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Original Article

EVALUATION OF SALIVA PARAMETERS AND CALCIUM CONCENTRATION ON ALMOND (*PRUNUS DULCIS*) STIMULATED SALIVA

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ABSTRACT

Objective: The pivotal role of saliva in maintaining oral environment is accomplished through regulation of its flow rate, Potential of Hydrogen (pH), and secretion of minerals, including calcium. The great number of calciums contained in Almond is beneficial for preserving oral health. The current study aimed to assess the effect of almond intake on salivary parameters and its calcium levels from individuals with and without caries.

Methods: The study was an experimental design with pretest and posttest. The subjects were divided into two categories, control (wax gum pieces) and treatment (almond) group, respectively. Sample was isolated for both unstimulated and stimulated saliva. The flow rate, pH, buffer capacity and calcium levels were determined. The data analysis were performed using a Statistical Package for the Social Sciences (SPSS) software and a t-test was selected to evaluate the statistical difference.

Results: Almonds significantly increased salivary flow rate, pH, buffer value and calcium ion levels ($p<0.05$). In addition, the greater change of pH, buffer capacity, calcium ions, excluding pH value were noticed in almond stimulation compared to wax gums ($p<0.05$). No significant difference of saliva flow, pH and buffers between caries and non-caries individuals was observed, but saliva from free decay teeth showed more calciums secretion than the decay teeth.

Conclusion: Almonds promoted the raise of salivary flow rate, pH, buffer capacity and calcium ions both in the healthy and caries subjects.

Keywords: *Prunus dulcis*, Saliva, Flow rate, Buffer capacity, Calcium

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INTRODUCTION

Saliva is a primary regulator in preserving oral homeostasis by protecting it from pathogenic bacteria and neutralizing acidic conditions [1]. It consists primarily of water mixed with small amounts of organic and inorganic components, which have distinct role to keep a balance in oral cavity [2, 3]. This oral fluid is secreted by two salivary glands, the major and minor glands [4, 5]. The fundamentals of saliva include the process of digestion for taste, preliminary food dissolution, mastication, ingestion, debris cleanser and even as antibacterial agent to protect teeth from diseases [6]. The salivary flow rate, pH, buffering ability, and inorganic elements that belong to saliva parameters may regulate the demineralization and remineralization processes. The rate of demineralization is affected by salivary pH. When the pH lower 5.5 remains in oral cavity, the crystals of hydroxyapatite are subsequently dissolved, accelerating the rate of demineralization [7]. However, the constant pH of saliva at normal levels can be attributed to a buffer capacity mechanism that promotes remineralization [8]. A persistently low pH resulting from a lower buffer capacity fails to neutralize plaque acids and hinders tooth remineralization, thus increasing the incidence of caries [9]. Salivary pH is higher in individuals with no caries or the Decayed, Missing, and Filled Teeth (DMFT) score less than 5 than in those with more than 5 [10-12]. The DMFT index is a key indicator for assessing the oral health status, which determines the number of decayed teeth requiring treatment and/or tooth loss due to caries [13, 14].

The flow rate is the primary factor that influences the composition of saliva. Raising saliva secretion results in an increase in pH and the quantity of particular components, including bicarbonate, protein, sodium, and chloride [15]. Hence, a decline in salivary flow lessens the protective elements in saliva, leading to a decrease in pH and accelerating demineralization, which ultimately fosters the formation of cavities on the tooth surface [12]. A previous study reported that the mean salivary flow rate in caries subjects was lower than that in caries-free individuals [12, 16], indicating the significance of saliva in caries development.

The presence of inorganic constituents, including calcium, plays a notable part in the oral environment in preventing caries by maintaining a balance between demineralization and remineralization [17-19]. Nutritional deficiencies, especially in calcium, phosphorus, and vitamin D, may lead to higher risk of caries occurrence [20]. Greater amounts of calcium and phosphate present in the saliva likely diminish the attachment of bacteria to the enamel and impede the growth of biofilms. An additional intake of calcium may improve the process of remineralization, mitigate demineralization, and inhibit alveolar bone destruction [21]. Therefore, this mineral is indispensable for oral equilibrium to suppress the initiation of caries.

Calcium intake is commonly associated with dairy products such as yogurt and cheese. The elevated salivary calcium was reported in the subjects who consumed the cheese [22]. In addition to dairy-based foods, nuts are a good source of calcium. Almonds are nuts that contain various vitamins and minerals, including calciums, proteins, dietary fibers and sugars. The almonds possess a variety of benefits for the body. The long-term intake of almonds not only induces the growth of favorable microbes in the gut, but also decrease the number of pathogens [23]. Several studies reported the advantageous influences of almonds on glycemic control [24], regulation of vascular function [25], and antioxidant property [26]. Calcium-rich diets increase the calcium ion levels in saliva, which benefit to prevent dental caries [27]. However, the influence of almonds on saliva indicators has not been clearly elucidated. Therefore, this study was to evaluate the effect of consuming almonds (*Prunus dulcis*) on salivary pH, flow rate, buffer capacity, and calcium levels.

MATERIALS AND METHODS

Study design and participants

This study was conducted using a pretest-posttest control group design to assess the effect of almond consumption on flow rate, pH, buffer capacity, and salivary calcium ion levels. Ethical approval was

obtained from the Health Research Committee of Universitas Sumatera Utara (no. 425/KEPK/USU/2023) on 31st August 2023, and informed consent was obtained from all study subjects. Thirty-two students from Universitas Sumatera Utara who met the inclusion criteria for caries and caries-free groups were selected. The participants were then divided into two groups in each category (healthy and caries subjects): the treatment group (almond) and the control group (wax gum pieces). The subjects met the inclusion criteria such as aged–18-24 y, physical and psychological health, DMF-T score 0 for caries-free, and a score above 5 for caries. In addition, the exclusion criteria were patients with gingivitis or periodontitis, systemic diseases, use of orthodontic appliances, radiotherapy, active smoking or alcohol, drug therapy, and allergy to nuts.

Salivary parameters measurement

An intraoral examination was performed to determine the DMFT scores for individuals. The DMFT index was based on the World Health Organization (WHO) criteria [28]. Subjects were restricted to eating, drinking, brushing teeth, or performing physical activities 60 min before the experiment. They were then instructed to rinse their mouths with water to remove any debris. Saliva was isolated before and 5 min after treatment. The subjects were requested to swallow 15 g of almonds following a 3 min chewing process, while the subjects in the control group (wax gum) were required to chew the wax for 3 min, then spitting it out. Stimulated saliva was obtained 5 min after consuming the almonds or chewing the wax gum. Saliva was stored at-20 °C until further assessment.

Salivary flow was measured using a digital scale. The collected saliva was weighed and subtracted from the weights of the saliva tubes. The saliva volume was divided by the length of time and expressed in ml/min units. The pH was evaluated using a digital pH meter and the buffer capacity was determined using a Saliva-Check Buffer kit (GC Check Buffer). The QuantiChrom Calcium Assay Kit (DICA-500) was used to quantify salivary calcium ion levels [29, 30]. The standard solution was diluted, and 5 µl** of the standard solution and diluted saliva sample were added to 96-well plates. Well plates containing a mixture of standard solutions and saliva samples were incubated for 3 min at room temperature. The optical density was measured using a spectrophotometer at a wavelength of 570-650 nm (peak absorbance at 612 nm). The sensitivity range was 0.08 mg/dl to 20 mg/dl with a detection limit of 0.08 mg/dl.

Statistical analysis

Data are presented as mean±SD. The analysis was performed using a statistical software program (IBM SPSS Statistics V22.0), and a t-test was used to compare the differences before and after treatment and between the two groups. Statistical significance was set at p-value<0.05.

RESULTS AND DISCUSSION

Saliva parameters, including flow rate, pH, buffer capacity and calcium levels measured before and after almond intake and or wax gums stimulation is presented in table 1 and 2. The current study demonstrated a significant increase of all indicators on the almond-stimulated saliva from the two groups, yet the wax gums only showed the significant rise from some of them. Gustatory stimulation or masticatory actions can contribute to saliva production [6]. The almond groups simultaneously applied the two stimuli which effectively elevating saliva volume. This result is corresponding to other study reported individuals who consumed more nuts had significantly higher salivary pH levels than those who had fewer nuts [31]. Previous study exhibited the feeding of date fruits as meal significantly promoted the flow rate of saliva as a result of its taste and masticatory stimuli and had no significant effect in reducing pH [32, 33]. Although the salivary flow was significantly greater in the wax gum-stimulated saliva, but it did not raise the level of pH. However, the mechanical stimulus from chewing directly triggered saliva, which elicited a simple salivary reflex [34]. The upregulation in salivary pH after almond stimulation is consistent with the increase in salivary flow, as a result of its function to maintain buffer capacity. A buffer is a solution that regulates pH levels [8]. Salivary buffers preserve the pH value by keeping acids and bases neutral, so that the pH returns to normal level. A high rate of saliva secretion increases the capacity of the buffer, thereby enhancing the pH of saliva [35]. The flow rate modulates the salivary Na+/HCO3 ratio, thus eventually enhancing the buffer capability [8].

Table 1: Evaluation of salivary flow rate, pH, Buffer capacity, and calcium ions level before and after stimulation in healthy subjects

Evaluation of salivary	Almond			Wax Gums		
	Before	After	p value	Before	After	p value
	*Mean±*SD	*Mean±*SD		*Mean±*SD	*Mean±*SD	
Flow Rate (ml/min)	0.39±0.07	0.6±0.07	0.003*	0.39±0.05	0.82±0.07	0.000*
pH	6.19±0.16	6.63±0.14	0.012*	6.09±0.16	6.05±0.15	0.505
Buffer Capacity	2.00±0.81	7.10±2.02	0.005*	1.90±0.99	3.30±1.25	0.010*
Calcium Ion Level (mg/dl)	4.25±0.27	7.11±0.31	0.000*	3.53±0.5	3.95±0.49	0.030*

*Significant (p<0.05), SD (standard deviation)

Table 2: Evaluation of salivary flow rate, pH, buffer capacity, and calcium ions level before and after stimulation in caries subjects

Evaluation of salivary	Almond			Wax Gums		
	Before	After	p value	Before	After	p value
	*Mean±*SD	*Mean±*SD		*Mean±*SD	*Mean±*SD	
Flow Rate (ml/min)	0.22±0.06	0.39±0.06	0.000*	0.24±0.03	0.54±0.1	0.012*
pH	5.73±0.15	6.27±0.2	0.000*	5.8±0.15	5.74±0.24	0.176
Buffer Capacity	1.30±0.67	6.20±2.2	0.005*	1.50±0.52	2.0±0.93	0.059
Calcium Ion Level (mg/dl)	2.14±0.72	3.75±0.69	0.001*	2.08±0.46	2.59±0.49	0.007*

*Significant (p<0.05), SD (standard deviation)

Furthermore, we evaluated the alteration of these parameters between the almond and wax gum groups. The mean difference was determined by subtracting the baseline values from the post-treatment values. Negative symbol indicates an increase of the value after stimulation, while the positive one denotes a reduction. As illustrated in table 3, the mean difference of almond induced an increase in salivary pH, buffer, and calcium ions was significantly higher than in the control, both caries and non-caries individuals. Almond-stimulated saliva had less increase of secretion compared to wax gums. The greater increase of salivary flow rate in wax gums is as a result of the continuous chewing activity. Chewing sugar-free gum for 3 to 6 min significantly enhanced saliva secretion [36, 37]. Interestingly, the alteration of salivary pH, buffer and calcium ions in almond almond-stimulated group was significantly greater than the

control group. The possible mechanism of this phenomenon is the presence of minerals content found in almonds. Almonds are abundant in nutrients as calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and other components [38, 39]. Calciums and phosphates are the principal elements made up hydroxyapatite crystals [40] and their amount serves as critical in sustaining salivary supersaturation for hydroxyapatite [6]. Despite the fact that the bicarbonate, pH, and buffer capacity enhance alongside salivary flow rate [6], yet the current study showed some contributing factors like the amount of nutrients or taste of the foods may regulate the change of those saliva parameters.

Consuming the high-calcium foods positively affected the calcium secretion [22]. Calcium ions from ingested food penetrate acinar cells from the salivary glands on the basolateral side via a CaT-like calcium channel or accumulate in the gingival sulcus, leading to the release of calcium ions into saliva. The increase calcium ions in saliva is initiated during mastication process. The ionised calciums are absorbed in the duodenum and transcellular transport occurs. The transported calcium ions into the membrane is facilitated by epithelial calcium channels (CaT1). The ions binding within the cell is achieved by calcium-binding proteins (calbindin) or by the active calcium pump, sarco/endoplasmic reticulum Ca^{2+} (SERCA), promoting the absorption of the ions into the endoplasmic reticulum. The calcium secretion from the cell is mediated by a primary active mechanism, namely the plasma calcium pump ATPase (PMCAs-1). The rise of cytosolic calsium leads to activation of PMCA to eventually release the ions into saliva [41]. Although the wax gums group elevated the calcium ions concentration, but almond showed a significant greater change compared to the control. This finding indicates that not only can mechanical stimuli induce the saliva thus promoting calcium levels, but also the amount of calcium contained in particular food or drink may also regulate the salivary calciums.

An adequate calcium intake is required to restore calcium loss during demineralization [40]. Calcium in the oral cavity contribute to facilitate remineralization, to regulate body fluid balance, and to activate salivary gland secretory cells [27]. Besides, saliva is also composed of a variety of proteins that are essential for the emergence of enamel pellicle, a thin coating of proteins and minerals on enamel surfaces. They exhibit a strong affinity for hydroxyapatite, binding calcium and hindering calcium phosphate salts deposition from saliva that is supersaturated associated with hydroxyapatite, subsequently protecting the teeth against demineralization and calculus development [6].

Table 3: The comparison of salivary flow rate, pH, buffer capacity, and calcium level following almond and wax gums stimulation

Variable	Stimulation	Healthy		Caries	
		***Mean diff*±SD**	**p value**	***Mean diff*±SD**	**p value**
Flow rate	Almond	-0.21±0.13	0.001*	-0.17±0.02	0.010*
	Wax Gums	-0.43±0.02		-0.3±0.1	
pH	Almond	-0.44±0.18	0.002*	-0.54±0.2	0.001*
	Wax Gums	0.04±0.15		0.06±0.11	
Buffer capacity	Almond	-5.10±1.52	0.000*	-4.90±2.02	0.000*
	Wax Gums	-1.40±0.96		-0.50±0.70	
Calcium ion level	Almond	-2.87±0.55	0.001*	-1.6±0.86	0.012*
	Wax Gums	-0.42±0.44		-0.51±0.38	

*Significant (p<0.05), SD (standard deviation)

In addition, we further evaluated whether almonds had a distinct impact on saliva in healthy or caries individuals. Table 4 demonstrates that among these parameters, only calcium levels were significantly different between saliva from caries and non-caries participants. The calcium was significantly higher in cavity-free teeth than in decayed teeth after almond stimulation, which indicated the essential role of salivary calcium in preserving oral homeostasis. This is consistent with other studies showing that the level of calciums was lower in the caries tooth compared to the no caries tooth [42, 43]. The acidic environment in caries patient has more concentration of hydrogen ions, causing tooth demineralization. As a result of continuous loss of minerals, small pores or porosity are formed on the surface of tooth enamel, leading to the risk of caries development [44]. Ca and P are related to dental caries with regard to the calcium-to-phosphorus ratio. It is commonly known dairy products are significant sources of Ca and P, alterations in the Ca/P ratio may come from other cariogenic or anticariogenic foods or substances [27]. In some conditions, individuals who are allergic to the sort of dairy products need to find other rich-calcium-containing foods/drinks to replace them. Hence, the variety of healthy diets should be proposed to bring any alternative to overcome this matter. Almond not only has a substantial amount of minerals in content, but also is composed of dietary fibers, proteins and secondary metabolites, which provides a fruitful impact to our body health [39]. Protein and fat-rich foods provide a more neutral pH for biofilms. Foods with high protein content elevate salivary urea level, which ureolytic bacteria can convert to ammonia. This raises the pH of the biofilm and is linked to a lower risk of dental cavities. By altering the nature and biological activity of dental biofilm, dietary components might have an indirect impact [45].

Table 4: The difference effect of almond intake in salivary parameters among healthy and caries subjects

Variable	Treatment	Mean±SD	p value
Flow rate	Caries-free	-0.21±0.13	0.188
	Caries	-0.17±0.02	
pH	Caries-free	-0.44±0.18	0.3
	Caries	-0.54±0.2	
Buffer Capacity	Caries-free	-5.10±1.52	0.643
	Caries	-4.90±2.02	
Calcium ion level	Caries-free	-2.87±0.55	0.001*
	Caries	-1.6±0.86	

*Significant (p<0.05), SD (standard deviation)

In our study, the subjects were instructed to eat 15 g of almonds at one time. Although the recommended portion for adults is about 30 – 42.5 g daily [46-48], but it was quite difficult to feed as much as 30 g almonds at once so the amount was reduced to half from its minimum serving suggestion. The hard and crunchy textures of almond require more active mastication than the soft and tender foods, therefore,

might affect the chewing and swallowing process [39, 49]. The harder the food texture has, the longer duration of chewing is needed which can cause pain on the jaws [49]. In this study, the almonds were consumed for only once, so that the knowledge on a long-term effect of the almond intake for caries inhibition is limited. Therefore, a longitudinal follow-up study is required to reveal this research gap. The other disadvantage is the absence of demineralization stage in the oral environment by decreasing the pH to investigate the effect of almonds in restoring the pH value back to neutral. However, this study demonstrated the almonds significantly upregulated the saliva parameters, suggesting its potential ability in improving oral health condition, particularly saliva. Future study is proposed to evaluate the almond consumption for a longer period of time according to the dietary guidelines to determine its optimum result on the remineralization process, thus considering it as a good meal to oral health.

CONCLUSION

Almonds acutely enhanced saliva characteristics associated with caries prevention. Nevertheless, these biochemical alterations must be verified through longitudinal clinical trials to determine whether they result in tangible oral health advantages. Future study is necessary to investigate a dose-response effect and a synergistic use with fluoride or other remineralizing agents.

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AUTHORS CONTRIBUTIONS

ARF: conceived and designed the study, performed data analysis, wrote the paper, Y: contributed data, performed data analysis, reviewed the paper, AP: contributed data, reviewed the paper, PS: performed the analysis, reviewed the paper, AFD: performed the analysis, reviewed the paper, PA: performed the analysis, reviewed the paper, RM: collected the data, reviewed the paper, NDS: collected the data, reviewed the paper.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest associated with the materials presented in this paper.

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