Microstructure and hardness of bovine enamel in roselle extract solution

M T Dame, A Noerdin and D J Indrani*
Department of Dental Material Science, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

*E-mail: deckyji@gmail.com

Abstract. The aim of this study was to analyze the effect of roselle extract solution on the microstructure and hardness of bovine enamel. Ten bovine teeth and a 5% concentration of roselle extract solution were prepared. Immersions of each bovine tooth in roselle extract solution were conducted up to 60 minutes. The bovine enamel surface was characterized in hardness and microscopy. It was apparent that the initial hardness was 328 KHN, and after immersion in 15 and 60 min, the values decrease to 57.4 KHN and 11 KHN, respectively. Scanning electron microscopy (SEM) revealed changes in enamel rods after immersion in the roselle extract solution.

1. Introduction
Indonesia is rich in various types of tropical plants; many of these are beneficial for the human body, therefore, they are used as traditional remedies. One plant that is currently widely used as a traditional remedy is the roselle calyx (Hibiscus sabdariffa L). The beneficial components in the roselle include phenols, anthocyanin, flavonoids, tannins, and vitamin C. Roselle extract has an acid component comprising citric acid, ascorbic acid, malate acid, and tartaric acid [1]. Because of the roselle’s beneficial components, many studies on this plant’s advantages have been conducted. Some studies have shown that roselle extract can reduce the blood pressure of hypertensive patients [1,2]. In addition, the extract has an anti-infection effect [3] and the ability to increase liver function [4], as well as, to reduce cholesterol and glucose levels [4,5]. The roselle has also been proven to reduce the diameter of oral ulcers [6], to hinder the proliferation of Candida albicans dentures, and to prevent C. albicans infection in oral cavities [2]. Because of its many beneficial functions and non-toxicity, the roselle has been widely studied.

The roselle is available in the form of dry calyces that are processed into various kinds of food and beverages. The roselle calyx contains organic acids—specifically, citric acid, ascorbic acid, malate acid, and tartaric acid [1]—and the extract used in previous studies had a pH level of 2.791. This low pH can influence the hard tissue of teeth, leading to demineralization and decreased hardness [7]. Many previous studies on the effect of acid solution on enamel’s hardness have been conducted. Rytomaa et al. (1988) found that soaking roselles in acid solution for 60 minutes with pH 2.96 caused erosion on the bovine tooth. Furthermore, Zheng (2011) demonstrated that soaking roselle for 10 minutes in an acid solution of pH 3.2 could significantly reduce hardness of bovine tooth. An initial study on roselle showed that the pH of roselle solution was 2.791.

Hardness of the enamel surface is one of the most important mechanical properties in the masticatory system, as the mastication load is directly influenced the tooth enamel surface. On the other hand, bovine teeth are widely used in experiments in place of human teeth, despite their different microscopic structures and mineral morphologies. This study, therefore, was to observe the effects of roselle extract for up to 60 minutes on the hardness of bovine teeth.
2. Materials and Methods

Ten specimens of incisal bovine teeth were employed in this study. Each tooth was embedded in a decorative resin with the labial surface facing upward. The labial surface was ground using #1500 and #2000 grinding paper in a grinding machine and polished using a polishing machine. On the other hand, roselle solution was prepared by extraction of dried roselle calyces and diluted to a concentration of 5%.

The polished specimens were soaked in roselle solution for 0, 15, 30, 45, or 60 min in an incubator at a temperature of 37°C, for which the specimens without soaking (0 min) was used as control. After soaking, the specimens were rinsed with aquadestilates and then tested in hardness. The hardness measurement was conducted using a Knoop hardness tester machine (Zwick/Roell ZHμ Microhardness Tester) with a load of 50 g and five indentation in 5 seconds for each indentation. The data were analyzed using repeated-measures analysis of variance (ANOVA) to determine whether there were significant differences in hardness values among groups that were exposed to different soaking times.

The Tukey post-hoc test was used to analyze the differences between the groups. Specimens that had been tested for hardness were then observed microscopically. Each specimen was cut longitudinally in 2x3 mm. They were then dried inside a vacuum tube and gold coated. Observation of the surface of bovine teeth was conducted using SEM (Zeiss, Germany).

3. Results and Discussion

The mean and standard deviation hardness values of bovine teeth enamel specimens without and with soaking in roselle extract solution. The highest value of bovine tooth hardness was 328.9±14.6 KHN, obtained from bovine teeth enamel specimens without soaking in roselle. After soaking in 0, 15, 30, 45, and 60 minutes, the specimens revealed with hardness values of 57.4±7.4, 22.9±7.4, 14.0±2.5, 11.0±1.5 KHN, respectively. The obtained data were tested for homogeneity, and the data variation was normal. Repeated-measures ANOVA indicated statistically significant differences ($p < 0.05$) among the enamel hardness values of all specimens. The Tukey post-hoc test found the significant differences between each hardness value. Results of the post hoc test showed significant differences ($p < 0.05$) between groups of soaking durations, as showed in Table 1.

Table 1. Significance differences of the enamel hardness between groups

| Soaking Duration | 15 minutes | 30 minutes | 45 minutes | 60 minutes |
|------------------|------------|------------|------------|------------|
| 0 minute         | **         | *          | *          | *          |
| 15 minutes       | -          | *          | *          | *          |
| 30 minutes       | -          | -          | *          | *          |
| 45 minutes       | -          | -          | -          | *          |

*p<0.05

SEM observations of the specimens without soaking and with soaking treatment for 15, 30, 45 and 60 minutes illustrated a change on the enamel’s surface. Figure 1(a) showed the bovine tooth surface without soaking treatment; here, the enamel prism was intact and parallel. Figure 1(b) showed the enamel’s surface after 15 minutes of soaking; the enamel prism was not parallel and some parts of the enamel were in continuity (black parts). Figure 1(c) showed the enamel’s surface after 30 minutes of soaking; the enamel was not intact and the black parts were wider. Figure 1(d) showed the enamel’s surface after 45 minutes of soaking; at this time, the enamel was scattered, and some parts of it fell apart, and Figure 1(e) showed the enamel’s surface after 60 minutes of soaking; the enamel surface was broken and diluted, leaving a rough surface, and micropores had formed.
Figure 1. Microstructure of the bovine tooth enamel specimens after (a) 0 min (without soaking), (b) 15 min, (c) 30 min, (d) 45 min and (e) after 60 min of soaking in roselle extract solution.

4. Discussion

This study observed significant differences ($p < 0.05$) in bovine tooth hardness between groups soaked in roselle extract solution in different time. When compared with human tooth enamel that has a hardness value of 343 KHN [8], the hardness value of enamel bovine 328.9 ± 4.6 KHN was close. This can be explained by the intact and parallel performance of the enamel prism.
The dropped of the enamel hardness of bovine tooth was caused by the acidity of the roselle extract solution. Of the components in rosella, citric acid has the greatest potential to cause erosion. As the roselle extract solution has a low pH of 2.791, soaking the specimens has causes a serial dissolution of the enamel structure. The enamel structure contains hydroxyapatite crystals, \( \text{[Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2] \), that dissociates into \( \text{Ca}^{2+} \), \( \text{(HPO}_4\text{)}^2- \), and \( \text{OH}^{-} \) ions that started when the oral cavity has a pH below the critical level pH of 5.5 [7]. This demineralization caused destruction of tooth structure that cause enamel’s prism leading to porosity [9,10]. After 60 min of soaking, the enamel’s prism demonstrated obvious changes of rough, discontinuity, broken, and microporous surface (see Figure 1) which illustrated the most effect in lowering the hardness value. Thorbjorget al. revealed that the erosion potential in beverages is influenced by pH due to the citric acid components [11]. This acid can bond directly with calcium, forming the complex molecule of citric calcium [12]. It was clear that the dissolution of the enamel surface caused a reduction in hardness value.

Bovine tooth enamel is characterized by larger crystal molecules and greater porosity, that cause the speed of water diffusion up to 2–3 times faster than that of human tooth enamel [13]. The greater the porosity, the more acid ions may have reached the deeper enamel layer and dilute the hydroxyapatite crystals. The oral cavity is a complex environment, and it is influenced by multiple factors. Further study, therefore, need to explore the influence of other low pH solutions on human teeth properties. Furthermore, hardness measurement should include nano-scale hardness testing to gain sensitive results and a more detailed view of the damaged enamel surface.

5. Conclusion
The roselle extract solution with a concentration up to 60 minutes decreased the hardness of bovine tooth enamel significantly. It was shown that there were different changes in the enamel prism structure of each immersion time.

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