Comparison of *Candida albicans* colony amount in heat-cured acrylic and thermoplastic nylon resin after immersion in *Ulee Kareng* coffee (*Coffea robusta*)

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ABSTRACT

Introduction: Heat-cured acrylic resin is the most often used material in the manufacture of denture base. Along with the development of science and technology, to overcome the shortcomings of heat-cured acrylic resin, repairment of denture base material was done, one of them is a thermoplastic nylon resin. On the use of denture often found *Candida albicans* attached to the denture. This study aimed to determine the comparison of the amount of *Candida albicans* colony on heat-cured acrylic and thermoplastic nylon resin after immersed in the *Ulee Kareng* coffee *(Coffea robusta)*. **Methods:** The number of specimens in this study were 8 specimens; 4 Meliodent® heat-cured acrylic resins and 4 Bio Tone® thermoplastic nylon resins with size of 10x10x2 mm. The methods of this study was experimental laboratory. The specimens were stored in a solution of *Ulee Kareng* coffee *(Coffea robusta)* for 7 days. Each specimen was contaminated with *Candida albicans,* then the number of *Candida albicans* colony was counted with Colony counter, from threshing results of heat-cured acrylic and thermoplastic nylon resins. Data were analyzed with unpaired t test. **Results:** Unpaired t test results showed that there were significant differences ($p<0.05$) between the number of colonies of *Candida albicans* in heat-cured acrylic resin (4.5 CFU/ml) and thermoplastic nylon resin (1.5 CFU/ml) after both immersed in the *Ulee Kareng* coffee *(Coffea robusta)*. **Conclusion:** The amount of *Candida albicans* colony on heat-cured acrylic resin was higher than on thermoplastic nylon resin.

Key words: Heat-cured acrylic resin, Thermoplastic nylon resin, *Ulee Kareng* coffee, *Coffea robusta*, *Candida albicans* colony

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INTRODUCTION

Acrylic resin was first introduced by Dr. Walter Wright and Vernon Brothers from Philadelphia in 1937. In 1940, 95% of denture base was made of acrylic resin with polymethyl methacrylate (PMMA) as basic materials. Acrylic resin that widely used as denture base material is heat-cured acrylic resin because of its esthetic, low toxicity, easily processed, and repaired, as well as economical

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reason. However, heat-cured acrylic resin also has drawbacks in its mechanical properties such as weak flexural and impact strength, low fatigue strength, brittle, low hardness, and high degree of porosity.[1-3]

Along with the development of dentistry science, conducted several studies to improve the mechanical properties of acrylic resin.[4] In the 1950s introduced polyamide or thermoplastic resin as new denture base material. The application of thermoplastic resins continued to increase, especially in the nylon material.[5] Examples of denture base materials made of nylon such as Bioplast®, Valplast®, Bio Tone®, Ulltimate®, and Lucitone FRS®.[6] Thermoplastic nylon has several advantages, which was excellent aesthetic, non-toxic properties, high flexibility, safe to use in patients who are allergic to metals and monomer resin, and has sufficient strength to be used as denture base material.[4] Despite of those advantages, thermoplastic nylon have drawbacks such as high water permeable, tendency of color changing, difficult to be manipulate, and increasing surface roughness after few weeks usage.[5] The surface roughness, strength, and hardness of denture base material can be affected by acidic food or drinks consumed by users such as coffee.[7]

Coffee contains carbohydrates, proteins, minerals, caffeine and some amount of pectin and tannins. There are 2 types of coffee plants often consumed, Arabica coffee and Robusta coffee. Robusta coffee prices relatively cheaper than Arabica coffee. Robusta coffee has caffeine composition higher than Arabica coffee, which was 1.7-4.0%, whilst the composition of the caffeine in Arabica coffee was only 0.8-1.4%. The caffeine contents in coffee is a source of energy for microorganisms growth, whilst pectin, and tannins accelerate the growth.[8,9]

Microorganisms often found in denture usage is *Candida albicans. Candida albicans* is a common flora in the oral cavity. The normal amount of *Candida albicans* in the oral cavity is less than 200 cells per ml saliva.[10] Research conducted by Zomorodian and Haghigh N[10] towards 114 subjects of denture user found the existence of as many as 41.5% of *Candida albicans,* 18.4% of *Candida glabrata,* and 12.9% of *Candida tropikalis*.[10]

The use of denture can cause other problems if the hygiene and treatment was unnoticed. Denture user who does not pay attention to oral hygiene, including the hygiene of the denture itself, will resulted in piling of food debris which was a predisposition to plaque formation. This situation will increases the prevalence of *Candida albicans* microorganism in patient's oral cavity.[12] Denture user that having unhealthy oral conditions will increases the prevalence of *Candida albicans* up to 47.5-55%.[13]

One of denture base material properties such as surface roughness also shown direct relationship between plaque accumulation and adhesion of *Candida albicans*. Research conducted by Abuzar et al[13] showed that the surface roughness of thermoplastic nylon resin was 3 times higher than polymethyl methacrylate acrylic resin, but still within the normal limits of acceptable surface roughness in the oral cavity which is 0.2 µm Ra.[13] The nature of porosity in heat-cured acrylic resin can triggers the emergence of *Candida albicans,* where the porous will be the accumulation place of food debris and microorganisms.[15] Increasing numbers of *Candida albicans* influenced by several factors, such as oral hygiene, denture usage and denture characteristics.[11]

Based on description above, this study was conducted to know the comparison of the amount of *Candida albicans* colony on heat-cured acrylic resins and thermoplastic nylon *heat cured* after immersed in *Ulee Kareng* coffee *(Coffea robusta)*.

METHODS

The research method was experimental laboratory with posttest only control group design. Meliodent® heat-cured acrylic resin making was performed at the Skill's Room of Dental Education Program Laboratory Faculty of Dentistry Syiah Kuala University. Whilst Bio Tone® thermoplastic nylon resin was booked through laboratory technicians. The brewing of *Ulee Kareng* coffee (*Coffea robusta*) was conducted at the Laboratory of Biology Faculty of Educational Science Syiah Kuala University, and the counting of *Candida albicans* colony amount was conducted at the Laboratory of Microbiology, Faculty of Veterinary Medicine Syiah Kuala University. The study was conducted in May 2016.

Samples of this study were ATCC 10231 *Candida albicans*, and the test material used

was the specimens of Meliodent® heat-cured acrylic resin and Bio Tone® thermoplastic nylon resin. The making of molds for the specimens of Meliodent® heat-cured acrylic resin was done by preparing a wax base plate with the size of 10x10x2 mm planted in a cuvette. After that, the wax elimination process was performed by inserting the cuvette into a pot of boiling water (100˚C) for 15 minutes. Then the surface of the mold smeared with Cold Mold Seal (CMS) using a small brush. The powder and liquid were mixed with the ratio of 23 gr:10 ml in a porcelain cup. The porcelain cup then closed and let stand until reached the dough stage phase (able to formed and not adhesive) and the packing was done into the mold *(*mold space*)*, pressed, and the acrylic resin excess was removed, get pressed back and the curing was done by being boiled. While the making of Bio Tone® thermoplastic nylon resin specimens was done by the booking through laboratory technicians. Each specimen consists of 4 pieces with the size of 10x10x2 mm (Fig. 1).

The specimens were immersed in *Ulee Kareng* coffee solution. The making of the coffee solution was done by dissolving 60 grams of *Ulee Kareng* coffee powder in 600 ml of boiling water with temperature of 100ºC then let stand until the temperature reaches 55ºC, then poured into immersing container. Before the immersion was done, the degree of acidity (pH) of *Ulee Kareng* coffee solution was measured. pH measurement was performed three times and then taken the average value. Heat-cured acrylic and thermoplastic nylon resin specimens respectively immersed in the coffee solution inside a container, then the container was closed and stored in an incubator at the temperature of 37ºC Immersion materials was replaced daily.

Candida albicans isolates was cultivated before by taking 1 ose of *Candida albicans,* then inoculated in a petri dish contained Sabouraud's Dextrose Agar (SDA). The scratches can be done 3-4 times to form horizontal lines in one cup. This step was continued until all four sides of the cup were scratched, and incubated for 24 hours at 37˚C afterwards. The dilution process was done after, by doing gradual dilution up to 10^{-6} dilution. The suspension results from gradual dilution was then cultivated in each SDA media. The cultivated results can be seen with Colony counter. The amount of *Candida albicans* colonies that grew were 30-300 colonies. The next colony was taken, and was made into suspension by physiological saline solvent.

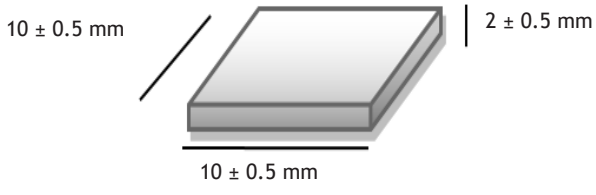

Figure 1. Resin specimens dimension

The suspension of *Candida albicans* that consisted of 30-300 colonies was taken each as much as 1 ml into 10 test tubes contained 10 ml physiological saline, and then incubated for 24 hours at the temperature of 37˚C. Then each heat-cured acrylic and thermoplastic nylon resin specimens that has been immersed in *Ulee Kareng* coffee solution was inserted into a test tube contained *Candida albicans* suspension and incubated for 24 hours at the temperature of 37˚C. Specimens of heat-cured acrylic and thermoplastic nylon resins which has been contaminated with *Candida albicans* suspension was inserted into 10 ml of physiological saline media, Then vibrated with Vortex for 30 seconds to release the *Candida albicans* attached to the specimen.

Candida albicans suspension was taken in the amount of 0.1 ml inserted into the SDA media, carried out by spread using L rod and incubated for 24 hours at the temperature of 37˚C then calculated the amount of *Candida albicans* colony by using a Colony counter.

The results of the research were analyzed using unpaired t test to compare the number of *Candida albicans* colony in heat-cured acrylic and thermoplastic nylon resin after immersed in *Ulee Kareng* coffee.

RESULTS

In this study, statistical analysis used was unpaired t test that consisted of two groups, *Candida albicans* colonies on heat-cured acrylic resin and *Candida albicans* colonies on thermoplastic nylon resion, after both were immersed in *Ulee Kareng* coffee.

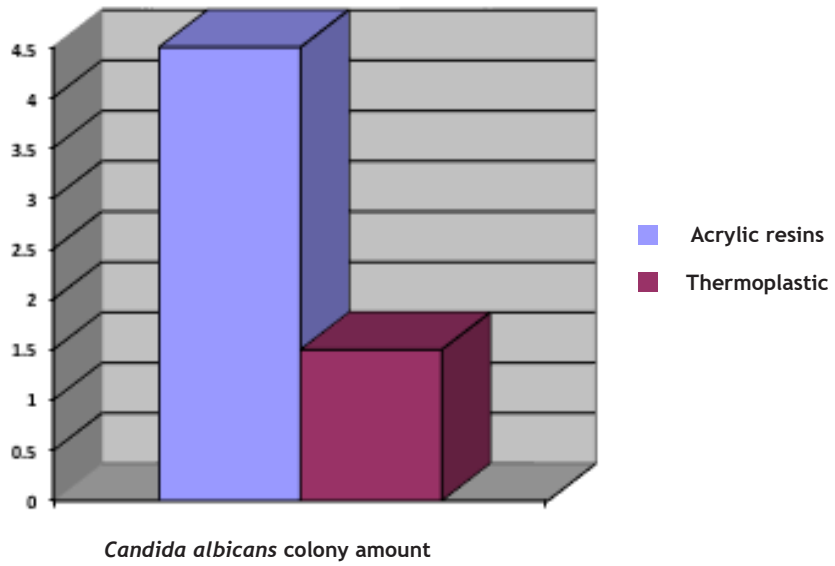

Figure 2. Diagrams of the amount of *Candida albicans* colonies between heat-cured acrylic and thermoplastic nylon resins after immersed in *Ulee Kareng* coffee solution

Table 1. Comparison of the amount of *Candida albicans* colonies on heat-cured acrylic and thermoplastic nylon resins after immersed in Ulee Kareng coffee solution

Specimens type	Amount of *Candida albicans* colony (± SD)	p
Heat-cured acrylic resin	4.5 ± 1.291	*0.035
Thermoplastic nylon resin	1.5 ± 1.732	

* Significance value was P < 0.05 with unpaired t test analysis

Table 2. PH Measurement of *Ulee Kareng* coffee solution

Days	Measurement			pH average
	1st	2n	3rd	
1	5.53	5.57	5.58	5.56
2	5.67	5.68	5.67	5.67
3	5.62	5.61	5.58	5.60
4	5.64	5.65	5.66	5.65
5	5.57	5.58	5.60	5.58
6	5.58	5.57	5.59	5.58
7	5.60	5.62	5.65	5.62
pH Averages				**5.60**

The average amount of *Candida albicans* on heat-cured acrylic resin was 4.5 CFU/Unit. Whilst the average amount of *Candida albicans* on thermoplastic nylon resin was 1.5 CFU/Unit showed in Table I. Unpaired t test results in both groups showed significant differences (p<0.05) in the average amount of *Candida albicans* colonies between heat-cured acrylic resins and thermoplastic nylon resins after being immersed in *Ulee Kareng* coffee solution. The data obtained tested with normality test by using *Shapiro-Wilk* test. The results indicated that the data was normally distributied with p>0.05.

pH measurement of *Ulee Kareng* coffee solution was done 3 times daily and the results were averaged. The average showed in Table 2 results of Ulee Kareng coffee pH measurement for 7 days was 5.60. This indicated that *Ulee Kareng* coffee has an acidic pH.

DISCUSSION

This study was conducted to see the comparison between the amount of *Candida albicans* colony on heat-cured acrylic and thermoplastic nylon resins after immersion in *Ulee Kareng* coffee. This study showed that the average amount of *Candida albicans* colony on heat-cured acrylic resin after immersion in *Ulee Kareng* coffee was 4.5 CFU/ml. Whilst the average value of the amount of *Candida albicans* colony on thermoplastic nylon resin was 1.5 CFU/ml. The results of unpaired t test data analysis showed that the difference was significant (p<0.05) where the amount of *Candida albicans* colonies was larger on heat-cured acrylic resin than thermoplastic nylon resin. The difference value of the average amount of *Candida albicans*

colonies in both groups might be caused by the porosity of heat-cured acrylic resin. Micro pores or porosity on the acrylic resin easily trigger the piling of food debris and microorganisms inside.[16]

In this study, heat-cured acrylic resin material was immersed in *Ulee Kareng* coffee solution, where 10% of Robusta coffee contained chlorogenic acid. During coffee bean roasting, chlorogenic acid decomposed into volantil and melanoidin compounds. In volantil compounds contained phenol compounds that increased after roasting.[3] Phenol compound is an aromatic hydrocarbon class that is expected able to penetrate the micro porous space and dissolve it.[17] Thus in this study, 5% of phenol solution was contacted with material which suspected influencing the degree of hydrophobicity of heat-cured acrylic resin.

Water absorption can occured due to the diffusion of water molecules through a gap contained in heat-cured acrylic resin. Liquid molecules were able to penetrate the density of PMMA and was mixed inside the macromolecular structure, resulted the acrylic resin polymer chains were pressed and separated, and changed the physical characteristics of the polymer. A separated polymer chains will also resulted to porosity. The high amount of porosity in heat-cured acrylic resin will resulted a rough surface.[18] In Deliga study showed that the value of surface roughness was higher in heat-cured acrylic resin than in thermoplastic resin after being immersed in *Ulee Kareng* coffee solution.[19] This condition can also trigger *Candida albicans* adhesions in denture base.

The elements contained in coffee are caffeine, trigonelin ($C_7H_7NO_2$), sucrose, monosaccharides, chlorogenic acid, and nicotinic acid. The caffeine contents in coffee is a source of energy for microorganisms growth, while pectin and tannins accelerate the growth.[8,9] The growth of *Candida albicans* can also be influenced by several factors, one of them is nutrition. Sucrose contents in coffee can also trigger the growth of *Candida albicans* because this species grows rapidly in media containing peptone, dextrose, maltose, or sucrose.

In this study, specimens of heat-cured acrylic and thermoplastic nylon resins was not immersed in artificial saliva, so the study conditions was not approached the oral cavity condition. The higher amount of *Candida albicans* colony in heat-cured acrylic resin could also caused by the structure differences. Acrylic resin is an amorphous polymer, where the molecular structure was arranged in irregular manner, so that the bond lengths and angles are also irregular, and influences the surface roughness and permeability.

Thermoplastic nylon is a crystalline polymer, where the structure of its constituent molecules are arranged regularly based on the length and angle of the bond and also has a strong hydrogen bonds in its chemical structure resulting to its low permeability, resistant to chemical solvents and high temperature condition.[20,21] Thermoplastic nylon also showed no color changing along the time with no porous, which causes the minimum microorganisms growth.[1] This condition resulted in the lower amount of *Candida albicans* colonies than found on the heat-cured acrylic resins.

CONCLUSION

The amount of *Candida albicans* colony on heat-cured acrylic resin was higher than on thermoplastic nylon resin due to different polymer structure characteristics.

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