Cumhuriyet Dental Journal, 28(2): 272-277, 2025
DOI: https://doi.org/10.7126/cumudj.1667575

Cumhuriyet Dental Journal

| cdj.cumhuriyet.edu.tr | Founded: 1998 Available online, ISSN: 1302-5805 Publisher: Sivas Cumhuriyet Üniversitesi

Comparative Impact of Chocolate and Chocolate- Raw Almond Intake on Salivary pH Dynamics and Stephan Curve

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Research Article

History

Received: 28/03/2025
Accepted: 26/05/2025

ABSTRACT

Objective: This study aimed to evaluate the effect of consuming chocolate alone versus in combination with raw almonds on salivary pH kinetics, focusing on their ability to prevent critical pH drops and their buffering efficiency over time.

Materials and Methods: This study assessed the Stephan Curve response to the consumption of chocolate alone and in combination with raw almonds in 34 healthy young adults, measuring salivary pH kinetics and buffering capacity. Saliva samples were collected at multiple time points using a standardized protocol, and pH was analyzed with a calibrated digital pH meter to evaluate the impact of each condition on oral clearance and acid neutralization.

Results: Both groups exhibited an immediate decrease in salivary pH post-consumption. The lowest pH values were observed at 1 minute for chocolate (pH = 5.1) and at 5 minutes for the chocolate-almond combination (pH = 5.6). The chocolate-alone group returned toward baseline within 30 minutes, whereas the chocolate-almond combination required approximately 45 minutes. Notably, the pH for the chocolate-almond combination remained above the critical pH threshold of 5.5 for hydroxyapatite, indicating a lower risk of enamel demineralization.

Conclusion: Adding raw almonds to chocolate slowed pH decline, keeping it above the critical threshold, indicating a potential cariostatic effect. Raw almonds may help reduce the cariogenic risk of high-carb foods.

Keywords: Saliva, chocolate, almond, dental caries

Çikolata ve Çikolata-Badem Kombinasyonunun Tükürük pH Dinamiği ve Stephan Eğrisi Üzerindeki Karşılaştırmalı Etkisi

Araştırma Makalesi

Süreç

Geliş: 29/03/2025
Kabul: 26/05/2025

ÖZET

Amaç: Bu çalışmanın amacı, çikolatanın tek başına tüketilmesi ile çiğ bademle birlikte tüketilmesinin tükürük pH kinetiği üzerindeki etkisini değerlendirmek ve kritik pH düşüşlerini önleme yetenekleri ile zaman içindeki tamponlama verimliliklerini karşılaştırmaktır.

Materyal ve Metot: Bu çalışmada, 34 sağlıklı genç yetişkinde çikolatanın tek başına ve çiğ bademle birlikte tüketilmesine bağlı Stephan Eğrisi yanıtı değerlendirilmiş, tükürük pH kinetiği ve tamponlama kapasitesi ölçülmüştür. Standart bir protokol kullanılarak çoklu zaman noktalarında tükürük örnekleri toplanmış ve pH, kalibre edilmiş bir dijital pH metre ile analiz edilerek her bir koşulun oral klirens ve asit nötralizasyonu üzerindeki etkisi incelenmiştir.

Bulgular: Her iki grupta da tüketim sonrası tükürük pH'ında ani bir düşüş gözlemlenmiştir. En düşük pH değerleri, çikolata grubunda 1. dakikada (pH = 5,1), çikolata-badem kombinasyonunda ise 5. dakikada (pH = 5,6) kaydedilmiştir. Çikolata tek başına tüketildiğinde pH 30 dakika içinde başlangıç değerine yaklaşırken, çikolata-badem kombinasyonunda bu süre yaklaşık 45 dakika sürmüştür. Önemli bir şekilde, çikolata-badem kombinasyonunda pH, hidroksiapatit için kritik pH eşiği olan 5,5'in üzerinde kalmıştır, bu da mine demineralizasyonu riskinin daha düşük olduğunu göstermektedir.

Sonuç: Çikolataya çiğ badem eklenmesi, pH düşüşünü yavaşlatarak kritik eşiğin üzerinde kalmasını sağlamış ve potansiyel bir kariyostatik etkiye işaret etmiştir. Çiğ badem, yüksek karbonhidratlı gıdaların kariyojenik riskini azaltmada yardımcı olabilir.

Anahtar Kelimeler: Tükürük, çikolata, badem, diş çürüğü

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How to Cite: Çelik ZC, Özkeleş SŞ, Tula EÖ. (2025) Comparative Impact of Chocolate and Chocolate- Raw Almond Intake on Salivary pH Dynamics and Stephan Curve, Cumhuriyet Dental Journal, 28 (2): 272-277.

Introduction

Saliva is a biological secretion with various functions, including chewing, swallowing, taste perception, speech, and the initial digestion of carbohydrate-containing foods. Under healthy conditions, the pH of saliva generally ranges between 6.7 and 7.4. In addition to its static protective effects, saliva also exhibits dynamic effects that are activated during a stimulus or challenge. The oral clearance effect and buffering capacity of saliva are among the key dynamic mechanisms that help prevent the demineralization of dental tissues.[1,2]

Oral clearance refers to the rate at which substances (e.g., drugs, foods, microorganisms) are removed from the oral cavity through mechanisms such as salivary flow, tongue movements, and swallowing [3] This mechanism plays a critical role in maintaining oral health. Oral clearance is influenced by various factors, including the quantity and quality of saliva, tongue mobility, swallowing function, and the structure of teeth.

When sugary foods are consumed, bacteria in the oral microbiota (particularly cariogenic species like *Streptococcus mutans*) metabolize these sugars to produce acids.[4] These acids lower the oral pH to levels that can cause demineralization of tooth enamel. Once the pH drops, minerals in the enamel begin to dissolve, increasing the risk of caries formation. This pH fluctuation is effectively illustrated by the Stephan Curve.[5]

Dairy products contain substances with natural buffering effects that play a crucial role in maintaining oral health. Milk proteins (casein), calcium, and phosphates can prevent pH drops and neutralize the acidic environment caused by sugary foods more rapidly.[6] However, like cheese and yogurt have the potential to reduce caries risk, they are not always practical to carry and consume during daily activities due to refrigeration requirements.

Nuts, such as walnuts and almonds, are portable and practical healthy snacks,[7] but their effects on oral pH changes and Stephan Curve kinetics in conjunction with cariogenic foods have not been thoroughly investigated.

Foods with high fiber content and alkaline properties, such as raw almonds, may buffer the acidic effects of cariogenic foods and influence oral pH kinetics. Cariogenic foods like chocolate are widely consumed, pairing them with healthy snacks such as raw almonds may alter their impact on oral pH.[8] Raw almonds, rich in fiber and alkaline properties, have been suggested to buffer pH changes, thereby reducing cariogenic risk.

While the Stephan Curve provides a framework for understanding pH dynamics following sugar consumption, the impact of co-consumption with healthy snacks on oral clearance and pH buffering remains underexplored. This study aims to investigate the effect of consuming fermentable sugar-containing cariogenic foods alone and in combination with practical, portable healthy snacks on oral clearance and Stephan Curve kinetics, focusing on their potential to mitigate cariogenic risks. The null hypothesis is that there is no significant difference in oral pH dynamics between the consumption of chocolate alone and the consumption of chocolate combined with raw almonds.

Materials and Methods

The study was approved by the Bursa Uludağ University Health Research Ethics Committee (Ethics Committee No: 2024-16/21). After providing an explanation in the local language, informed consent was obtained from the participants. A power analysis was conducted prior to the study to determine the minimum sample size required to detect a significant difference in salivary pH changes between groups, with a power of 80% and an alpha level of 0.05. Based on a previous related data[6] and an expected medium effect size (Cohen's d = 0.5), the required sample size was calculated to be 32 participants. The study included young adults (n=34) aged 18-35 who were non-allergic to chocolate, chocolate derivatives, raw almonds, or any type of nuts; non-smokers; free of systemic diseases, and had not received antibiotic treatment in the past month.

A series of in vivo experiments were conducted to assess the Stephan Curve response to the consumption of 4.25 g of milk chocolate (Milk chocolate, 33 g, Napoliten™, ÜLKER, Türkiye) alone and in combination with ~1.5 g of raw almonds (Raw almond, Peyman, USA)

Participants were instructed to refrain from oral hygiene practices for a set duration prior to each trial.

The primary outcome measures were the minimum pH reached, the time taken to reach the minimum pH, and the overall area under the Stephan Curve. Secondary outcomes included the time required for pH to return to baseline levels and the buffering capacity of the different test conditions.

Saliva samples were collected between 9:00 am and 11:00 am to avoid the effects of circadian rhythm. Participants were instructed to refrain from eating, drinking, or chewing gum for at least two hours prior to sample collection. The sampling procedure included the following steps:

1. 5 minutes of rest.
2. Baseline: Collection of unstimulated saliva for 5 minutes.
3. Consumption of a 4.25 g of chocolate.
4. T0: 1 minute after consumption, collection of unstimulated saliva for 5 minutes.
5. T1: Collection of unstimulated saliva 10 minutes post-consumption for 5 minutes.
6. T2: Collection of unstimulated saliva 20 minutes post-consumption for 5 minutes.
7. T3: Collection of unstimulated saliva 30 minutes post-consumption for 5 minutes.

On a separate testing day, participants underwent the same procedure with an additional step: consuming a 4.25 g of chocolate, followed by the ingestion of one raw almond.

Salivary pH was directly estimated using a digital pH meter calibrated with pH 4 and 6.86 buffers at 25ºC. The accuracy of the pH meter was regularly checked to ensure precise measurements. A pH-sensitive electrode was immersed in 1 ml of saliva, and the digital reading were recorded once it stabilizes. Between measurements, the electrode was cleaned with a drop of distilled water and placed in a pH 6.86 standard solution to maintain stability and monitor for potential drift.

Salivary pH was measured as soon as possible, but no later than 30 minutes after sample collection. Oral clearance time were estimated based on the time taken for salivary pH to return to baseline levels.

Results

The consumption of chocolate alone resulted in a significant and prolonged drop in salivary pH, with a minimum of 5.2 reached within 10 minutes. In contrast, the combination of chocolate and raw almonds led to a less pronounced pH decline, with a minimum of 5.59 observed at 15 minutes, which remained above the critical pH of 5.5 for hydroxyapatite crystals.

The area under the Stephan Curve was also considerably smaller for subjects consuming the chocolate-almond, indicating a more favorable pH profile and reduced cariogenic potential.

The baseline pH was not reached within 30 minutes in either condition. The time required for pH to return to baseline levels was approximately ~35 minutes for chocolate alone, compared to ~45 minutes for the chocolate-almond combination.

The Stephan Curve graph represents these phases, showing a rapid decline in pH after the intake of cariogenic foods, followed by a recovery period. Tooth demineralization occurs during the critical phase when pH falls below 5.5. Prolonged or frequent exposure to this phase increases the risk of caries as enamel minerals are continuously dissolved without adequate recovery time. By visualizing this process, the Stephan Curve highlights the dynamic relationship between diet, oral pH, and caries risk.

There was no significant difference in baseline salivary pH between the two groups. However, immediately after consumption (T0), the chocolate group exhibited a significantly greater drop in pH compared to the chocolate + raw almond group ($p < 0.001$). This difference remained significant at 5 minutes (T1), favoring the combination group ($p = 0.006$). At 20 minutes (T2), the pH levels in both groups showed recovery and were not statistically different ($p = 0.191$). By 30 minutes (T3), the chocolate group had a significantly higher pH than the combination group ($p = 0.0021$).

In terms of percentage change, the pH drop from baseline to T0 was significantly more pronounced in the chocolate group ($p = 0.0003$). Between T0 and T1, the chocolate group showed a slight increase in pH, while the combination group experienced a further decrease ($p = 0.0026$). From T1 to T2, pH recovery was significantly greater in the chocolate group ($p = 0.0012$). However, the additional increase between T2 and T3 was comparable between groups ($p = 0.161$). (Table 1)

Figure 1 presents the salivary pH changes over a 30-minute period following the consumption of chocolate alone and chocolate combined with raw almonds. The data illustrate the initial drop in pH and the subsequent recovery, highlighting the potential buffering effect of raw almonds. The critical pH threshold (5.5), below which enamel demineralization may occur, is also considered.

Table 1. *Changes in measured parameter values (mean values) at different time points for Chocolate and Chocolate + Raw Almond groups.*

	Chocolate	Chocolate +Raw Almond	P
Baseline	6.32 ± 0.70	6.71 ± 0.32	0.523
T0	5.16 ± 0.59	6.02 ± 0.49	**< 0.001**
T1	5.29 ± 0.62	5.65 ± 0.51	**0.006**
T2	5.99± 0.55	5.79± 0.36	0.191
T3	6.35 ± 0.47	5.97 ± 0.44	**0.0021**
Δ(T0-Baseline)	-%18.31	-%10.19	**0.0003**
Δ(T1-T0)	+%2.51[b]	-%7.24	**0.0026**
Δ(T2-T1)	+%13.11	+3.77	**0.0012**
Δ(T3-T2)	+%6.01	+%3.11	0.161

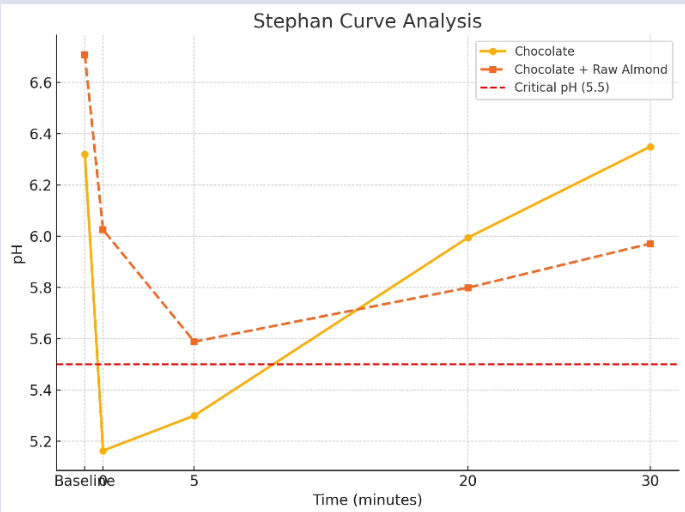

Figure 1. *Effect of chocolate and chocolate with raw almond on salivary pH over time*

Discussion

This study aimed to evaluate the effect of consuming chocolate alone versus with raw almonds on pH kinetics, focusing on their ability to prevent critical pH drops and their buffering efficiency over time.

The increase in salivary pH may enhance the competitive potential of less harmful oral bacteria, thereby inhibiting the growth of acidophilic bacterial species such as streptococci and lactobacilli.[9] It can be anticipated that, with a higher pH, less cariogenic bacteria metabolize the available substrates to produce weaker acids, which may contribute to maintaining an elevated pH level.

When cariogenic products are consumed, the initial response of dental plaque is a rapid decrease in pH, often dropping below the critical threshold of 5.5, which is conducive to the demineralization of tooth enamel. For instance, studies have shown that after the consumption of candies, there is a sharp decline in salivary pH within minutes due to the production of organic acids by plaque bacteria metabolizing these sugars.[10,11] This phenomenon aligns with studies shown a direct correlation between the consumption of fermentable carbohydrates and the acidogenic response of dental plaque.[12,13]

Chocolate consuming dropped pH 5.2 but pH increased to 5.3 in five minutes and reached above critical pH level in 10 minutes. Moreover this was faster in 30 minutes even to higher pH 6.3 than chocolate and raw almond. Because of either cacao or theobromine which both of them have cariostatic properties, chocolate consumption here, reached to neutral pH even in ten minutes according to Figure 1. Similarly to our study observed a more pronounced decrease in pH in the group consuming chocolate, with post-consumption pH values being lower for chocolate (5.54) compared to milk (5.98).[11] Furthermore, König et al. emphasized in their study that the decrease in salivary pH following food consumption is subsequently followed by a pH increase.[14] These results suggest that the chocolate-almond combination creates a more prolonged buffering effect in the oral environment, delaying the return of pH to baseline. The chocolate-almond combination required a longer buffering period (45 minutes vs. 30 minutes for chocolate alone), suggesting that the fat and protein content of almonds may influence the solubility of chocolate, slowing down the salivary neutralization process. The fat content of almonds might also contribute to prolonged food retention on tooth surfaces, further delaying pH recovery.

Moreover, the extent of the pH drop and the duration of the acidic environment can vary based on individual factors such as metabolic conditions. For example, research indicates that diabetic children exhibit a more pronounced acidogenic response compared to their non-diabetic counterparts, suggesting that underlying health conditions can exacerbate the cariogenic potential of dietary sugars.[15] This highlights the importance of considering individual health status when evaluating the effects of cariogenic products on the Stephan curve. The area under the curve (AUC) of the Stephan curve is also a critical metric for assessing caries risk. A higher AUC indicates a greater cumulative exposure to acidic conditions, which correlates with an increased risk of dental caries.[15] Interventions aimed at reducing the cariogenic potential of diets, such as the inclusion of probiotics, have shown promise in mitigating the acidogenic response, as evidenced by a significant reduction in AUC following probiotic intake.[16] This suggests that dietary modifications can influence the dynamics of the Stephan curve and potentially lower caries risk. In our study, the addition of raw almonds to the diet slowed the decrease in salivary pH and prevented it from dropping below the critical threshold, supporting this perspective.

However, since the minimum pH for the chocolate-almond combination remained at 5.59, staying above the critical pH threshold of 5.5 for hydroxyapatite, this combination may result in less demineralization compared to more acidic conditions. While the pH drop was less pronounced, the prolonged buffering requirement suggests that oral hygiene considerations are still necessary. Therefore, chocolate alone is neutralized more quickly, while the chocolate-almond combination has a longer-lasting effect in the oral cavity. This finding highlights the need for further research to understand the long-term impact of this combination on caries risk.

Studies on the oral clearance of sugary foods and beverages are well-documented.[17,18] Additionally, research has shown that consuming dairy products accelerates oral clearance.[6] Dairy products contain substances with natural buffering effects that play a crucial role in maintaining oral health. Milk proteins (casein), calcium, and phosphates can prevent pH drops and neutralize the acidic environment caused by sugary foods more rapidly. While dairy products like cheese and yogurt have the potential to reduce caries risk, they are not always practical to carry and consume during daily activities due to refrigeration requirements. Nuts, such as walnuts and almonds, are portable and practical snacks, but their effects on oral pH changes and Stephan Curve kinetics in conjunction with cariogenic foods have not been thoroughly investigated. While the buffering effects of dairy products are well-known, clinical studies exploring the potential buffering effects of nuts like walnuts and almonds are limited.

The findings of this study support the hypothesis that the inclusion of raw almonds with chocolate can mitigate the adverse effects of the cariogenic food on oral pH dynamics. The alkaline properties and high fiber content of raw almonds appear to counteract the acidogenic response triggered by the fermentable carbohydrates in chocolate, leading to a less severe pH drop that is also resolved more quickly.[7] The fiber in almonds may help stimulate saliva flow, which can further aid in buffering the pH changes and clearing the oral cavity of residual food particles and acids. Additionally, the alkaline minerals present in almonds, such as calcium and magnesium, can directly neutralize the acidic byproducts of carbohydrate fermentation, helping to maintain a more favorable pH balance.

These results are consistent with previous research on the pH-modulating effects of non-cariogenic foods[19,20] and the specific buffering potential of nuts.[21,22] The combination of chocolate and raw almonds may thus represent a practical and readily available approach to reducing the cariogenic risk associated with popular snack choices. By pairing these items, consumers can enjoy cariogenic treats like chocolate while potentially mitigating their adverse impacts on oral health through the buffering and pH-modulating effects provided by the alkaline and fibrous raw almonds.

In a recent study, roasted almonds contained the highest carbohydrate levels, whereas raw almonds had the lowest. Notably, raw almonds have been reported to possess an anti-cariogenic effect, which may be attributed to their low carbohydrate content and the presence of polyphenols that inhibit bacterial adhesion and acid production[23] by cariogenic microorganisms such as *Streptococcus mutans*. The lower carbohydrate content of raw almonds compared to their roasted counterparts may further contribute to their potential role in caries prevention. These findings suggest that roasting may alter the carbcfohydrate composition of nuts, potentially due to moisture loss and structural changes in macronutrient composition.[7]

Conclusions

The consumption of chocolate with raw almonds resulted in a reduced rate of salivary pH decline, prevented the pH from dropping below the critical threshold, and led to a subsequent increase in pH. However, the basal pH level was not restored within the 30-minute period. These findings support the notion that raw almonds are non-cariogenic and to some extent cariostatic, suggesting that their inclusion in the diet following the consumption of high-carbohydrate sweets and snacks may help reduce the risk of dental caries. Additionally, raw almonds are a convenient dietary option as they require no refrigeration and may serve as a suitable alternative for individuals with intolerance or allergies to dairy products, as well as for vegan individuals.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors would like to thank all participants for their involvement in this study.

Authors' Contributions

Design of study: Z.C.C.; Data collection: E.O.T & Z.C.C.; Interpretation of data: Z.C.C. & S.S.O. & E.O.T.; Literature search: Z.C.C. & E.O.T.; Writing-original draft preparation: Z.C.C.; All authors read and approved the final manuscript.

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