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Mia Damiyanti,
Department of Dental Materials,
Faculty of Dentistry,
University of Indonesia,
Indonesia

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Effect of Mangosteen (*Garcinia mangostana*) Peel Solution on Human Enamel Surface Color

Mia Damiyanti, Andy Soufyan, E. Yosi Kusuma and S.U. Putu Ayu Ditta

In recent years, pericarp (peel) of *Garcinia mangostana* (known as mangosteen) has been used as a traditional medicine for the treatment of oral malodor, mouth apthae and chronic ulcer or as anti-inflammatory agent and antibacterial substances. This study was conducted to investigate the effect of immersion in pericarp of *G. mangostana* solution on tooth surface color. Fifteen premolar teeth were immersed in 1, 2 and 3% pericarp of *G. mangostana* solution (n = 5). Duration of immersion was 60 min (equal to 1 min day^{-1} gargling for 2 months), 120 min (equal to 4 month gargling) and 180 min (equal to 6-month gargling). The specimen's color were measured with CIEL*a*b* method (intraoral spectrophotometer, Vita Easyshade, USA). There were significant changes of lightness (L*) and redness (a*) of specimen after immersion in 1, 2 and 3% solution of *G. mangostana* pericarp for more than 60 min ($p>0.05$). The tooth color change (ΔE) was clinically perceptible and beyond clinical tolerance ($\Delta E>3.50$) in all immersion time. It was concluded that *G. mangostana* solution can affect tooth color and was clinically unacceptable. The highest color change was in group that immersed on 2% mangosteen solution for 180 min ($\Delta E = 20.35$), it was equal to gargling for 6 months/day/minutes.

Key words: *Garcinia mangostana* solution, color changes of enamel

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Department of Dental Materials, Faculty of Dentistry, University of Indonesia, Indonesia

INTRODUCTION

Garcinia mangostana is a tropical evergreen tree, also known as 'the queen of fruit', a fragrant fruit with a sweet tangy flavor. *Garcinia mangostana* is commonly known as Mangosteen or Manggis (in bahasa Indonesia). The mangosteen plant grows 7-12 m high and has a straight trunk and dark brown bark (Fig. 1). It is cultivated principally in Indonesia, Malaysia, the Philippines and Thailand (Chin and Kinghorn, 2008). Mangosteen fruit has a thick smooth pericarp (peel) and is dark purple in color (Fig. 2). It has 4-8 triangular flesh which is snowy white in color (Lanny, 2012). Genus *Garcinia* is a rich source of secondary metabolites, include the xanthones, flavonoids, benzophenones and phenolic acids (Mitra *et al*., 2007).

The pericarp of mangosteen has been used for a long time in Southeast Asia as a traditional medicine for skin infection, inflammation, wounds, dysentery and diarrhea (Morton, 1987; Pedraza-Chaverri *et al*., 2008). In recent years, consumption of *G. mangostana* increased since people have used *G. mangostana* as a traditional medicine solution for the treatment of oral malodor and chronic ulcer and as antibacterial substances. Pericarp of *G. mangostana* has an antioxidant property obtained from xanthone substance which is able to block free radical substance (Fu *et al*., 2012; Reanmongkol and Wattanapiromsakul, 2008). In comparison to other fruits, the antioxidant capability of *G. mangostana* is considered high oxygen radical absorbance (capacity/100 g). The fruit also contains folic acid, niacin and pantothenic acid which makes it acidic (Rassameemasmaung *et al*., 2007).

Garcinia mangostana as herbal medicine is usually prepared in a gargling solution which is used frequently. The solution is usually gargled for some times and then swallowed. As it is gargled for quite some times in the mouth, it interacts directly with the most outer part of teeth, i.e., enamel. Since the solution is acidic in nature, demineralization, the process where tooth mineral dissolves due to acidic environment, might occur when intraoral pH is not balanced which results in the destruction or dissolution of enamel layer (critical pH of enamel is 4.5-5.5).

Garcinia mangostana also contains tannin which is why it has been widely used as a natural colorant. The presence of the substance might also interact with tooth color since tooth color is an inherent color for which its perceptibility is greatly affected by enamel transparency, dentine color and the presence of stain between enamel and dentine layer (Nordbas, 1977; Nathoo, 1997).

Tooth color is determined by the combination of intrinsic effect (the inner color) and extrinsic effect (colorant agent in contact with teeth). Extrinsically, tooth color is related to absorption of certain substances, such as those from tea, red wine, chlorhexidine, etc., in enamel surface especially in pellicle layer which produces extrinsic stain on teeth. Extrinsic stain tends to form in tooth region difficult to clean during tooth brushing activity or by abrasive activity of toothpaste (Watts and Addy, 2001).

Fig. 1: *Garcinia mangostana* tree (7-12 m high)

Fig. 2: Mangosteen fruits, has 4-8 triangular flesh, white in color

Formation of stain is also greatly provoked by habit of smoking, high consumption of food with high concentration of tannin and frequent use of cationic agent like chlorhexidine. Quality and level of staining is affected by type, frequency, duration and quality of exposure of the causal substance.

Thus, the aim of this study was to investigate the effect of duration of immersion in pericarp of

G. mangostana solution on tooth surface color and later to be able to find the way to overcome the discoloration.

MATERIALS AND METHODS

Specimen preparation: All experimental protocols were approved by the Ethical Clearance Committee, Faculty of Dentistry University of Indonesia. Specimens were human premolars (n = 15). Based on Federer formula, each group consisted of 5 specimens. Specimens were firstly prepared by varnishing the apical foramens of teeth to prevent penetration of solution intrinsically from dentine tubuli. Each specimen were then placed in plastic pot and numbered according to its group.

Extract preparation: The fresh pericarps of *G. mangostana* Linn. were collected in February 2013 from Bogor, West Java. Extraction was performed in Balitro Laboratory, Bogor in the following steps. The pericarps were mashed and macerated with water solvent (ratio, 1:10) and mixed with stirrer for 3 h. The mixture was then settled for 24 h to separate dregs and filtrate. Dregs were macerated with water and mixed for an hour, then sieved with sieving paper to get another filtrate. The first and second filtrate was then mixed on rotavapor to evaporate the solvent to get thick extracts. Thick extracts were then freeze dried to get the extraction powder. Table 1 shows substance analysis on pericarp of mangosteen extracts, was acidic with pH of 5.28. But in this study, 1, 2 and 3% solution acidic was pH 5.6, pH5.5 and pH5.4. Tannin, shows relative high in *G. mangostana* extract (13.52%).

Garcinia mangostana extract was weighed according to the amount needed to make expected concentration in 75 mL aquadest; 0.75 g for 1%, 1.5 g for 2% and 2.25 g for 3%. Powder and solution were then mixed with magnetic stirrer (Nuovo, Thermolyne, Oral Biology Lab, Jakarta).

Specimen treatment: In this study specimens were divided into 3 groups which are as follows:

- **Group A (n = 5):** Immersion in 1% solution for 60 (mimicking 1 month use of the solution as mouth wash), 120 (2 month use) and 180 min (3 month use)
- **Group B (n = 5):** Immersion in 2% solution for 60, 120 and 180 min
- **Group C (n = 5):** Immersion in 3% solution for 60, 120 and 180 min

Table 1: Substance analysis on pericarp of mangosteen (*Garcinia mangostana*) extracts

Substance analysis	Result (%)	Method
Tannin	13.52	Spectrophotometer
N	1.19	Kjeldahl
P	0.18	Spectrophotometer
K	0.44	AAS
Mg	0.06	AAS
Ca	0.06	AAS
pH	5.28	pH meter

Color measurement: Specimen's color from all group were firstly measured using intraoral spectrophotometer (Vita Easyshade, USA). After each treatment specimen's color was also measured using the same device. The specimen's color was measured according to International Commission of I'Eclairge (CIE), known as CIEL*a*b* method (Powers and Sakaguchi, 2006; Volpato *et al*., 2010).

The L* value is a measure of the lightness of an object and is quantified on a scale such that a perfect black has an L* value of zero (0) and a perfect reflecting diffuser an L* value of (100) represent absolute white.

The a* value is a measure of redness (positive a*) or greenness (negative a*). The positive a* is tends to red and negative a*, tends to green.

The b* value is a measure of yellowness (positive b*) or blueness (negative b*) (Joiner, 2004). The positive b* indicates to yellow and negative b* tends to blue.

The color difference, defined ΔL^*, Δa^*, Δb^* are the differences between CIE L*a*b* color paremeter of two samples. Clinical color matching may be rated according to Δa^* values (the change of redness on enamel surface before and after immersion (O'Brien, 2002; Volpato *et al*., 2010).

Statistical analysis: Data was analyzed using *post hoc* Mann-whitney to see significant difference between different concentration groups. Significant differences between duration of immersion were analyzed using repeated ANOVA test ($p>0.05$).

RESULTS AND DISCUSSION

Effect of immersion in pericarp of mangosteens to Lightness (L*) value: Table 2 shows L* value (lightness) of enamel surface before and after immersion in 1, 2 and 3% *G. mangostana* solution for 60, 120 and 180 min. Decreased in lightness score (L*), indicates a color change toward darker color, though still considered white clinically since L* value was still between 78.58-81.97 (L* value of white is 100) (Table 2, 3).

Table 2: Lightness (L*) change after immersion human enamel surface in 1, 2 and 3% mangosteen solution

		Lightness before immersion			After immersion decrease in lightness		
Solution (%)	Control	L* 60'	L* 120'	L* 180'	ΔL* 60'	ΔL* 120'	ΔL* 180'
1	79.98	81.97	79.85	80.39	1.99	-0.13	-0.41
2	80.09	83.53	78.72	78.78	3.44	-1.37	-1.31
3	78.58	81.93	80.82	80.67	3.35	2.24	2.09

Table 3: Statistical analysis after immersion human enamel surface in 1, 2 and 3% *G. mangostana* solution

	Concentration (%)		
Time (min)	1	2	3
60	-	p = 0.017*	p = 0.000*
120	p = 0.00*	p = 0.00*	p = 0.00*
180	p = 0.00*	p = 0.00*	p = 0.00*

*Significant results

Table 4: Redness score (a*) after immersion human enamel surface in 1, 2 and 3% mangosteen solution

		Redness before immersion			After immersion change in redness		
Solution (%)	Control	a*60'	a*120'	a*180'	Δa* 60'	Δa* 120'	Δa* 180'
1	-0.13	1.64	9.83	11.31	1.77	9.96	11.43
2	-0.63	2.19	8.73	10.69	2.83	9.37	11.33
3	-0.22	2.33	8.26	9.40	2.55	8.48	9.62

Table 5: Statistical analysis (a*) after immersion human enamel surface in 1, 2 and 3% mangosteen solution

	Concentration (%)		
Time (min)	1	2	3
60	NS	p = 0.017*	p = 0.000*
120	p = 0.00*	p = 0.00*	p = 0.00*
180	p = 0.00*	p = 0.00*	p = 0.00*

*Significant results, NS: Not significant

Table 6: Yellowness score (+b*) after immersion human enamel surface in 1, 2 and 3% mangosteen solution

		Yellowness before immersion			After immersion change in yellowness		
Solution (%)	Control	b*60'	b*120'	b*180'	Δb*60'	Δb*120'	Δb*180'
1	27.07	29.29	40.19	40.75	2.21	13.12	13.68
2	24.70	31.89	38.95	41.56	7.19	14.25	16.86
3	24.60	31.31	38.30	39.40	6.71	13.70	14.80

In group immersed in 1% solution, lightness change happened after immersion for 120 and 180 min, whereas, in group immersed in 2 and 3% solution, significant lightness change occurred after 60, 120 and 180 min ($p<0.05$).

Figure 3 shows the highest lightness change also occurred to groups immersed with 2 and 3% solution after 60 min (Table 3).

Decrease in lightness was also found in a study by Nordbas (1977) who stated that immersion in tannic acid 0.2% caused brownish discoloration on tooth surface (Nordbas, 1977). In this study, *G. mangostana* solution was used that contains quite large concentration of tannin (13.52%) based on the analysis done by Balitro Laboratory.

Effect of immersion in pericarp of mangosteens to redness (a*) score: The a* values indicates that positive of a* (a*>0) indicates that there is a color change toward redness and negative of a* (a*<0) indicate the color change tends to greenness.

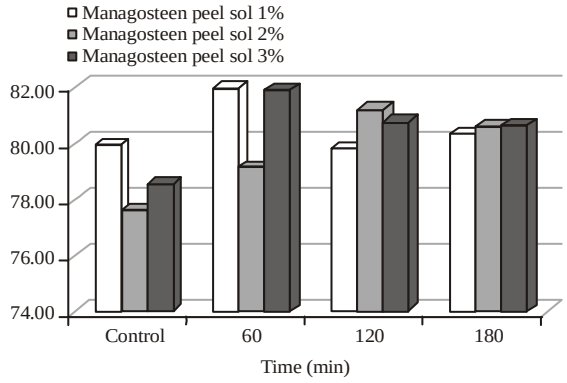

Fig. 3: Lightness (L*) after immersion of 1, 2 and 3% mangosteen solution

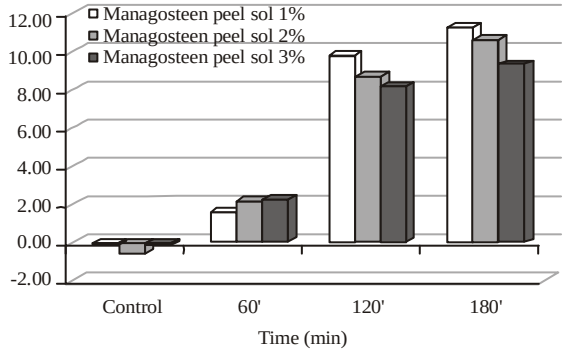

Fig. 4: Increased of Redness (a*) after immersion of 60, 120 and 180 min

Table 4 shows that control score has tends to green (- a*) but after immersed in mangosteen solution 1, 2 and 3% for 60, 120 and 180 min, the enamel surface color tends to red (+ a*).

From statistical analysis, immersion in 1% solution produced significant increase in a* value after 60, 120 and 180 min (p = 0.00) and between those immersed after 60 and 180 min (p = 0.00). Significant difference was also seen between groups immersed after 120 and 180 min (p = 0.00). Immersion in 2% mangosteen solution also shows significant change of surface enamel after 60 min was (p = 0.017), 120 min (p = 0.00) and 180 min (p = 0.00), as well as between 60, 120 and 180 min after immersion (p = 0.00). Immersion in 3% solution produced significant increase in a* value after 60 min immersion (p = 0.002), 120 min (p = 0.00) and 180 min (p = 0.00). Score after 60, 120 and 180 min immersion also seems to be significant (p = 0.00) (Table 5, 6).

The highest increase of redness (a*) is immersion of 1% solution after 180 min and immersion of 2% after 180 min (Fig. 4).

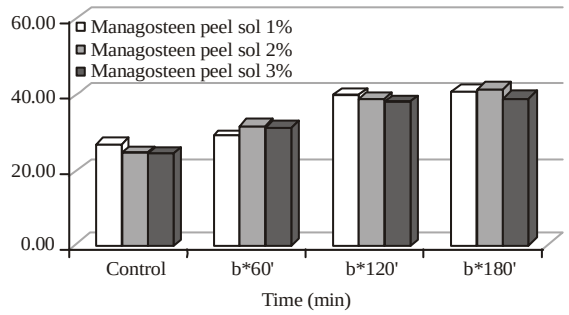

Fig. 5: Increased of yellowness (b*) after Immersion of 60, 120 and 180 min

Table 7: Statistical analysis (b*) after immersion human enamel surface in 1, 2 and 3% mangosteen solution

	Concentration (%)		
Time (min)	1	2	3
60	NS	p = 0.017*	p = 0.02*
120	p = 0.00*	p = 0.00*	p = 0.00*
180	p = 0.00*	p = 0.00*	p = 0.00*

*Significant results, NS: Not significant

Table 8: ΔE score after 60, 120 and 180 min immersion in *G. mangostana* solution

Concentration (%)	ΔE* 60'	ΔE* 120'	ΔE* 180'
1	3.46	16.47	17.83
2	8.46	17.10	20.35
3	7.92	16.27	17.77

Effect of immersion to yellowness score (b*): Table 6 shows b* value before and after immersion in 1, 2 and 3% *G. mangostana* solution for 60, 120 and 180 min.

Figure 5 shows that the b* values is measure of positive b* (indicates that there is a color change toward yellowness).

In Table 7, significant analysis shows that immersion in 1% solution produced significant increase in yellowness score (b*) before treatment and after 120 (p = 0.00) and after 180 min (p = 0.00). Immersion in 2% solution produced significant increase in b* value were found between before treatment and after 60 (p = 0.017), 120 (p = 0.00) and 180 min immersion (p = 0.00). Immersion in 3% solution also produced significant increase in b* value between before treatment and after 60 (p = 0.002), 120 (p = 0.00) and 180 min immersion (p = 0.00).

Differences between L* and a*, b* (ΔE Score): The ΔE is a single value which takes into account the differences between the L* and a*.

Table 8 shows the color difference (ΔE*), defined as:

$$\Delta E^* = [(\Delta L^*]^2+(\Delta a^*)^2+(\Delta b^*)]^2 1/2$$

The ΔE* can serve as a tolerance for color matching. According to classification of American Dental Association, color change (ΔE*) that is clinically acceptable is below 3.5 (Joiner, 2004; O'Brien, 2002).

This study showed that except group 1% solution for 60 min (ΔE* = 3.46), immersion in the solution for 60 min or more produced clinically unacceptable color difference (ΔE>3.50) since ΔE score should be below 3.50 to be considered not perceptible and thus clinically acceptable. The highest color difference was produced by group 2% solution immersed for 180 min or equal to use in 6 months (ΔE = 20.35).

Thus, color change occurring in group immersed in 1% solution for 60 min is still acceptable. However, if concentration of solution and the duration of immersion (or gargling) were increased, undesirable color change would be expected. A study previously done also indicated the same result (Watts and Addy, 2001).

Another study suggested that color intensity from material containing tannin increased along with increase of duration of immersion and temperature (Nordbas, 1977).

This study showed that immersing enamel surface in mangosteen solution for 60 min or more produced clinically unacceptable color difference (ΔE>3.50). The highest color difference was produced by group 2% immersing in mangosteen solution for 180' (ΔE = 20.35) equal to use in 6 months.

Quality and level of staining is affected by type, frequency, duration and quality of exposure of the causal substance. Since the solution is acidic in nature (5.6 for 1%; 5.5 for 2% and 5.4 for 3%), demineralization, the process where tooth mineral dissolves due to acidic environment, might occur when intraoral pH is not balanced which results in the destruction or dissolution of enamel layer (critical pH of enamel is 4.5-5.5). In condition below critical pH, chemical bond of hydroxyl apatite on tooth surface and thus enamel will dissolve and become porous. This condition enable deposition of tannin from the solution on enamel surface and thus produce more reddish tooth surface.

Extrinsic stain tends to form in tooth region difficult to clean during tooth brushing activity or by abrasive activity of toothpaste which is also greatly by high consumption of food with high concentration of tannin.

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