4425030.750	1	4425030.750	2393.742	.000
Temperature * Sample	116618.000	2	58309.000	31.543	.001
Error	11091.500	6	1848.583		
Total	8934813.000	12			
Corrected Total	4682942.250	11			

a. R Squared = .998 (Adjusted R Squared = .996)

*Corrected model sig < 0, 05

Table 3 shows the results of ANOVA test on the effect of the temperature of the solvent effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) in inhibiting the growth of *Candida albicans*. Overall, the model is significant because it was obtained p-value of 0,000 which is smaller than 0.05. Table 5.3 also shows that the temperature has a significant effect on the test model carried out, because it has a p-value of 0.000 which is smaller than 0.05, so overall it is concluded that, there is a significant influence of solvent temperature of 5-10⁰C, 20-25⁰C, 25-31⁰C effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) on the growth of *Candida albicans*, to determine the temperature of the solvent that most influencing the growth of

Candida albicans, advanced testing was done by using the Tukey Test. The test results are shown in table 4.

Table 4 Results of advanced different tests of the average decrease in the number of *Candida albicans* (CFU / mL) colonies at each temperature

(I)Temperature	(J)Temperature	Mean Difference (I- J)	P-value	95% Confidence Interval Lower Bound	Upper Bound
5-10 ⁰ C	20-25 ⁰ C	111.5000*	.024	18.2178	204.7822
	25-31 ⁰ C	254.5000*	.000	161.2178	347.7822
20-25 ⁰ C	5-10 ⁰ C	-111.5000*	.024	-204.7822	-18.2178
	25-31 ⁰ C	143.0000*	.008	49.7178	236.2822
25-31 ⁰ C	5-10 ⁰ C	-254.5000*	.000	-347.7822	-161.2178
	20-25 ⁰ C	-143.0000*	.008	-236.2822	-49.7178

*Post Hoc Test: Tukey's HSD; p < 0, 05: significant

Table 4 shows the results of advanced testing can be seen the comparison among the overall temperature observed, the ratio between the temperature of 5-10⁰C with 20-25⁰C has an average difference in the decrease in the number of bacterial colonies by 111.50 this shows that the decrease in the number of bacterial colonies at temperatures of 20-25⁰C slightly compared to the temperature of 5-10⁰C, so there is a significant difference between the temperature of 5-10⁰C with 20-25⁰C because it has a p-value of 0.024. Temperatures between 5- 10⁰C with 25-31⁰C have an average difference in the decrease in the number of bacterial colonies by 254.50 this shows that the decrease in the number of bacterial colonies at 25-31⁰C is less than the temperature of 5-10⁰C, so there is a significant difference between temperatures 5-10⁰C with 25-31⁰C because it has a p-value of 0.000.

Discussion

The study conducted was aiming to find out the effect of the solvent temperature of the effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) on the growth of *Streptococcus mutans* and *Candida albicans*. This research was carried out by immersing the acrylic resin plate into effervescent granules dissolved in the solvent temperature of of 5-10⁰C, 20-25⁰C, 25-31⁰C which had previously been immersed in *Streptococcus mutans* and *Candida albicans* suspensions, then after that the number of *Streptococcus mutans* and *Candida albicans* was calculated.

Tables 1 and 3 explain that there is a significant effect of solvent temperature of 5-10⁰C, 20-25⁰C, 25-31⁰C effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) on the growth of *Streptococcus mutans* and *Candida albicans* because based on statistical test results using Anova test, obtained p-value that is p < 0.05 thus, it was stated that Ho was rejected and Ha was accepted, which means that there was an effect of solvent temperature for effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) on the growth of *Streptococcus mutans* and *Candida albicans*. This study explains the solvent temperature has a very big role in dissolving effervescent granules with cocoa pod extract, because with the temperature of the solvent used, then effervescent granules can extract carbon dioxide (CO₂) which functions to break down effervescent granules when effervescent granules are dissolved into water , so that the 6.5% cocoa pod extract contained in the effervescent granule can work well in inhibiting the growth of *Streptococcus mutans* and *Candida albicans* on the acrylic resin plate.

Table 2 shows that the most influential solvent temperature in inhibiting the growth of *Streptococcus mutans* is the temperature of 20-25⁰C and table 4 shows the solvent temperature which is the most influential in inhibiting the growth of *Candida albicans* is the temperature of 5-10⁰C. Research

conducted explains that in addition to the 6.5% cocoa skin extract which can inhibit the growth of *Streptococcus mutans* and *Candida albicans*, temperature also has a role in inhibiting the growth of a microorganism. Temperature depends on chemical reactions and the rate at which these reactions are influenced by temperature, bacterial growth is strongly influenced by temperature because temperature is one of the most important environmental factors affecting the life and growth of microorganisms, in the research conducted it can be seen when the temperature drops then the metabolic rate of microorganisms also decreases and growth is slowed, and the rate of growth may be stopped, cell components become inactive and cells can die^(12,13)

This research in line with the research conducted by Amanda et al., She stated that immersion of the denture at solution temperature ($37 \pm 2^{\circ}\text{C}$) effervescent alkaline peroxide tablets and 0.5% sodium hypochlorite solution at temperature ($23 \pm 2^{\circ}\text{C}$) significantly removed biofilms at denture.⁽¹⁴⁾

The research carried out is also in line with research conducted by Richard which states that the effect of solvent temperature has a role in the decomposition process of chemical reactions from 2.5% effervescent *Sargassum polycystum* granules where the reaction effect produced is CO_2 , due to the form of gases that are in the effervescent granule solution cause an increase in pressure by reducing the volume will increase the concentration of the solution, this causes the gradient concentration in the effervescent granule solution so that when the acrylic resin plate is submerged there will be a push/ diffuse force causing the effervescent granule solution *Sargassum polycystum* 2.5% is absorbed into the acrylic resin plate and inhibits growth *Streptococcus mutans* and *Candida albicans* on acrylic resin plates, the thrust that occurs on the surface of acrylic resin can also cause *Streptococcus mutans* and *Candida albicans* to be separated from the acrylic resin plates.⁽⁸⁾

Based on the results of the study, it can be concluded that the solvent temperature had a role in extracting 6.5% effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) to inhibit the growth of *Streptococcus mutans* and *Candida albicans* while for the application of effervescent granules denture cleanser with cocoa pod extract (*Theobroma cacao* l) 6.5% as a removable denture remover made from acrylic resin, it is recommended to use the temperature of 20- 25°C, because the temperature of the solvent 20-25°C is the most influential temperature in inhibiting the growth of *Streptococcus mutans*, the solvent temperature of 20-25°C can also be used to inhibit the growth of *Candida albicans* (Table 4), the use of a solvent temperature of 20-25°C can facilitate the patient to dissolve effervescent granules denture cleanser with cocoa pod extract 6.5%, without the need for tools or other materials that must make the water cool first.

If the temperature of the solvent can inhibit the growth or eliminate *Streptococcus mutans* on the acrylic resin plate, then the bacteria and other fungi will not adhere and develop on the acrylic resin plate, this is in line with the research that says that *Streptococcus mutans* produces a substrate namely extracellular polysaccharides (PSE) which is not owned by other bacteria. The substrate provides a pathway for bacteria and other fungi to attach to the base of the denture.^{(4) (15) (16) (17) (18) (19)}

Conclusion

There is a significant influence among the temperature of solvent at 5-10°C, 20-25°C and 25-31°C of effervescent granules denture cleanser with cocoa pod extract (*Theobromacacao* l) on the growth of *Streptococcus mutans* and *Candida albicans*. The most influential temperature in inhibiting the growth of *Streptococcus mutans* and *Candida Albicans* is 20-25°C.

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