

Remineralization Evaluation of Cocos Nucifera Extracts via Element Quantification

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Abstract–Demineralization causes plaque calcium (Ca) to dissolve out from the enamel. Prolonged condition would allow enamel caries to worsen. To reverse the condition via remineralization, abundant of free available Ca and phosphorus (P) in the saliva are required. Thus, daily diet with high Ca and P content are important to ensure the continuous supply of Ca and P. Being one of Malaysia’s major component of medical applications and daily consumptions, virgin coconut oil (VCO), coconut milk (CM), and coconut water (CW) are yet to be discovered their mineral content especially Ca and P. Hence, this study aims to evaluate the Ca and P content in VCO, CM, and CW. Inductive coupled plasma-mass spectrometry (ICP-MS) was used in analyzing the Ca and P content in VCO, CM, and CW. After acid digestion (Method 3051a) using Teflon vessels under 170°C, the samples were diluted and the readings were compared. The Ca concentration in descending order VCO (0.47±0.15), CW (0.45±0.05) and CM (0.44±0.15), while P concentration in descending order is as follows CM (0.25±0.05), CW (0.09±0.05), and VCO (0.05±0.06). With no huge difference in Ca content, CM is considered to exhibit a high Ca and P content in total. In conclusion, CM exhibits a promising remineralization potential on enamel caries with high Ca and P content.

Keywords–*demineralization, remineralization, calcium, VCO, coconut milk*

I. INTRODUCTION

Coconut or *Cocos nucifera* is one of the most important crops worldwide and familiarly known as the “tree of life”. It is known as such because of its wide-ranging and all-inclusive products that supports many communities from different countries. Coconut is an ancient plant which existed since pre-historic times, spreading from Southeast Asia, eastwards to central Pacific, westwards to coastal India, East Africa and the Indian Ocean and possibly native to the Pacific coast of Central America [1]. As the “tree of life”, every part of the tree is useful for productivity and resources, starting from its roots up to its fruits. It is considered as an important survival element in many parts of the world

which it provides food, shelter and also transportation [2,3]. However, most of the tropical communities sought goods from the coconut fruit. Its fruit alone can be used to extract many resourceful needs such as oil, water, cream, including the coconut husk itself [3,4]. In many countries especially in Southeast Asia community, many of them extract their own oil from the coconut copra and use it as cooking oil. Apart from that, the coconut oil also used as hair oil therapy, skin care and prevent diseases [2]. Besides its major application in foods and food preparation, coconut fruits hold plenty of medicinal properties particularly coconut oil and coconut water [1].

The recently emerging virgin coconut oil (VCO) has certainly took the stage. The VCO is widely known for its antioxidant, antimicrobial, and antifungal properties. Its high concentration of medium-chain fatty acids (MCFA), lauric acid, and polyphenols enhances its medicinal properties [5-7]. Consequently, VCO was found to reduce low density lipoprotein (LDL) cholesterol, cardiovascular diseases, and inhibiting various pathogenic bacteria [8-10]. Previous study demonstrated that dietary VCO is able to reduce the risk of coronary heart disease. It was done so by VCO modulating the synthesis and degrading fatty acids by down-regulating the mRNA expression and transcription [11]. Coconut water (CW) on the other hand, has been practically used in medicine since the earliest of time. In Peninsular Malaysia, CW was consumed orally to treat fever whilst applied externally for hemorrhoids, bone ulcerations and ulcerative colitis in Java [1]. Although CW is commonly used to reduce dehydration and its cooling effect, it is also proven to potentially act as anti-cancer and antioxidant prior its ascorbic acid and cytokinin content [12]. Previous study by Anurag & Rajamohan (2003) [13] exhibited positive protection against induction of myocardial infarction and reduced total cholesterol concentration. This impact might be due to the presence of potassium, calcium, magnesium, and L-arginine. Unlike coconut oil, VCO,

and CW, coconut milk (CM) is less used in medicinal practice. Traditionally, CM was used as a vehicle for Malay medicaments which is applied internally and externally [1]. Later, a study was conducted on male Wistar albino rats to investigate the effect of coconut extracts on ulcer. The result showed that CM displayed better antiulcerogenic effects than CW, even similar effects of sucralfate [14]. However, it is not known the active compound contained in CM that imposed such effect.

Nevertheless, the potential of coconut extracts in supporting oral health has come into spotlight when Indians practice of oil pulling was studied and later studies on storage of avulsed teeth [15]. Oil pulling is a practice originating from India which is adapted from Ayurveda medicinal system [16]. It requires an individual to use oil as mouth rinse for 3-20 minutes [17,18]. Studies have been done on oil pulling and the results showed proves that the method is able to prevent many oral conditions by combating microbial growth in the mouth environment. In a study conducted [18], VCO used in oil pulling had reduced 22.79% colony growth of *Streptococcus mutans* (*S. mutans*) while chlorohexidine showed 25.72% reduction of colonial growth. Among few studies conducted on coconut oil, sunflower oil, sesame oil, and other edible oils against chlorhexidine, results showed that oil pulling is able to reduce halitosis and reduce the bacteria count which causes it to drop to a significant amount, reducing gingivitis and plaque as effectively as chlorhexidine [18-21]. Furthermore, oil pulling has advantages over chlorhexidine that oil pulling does not leaves staining, no lingering aftertaste, and most importantly no allergy [19]. Although coconut oil exhibited a significant reduction in *S. mutans* count after oil pulling [18,21], the coconut oil needs to be pre-digested before consumption to make it more effective [19]. Against oral microflora, oil pulling is found to be effective due to the abundant presence of MCFA in coconut oil and VCO. In a different study, Gopikrishna et al., (2008) [15] demonstrated that CW maintains periodontal ligament cell of avulsed teeth more viable than Hank's balanced salt solution (HBSS) or milk. Later he compared the efficacy of CW with HBSS, milk, and propolis. Similar result obtained indicating CW is more superior to propolis, HBSS, and milk in maintaining periodontal ligament cells of avulsed teeth [22]. Accordingly, he concluded that the electrolyte in CW helps in storing avulsed teeth, resembling intracellular fluid more than extracellular plasma. The solution is a hypotonic solution containing predominant cations of potassium, calcium, and magnesium while sodium, chloride, and phosphate are found in much lower concentration.

Study showed that there are significant amount calcium and phosphorus in CW and CM which mainly consists of 24 mg calcium (Ca), 20 mg phosphorus (P) and 16 mg Ca, 100 mg P respectively [1]. Component concentration in VCO usually focusing on its fatty acids

and protein content, thus little knowledge is known on Ca and P concentrations in VCO.

Remineralization is as an effective way to combat dental enamel caries by restoring the crystals structure. In fact, it is a natural oral mechanism in maintaining the absorption and released of dental minerals into and out of the dental enamel and saliva. Naturally, the saturation of the saliva is maintained by the released of Ca ions and P ions from enamel crystals into saliva. With lower saturation of saliva, more Ca and P ions will be released to ensure the saturation of saliva and enamel is in equilibrium. In consequence with other caries challenge such as xerostomia, low salivary flow rate and continuous low pH in oral environment, the possibility to reverse the effect and inducing remineralization is low. Hence, this event if allowed prolonged, will cause enamel to lose its structure through demineralization and consequently forming caries. Up until now, fluoride, caseins phosphopeptide-amorphous calcium phosphate (CPP-ACP), and xylitol are the effective anticaries substance and promote remineralization. However, in order for each of the anticariogenic agent to perform effectively, free Ca and P ions are also required to improve remineralization process and exhibiting best results. Thus, diet sources with high Ca and P concentration are the best option for cariogenic individuals to consume.

The aim of this study is to evaluate the presence of Ca and P in CW, CM and VCO to determine their potential in remineralization of enamel subsurface.

II. MATERIALS AND METHODS

A. Sample preparation

The coconut samples were mature coconut of kelapa MAWA and green coconut of kelapa pandan, were supplied by MARDI, Sungai Baging, Kuantan, Pahang, Malaysia. Mature coconut was used to extract coconut cream and the VCO while CW was extracted from the green coconut. The mature coconut was processed in a local store to extract the coconut cream. The CW was stored in -20°C until further use.

B. Analytes preparation procedure

High purity deionized water (18.2 mΩ cm⁻¹) was used in preparing all chemicals reagents and sample preparations obtained from a Milli-Q water purification system. All glassware was cleaned and then was soaked overnight with 2% (v v⁻¹) nitric acid (HNO₃, 65% w w⁻¹) for at least 24h. Then, they were rinsed and let dry before use.

For determination of elemental concentration of Ca and P, method digestion was referred to Method 3051A for CM and VCO, whilst for CW digestion method was prepared as [25]. For CW, an aliquot of 2 mL CW was transferred into Teflon vessels and 2 mL of 30% hydrogen peroxide (H₂O₂) and 6 mL of 1M nitric acid were added into the vessels. Then, it was digested for 3h at 140°C of microwave oven. For each sample of CM and VCO, 0.5 mL aliquots were inserted into Teflon vessels and 3.0 ml of concentrated nitric acid

was added to each vessel. The vessels were then placed into microwave oven to be digested at 170°C for 4h. After obtaining a completely clear and colorless digests, all digests were then diluted with deionized water to 50.0 mL. After the digestion procedure is completed, the analytes were determined by ICP-MS. The samples were intended to be analyzed as such, hence no pre-treatment performed on samples as to retain their properties.

C. Element quantification

Inductively coupled plasma-mass spectrometry (ELAN 9000, Perkin Elmer) was used to determine the micronutrient minerals. The instrumental setting details for the elemental analysis are as in Table I. A combination of high-purity analytical stock solutions (Perkin Elmer) with concentrations of 100, 200, 500, and 1000 ppb were used as the multi-element reference analytical solutions. The mixture was also used in preparing calibration curves of Ca and P before the elemental analysis.

TABLE I. INSTRUMENTAL SETTING FOR (ELAN 9000, PERKIN ELMER) ICP-MS BASED ANALYSIS OF CA AND P FOR CW, CM AND VCO

ELAN 9000, Perkin Elmer	
Parameters for the analysis of Ca and P of CW, CM, and VCO	
RF power	: 1135 W
Nebulizer gas flow	: 1.01 L/min
Dual detector mode	: Pulse
Acp. dead time	: 55 ns
Current dead time	: 55 ns
Lens voltage	: 6.50
Analog stage voltage	: -1550
Pulse stage voltage	: 900
Discriminator threshold	: 90
AC rod offset	: -3

III. RESULTS

A. Elemental quantification

Figure 1 shows the concentration of Ca and P found in VCO, CM, and CW respectively which directly acquired from ICP-MS. The result shows that VCO contains the highest Ca concentration with 0.47 mg/mL while CM contains the least Ca concentration with 0.44 mg/mL. For P concentration, CM contains the highest amount with 0.25 mg/mL and VCO contains the least P concentration with 0.05 mg/mL. However, there is only slight difference of Ca and P concentration between the samples. Cumulatively, CM contains considerably high concentration of Ca and P compared to VCO and CW. Referring to the calibration curves of the ICP-MS, provided it is the determination factor of the elemental quantification, low detection of P is expected. With limited study conducted on macro element composition of coconut especially on VCO, below results are the first elemental quantification done on VCO focusing on calcium and phosphorus content. The assumption for VCO to contain Ca and P is based on the finding reported by Yalegama *et al.*, (2013) [26] which reported 8.2±0.2 by percentage ash constituents of VCO residue. Table II suggests the range of Ca and P concentration in

CM and CW as demonstrated by previous studies. In comparison, the finding of previous studies supports the result, which the concentration of Ca and P in current study are in range of suggested minerals content enlisted in Table II.

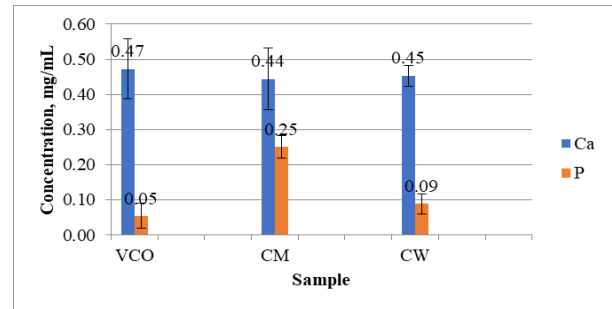


Figure 1. Concentration of Ca and P in VCO, CM, and CW with ± standard error means (n=3).

TABLE II. CA AND P CONTENT IN CM AND CW

Component	Ca (mg/mL)	P (mg/mL)	Reference
Young coconut water	0.24	0.20	[27]
Young coconut water	0.27	0.05	[28]
Coconut water	119-258	80-432	[29]
Coconut skim milk	1.70	5.00	[30]
Coconut milk	0.0058-0.131	0.026-0.341	[31]
Coconut milk	26.00	36.00	[5]
Coconut milk	94.00	21.40	[32]

B. Total content in VCO, CM, and CW against milk and dairy products

Descriptive statistics of the mean concentration element of Ca and P in VCO, CM, and CW are as reported in Table III as directly determined by ICP-MS and are compared to breast milk, milk, cheese, dairy products, and goat, sheep, and cow milk. The Ca and P concentration in every sample are found promising as they are only compared to blank. Total mineral content (v/v) was calculated using following formula in Figure 2.

$$\text{Total mineral content} = \frac{[(a - b) \times \text{sample volume} \times \text{DF}]}{\text{sample dry weight}}$$

Figure 2. Total mineral content (v/v) formula

Given *a* is ICP-MS reading of sample, *b* be the ICP-MS reading of blank, and DF the dilution factor. From the tabulated data, hard cheese contains the highest Ca and P concentration (mg/mL) with concentration range of 12.0-7.20 and 8.10-4.90 respectively. If arrange from highest to least concentration, hard cheese will be the highest, followed by dairy products, sheep milk, goat milk, cow milk, pasteurized skim milk, UHT milk, VCO, CM, CW, and followed by the least Ca and P concentration content, breast milk. Although breast milk recorded as the least Ca and P concentration, its Ca:P ratio is high which ranged from 6.0 to 0.8 while VCO with 3.25 ratio, CW with 2.52 and CM with the least ratio of 1.29. The data was used as benchmark for the assessment of VCO, CM, and CW in determining

their possibility as remineralization instrument for enamel caries.

TABLE III. DIFFERENT SALIVARY CHARACTERISTIC IN SECC AND CARIES-FREE CHILDREN

Salivary characteristic	SECC	Caries-free	P
Flow rate (ml/min)	0,37 ± 0,12	0.80 ± 0.21	< 0.001 ^d
Salivary pH	5,86 ± 0,51	7.02 ± 0.39	< 0.001 ^d
Buffer Saliva	4,93 ± 1,38	9.30 ± 1.23	< 0.001 ^d

^a. Unpaired t-test

IV. DISCUSSION

Milk and its dairy products are used as benchmark in determining the Ca and P concentration in VCO, CM, and CW for remineralization assessment is due to their high Ca and P content. Furthermore, environmental factors or constitutional factors do not consistently affect the milk quality in terms of Ca and P concentration [33]. Thus, the yield and quality of breast milk is sustained and consistent among lactating women. Milk yield and quality of cow, sheep, and goat milk on the other hand are vastly affected by their environmental factors [34].

According to Hagenmaier et al., (1974) [30], amount of minerals content in food sources dependent on its amount of ash that higher ash content determines a high mineral content. The quality of yield and organic component in plants differs in every region and hence the wide range of minerals concentration in different coconut components [34-36]. This may due to the factors that affect the yield quality of plants or the post-harvest quality such as the soil quality, influence of irrigation, temperature, altitude and sunlight exposure [34,37]. The elemental component in CM and CW are closely in range according to few studies as in Table IV. A range of minimum to maximum concentration of Ca and P in CW and CM can be concluded from the findings collected from previous studies that are 0.24–258 mg/mL Ca and 0.2–432 mg/mL P in CW and 0.0058–94 mg/mL Ca and 0.026–36 mg/mL P in CM. Previous studies support the findings of current study which falls within the ranges of concentration. The results implying VCO, CM, and CW as promising food sources in supplying free Ca and P ions into oral environment. With sufficient concentration of Ca and P in every samples, all samples have the potential to remineralize enamel caries with CM being the most likelihood in performing remineralization with its ample concentration of both Ca and P. Albeit low, the concentration of Ca and P in respective samples are considered adequate in comparison with breast milk. According to Greer (1989) [38] focusing in infant's formula milk, he suggested that the limit for the minerals should remain at the present concentrations: 45-50 mg/dl (65-75 mg/100 kcal) for Ca, and 30-40 mg/dl (48-58 mg/100 kcal) for P. This further supports the hypothesis for VCO, CM, and CW positively able to supply sufficient Ca and P in daily intake and hence increasing its probability for remineralization.

In promoting remineralization, high supply of Ca and P are vital to ensure remineralization process to

take place. In a caries challenge condition, dental plaque will release its Ca and P ions into its immediate environment to increase salivary saturation to reach equilibrium, thus inducing instant demineralization of enamel. The loss of Ca and P from plaque into the saliva, if not reversed, invites more harm to the enamel in cariogenic attack condition hence causing dental caries. One of the methods to reduce demineralization is through high supplement of Ca and P. The free Ca and P ions if presence, will bind to plaque binding site and hence reversing the demineralization process and remineralize the enamel simultaneously [39]. When leaving the binding sites empty, the abundant presence of Ca and P ions would probably compete for the binding sites. Provided that the Ca:P ratio of VCO, CM, and CW are in adequate amount in comparison to breast milk, VCO, CM, and CW could be another sources for Ca and P supply. This was supported by Flynn (1992) [40] that stated the Ca:P ratio suitable for artificial infant formulas was as close to human milk which was approximately 2.2:1.

For a xerostomia individual which has low salivary rate and high probability of low resting pH and buffering capacity, remineralization is especially hard. For this type of individual, best possible treatment to reduce the effect is through increasing hydration or to stimulates salivary production and exercises calcium phosphate therapy [41]. Many have suggested to increase consumption of milk and its dairy products in combating xerostomia and dental caries. This is so because milk and dairy products have high concentration of Ca and P ions in spite high content of CPP-ACP. It has been demonstrated in previous study that cheese able to reduce demineralization effect of xerostamic patient and help remineralization of enamel by releasing its Ca and P ions [42]. However, excess uptake of Ca and P daily would cause reverse effect to consumers health.

Excess calcium intake might cause damage to the kidneys and impairment in the absorption of magnesium, iron and zinc [43] while excess in phosphorus will cause bone resorption which with high serum phosphate concentration reducing serum-ionized calcium that important in bone formation [44]. Despite that, it was recommended to consume daily dosage of 1000 mg of Ca and P plant minerals respectively [45,46]. Presence of minerals do not guarantee the bodily uptake, this depends on the bioavailability of the minerals and the absorption rate and percentage.

The concentration of Ca and P in VCO, CM, and CW are comparable as to Ca and P concentration in breast milk. Theoretically, current study suggesting that VCO, CM, and CW are highly capable in supplying free Ca and P ions into oral environment and hence, assisting in remineralization process. More studies need to be conducted to validate the theory and also to understand further the mechanism of action holds by VCO, CM, and CW in assisting remineralization.

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