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EFFECT OF EDIBLE VEGETABLE JUICES ON THE PH OF CARIOGENIC ORGANIC ACIDS: AN IN VITRO STUDY

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Abstract

Objective: PH elevation of cariogenic organic acids is one of the most important ways for reducing dental caries. The purpose of this study is to investigate the effect of five edible vegetable juices Parsley (Petroselinum crispum), Tarragon (Artemisia dracunculus), Garden cress (Lepidium sativum), Summer savory (Satureja hortensis) and Mint (Mentha piperita) on the PH of cariogenic organic acids (formic acid, lactic acid and citric acid).

Materials and Method: The leaves of the five mentioned edible vegetables were freshly collected and after removal of non-edible parts, the juice of all vegetables were extracted by squeezing and filtering separately in the laboratory. Organic acid solutions including formic acid, citric acid and lactic acid in concentration of 1 mM and volume of 360 ml were prepared.

The initial PH of each acid was measured and 500 µl of each vegetable juice were then added to 10 ml of each acid step by step, until the PH was fixed on a certain value. Finally, the mean stable PH was measured for each group. Data were analyzed by SPSS software (ver.19).

Results: The mean PH values showed an immediate increment after adding the first 500 µl of vegetable juices. Tarragon and Mint altered the PH of lactic acid, which is the most effective organic acid in caries process before the two other ones. Other vegetables including Parsley, Garden cress and Summer savory changed the PH of formic acid faster than lactic and citric acids, then sustained in a stable level. Mint was able to increase the initial PH of all three acids to almost neutral level. Summer savory was able to increase the initial PH of formic acid only to almost neutral level. In total, these two vegetables, specially Mint, had a significant difference with other three vegetables (Tarragon, Garden cress and Parsley) in elevating the PH level of cariogenic organic acid solutions ($P < 0.05$).

Conclusion: Among the five edible vegetables, Mint and then Summer savory have the highest buffering properties against organic acids. Due to the herbal nature, availability and inexpensiveness of these vegetables in most countries, they can be used among meals to reduce dental caries injuries.

Keywords: Cariogenic organic acids, Edible vegetable juices, PH elevation, Neutral level

Introduction:

Dental caries is one of the most common infectious diseases in developing countries (1).Tooth decay is actually the local destruction of dental tissues with the acidic products derived from the fermentation of dietary carbohydrates by the activity of oral pathogenic bacteria(2, 3). The factors involved in the caries process include teeth, dental plaque, diet, and the contact time of acidic compounds with the teeth(3, 4) .Two types of bacteria including Streptococcus mutans and Lactobacillus groups are assumed to be the most important cariogenic and causative microorganisms in initiating and development of dental caries(5). The large number and vast activity of these two types of bacteria in the mouth are probably the main cause for high levels of carbohydrate fermentation, leading to reduced levels of PH values in the dental plaque(6).

Diminished PH in the mouth can cause the beginning of enamel destruction and eventually caries incidence(7). Studies have shown that the production of acids by pathogens, especially Streptococcus mutans, is the main cause of oral decays(8). Lactic acid, formic and citric acids are three important organic acids that are produced during fermentation of carbohydrates by oral pathogenic microorganisms in the caries process and cause demineralization of the teeth(9, 10).

In a mineralized tissue like enamel; Calcium and phosphate as the main mineral substances degrade when the peripheral PH decreases. Compensating the peripheral PH reduction may cause the dissolved calcium and phosphate to be deposited again on the remaining enamel crystals in a remineralization process. Several failures of remineralization process may be involved in occurrence and progression of dental lesions(11). Dietary carbohydrates are essential for bacteria to produce organic acids that initiate teeth demineralization. PH reduction is the most important factor promoting caries process after food intake, which may last for hours. Consequently, prohibiting the PH reduction will greatly help to prevent caries(12).

Dental care services are often associated with service cost and rareness of dental health materials(13).Alternative caries prevention programs and treatment options are required which should be safe, effective, and economical(14,15).

However several chemical agents are commercially produced and consumed in dental care services in different countries but they can alter oral microflora and may have undesirable side-effects such as allergic reactions, vomiting, diarrhea, and tooth staining (16). Therefore, alternative products especially herbal medicines and natural phytochemicals isolated from herbs used in traditional medicine are considered as good alternatives for synthetic chemicals with less side effects.

In a study by Haghighati et al. (2003), antimicrobial effects of ten species of fruit and plant extracts were evaluated on three types of oral microorganisms, including actinobacillus actinomycetemcomitans, Candida albicans and streptococcus mutans. The results showed that Thyme, Clove, oak apple, Pomegranate peach and black myrobalan have significant antibacterial and antifungal effects (17).

Abbaszadegan et al. (2015) have shown that essential (volatile) oil of Cinnamon has strong antimicrobial effects against E.Faecalis in both planktonic and biofilm forms(17).

JING-SHU XU et al. (2014) investigated the effect of Emodin, an active ingredient found in Senna, Rhamnus and Polygonum plants on Streptococcus mutans degeneration rate. They reported that Emodin suppressed the production of acid by streptococcal bacteria in the culture medium and also significantly inhibits the growth of these microorganisms(18).

Material and methods:

Fresh edible vegetables including Parsley (Petroselinum crispum), Tarragon (Artemisia dracunculus), Garden cress (Lepidium sativum), Summer savory (Satureja hortensis) and Mint (Mentha piperita) were purchased from a local market in Shiraz, Iran. All vegetables were transported to the laboratory simultaneously and after cutting manually the non-edible parts, were washed with distilled water. Vegetables were then mildly air-dried and were kept at the refrigerator temperature (4°**C**). To extract the juice, the leaves were crushed with mill, then a certain volume of them were weighed. The undiluted juice was obtained by squeezing the vegetables directly, then filtered by paper. (In this study, there was no need for solvent and direct vegetable squeezing was provided).

Organic acids including formic acid, citric acid and lactic acid in an exact concentration of 1 mM and 360 ml were prepared. Vegetable juices were incubated in 37°C for two hours and 500 µl of each were then added to containers having 10 ml of each acid. Then a magnet was put inside each container and placed on a stirrer to dissolve the contents equally well. The electrode of digital PH meter (HM digital PH – 80, USA) was then placed in the corresponding container at the beginning and each time 500 µl of vegetable juices were added to acid solutions, then the PH was recorded for three times. Juice adding process was continued until the PH was fixed in a stable degree. Data was analyzed by SPSS (ver.19) software and graph and the analysis of variance (Two-way ANOVA) was used to compare the effect of each vegetable on acid PH changes. The significance level was considered when $P< 0.05$.

Parsley Tarragon Garden cress Summer savory Mint

Results:

The results of analysis of two way ANOVA showed that there is a significant difference between the PH changes of three cariogenic organic acids in contact with vegetable juices (Parsley, Tarragon, Garden cress, Summer savory and Mint) ($P < 0.05$).

The PH changes of three organic acids after adding Parsley juice is shown in figure 1.

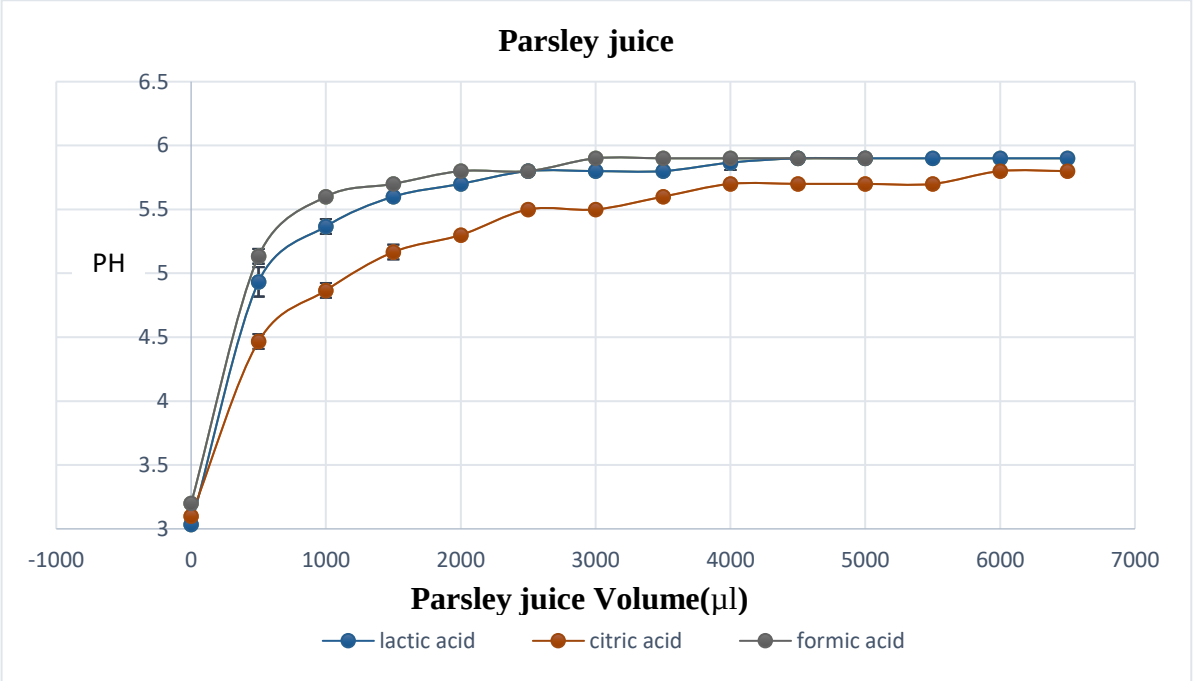

Figure 1. Mean PH changes of cariogenic organic acids against different volumes of Parsley juice

The PH of formic acid after adding 5000 µl of Parsley juice reached to the stable level of PH = 5.9 before the two other acids, while the PH of lactic acid and citric acid after adding 6500 µl of Parsley juice reached to 5.9 and 5.8, respectively.

The PH changes of organic acids after adding Garden cress juice is shown in figure 2.

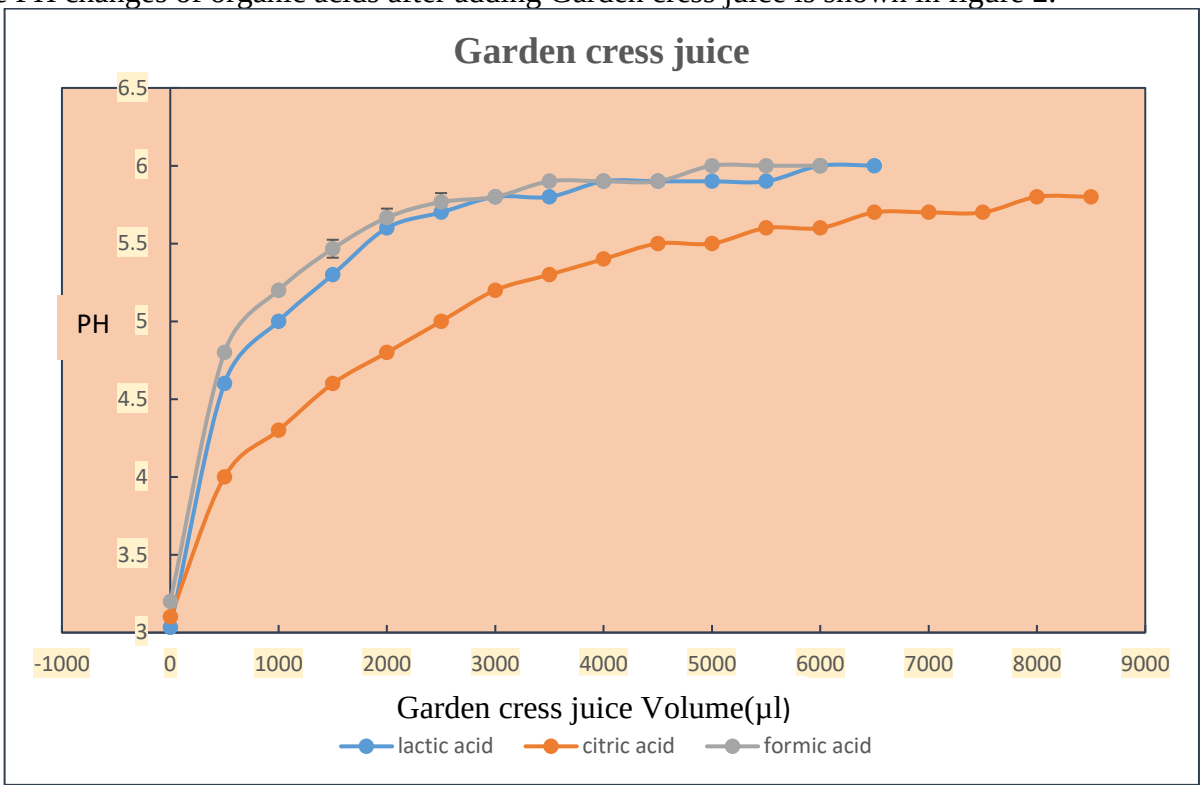

Figure 2. Mean PH changes of cariogenic organic acids against different volumes of Garden cress juice

PH changes of formic acid after adding 6000 µl of Garden cress juice reached to stable level of PH = 6, while PH of lactic acid after adding 6500 µl of Garden cress juice reached to 6 and of citric acid after adding 8500 µl of Garden cress juice reached to 5.8. The PH of formic acid is fixed earlier than the two other acids.

The PH changes of organic acids after adding Tarragon juice is shown in figure 3.

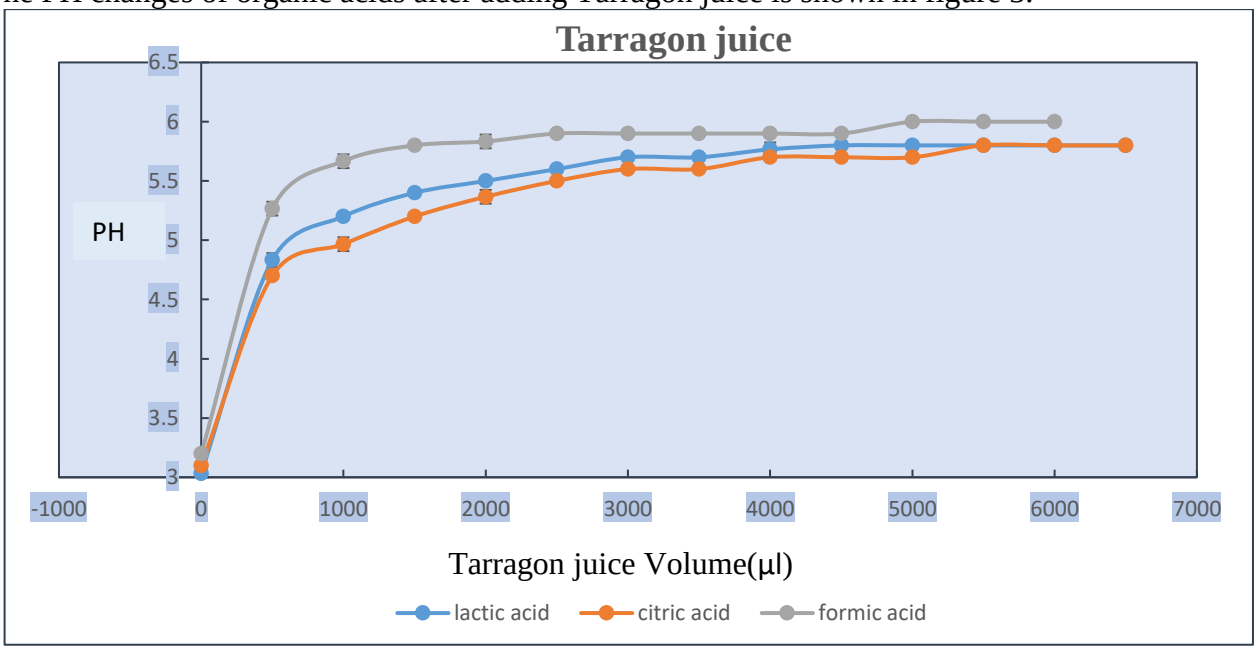

Figure 3. Mean PH changes of cariogenic organic acids against different volumes of Tarragon juice

The mean PH of lactic acid has been fixed after adding 4500 µl of Tarragon juice before the two other acids, at a value of 5.9 .PH changes of formic and then citric acids were stabilized later.

The PH changes of organic acids after adding Summer savory juice is shown in figure 4.

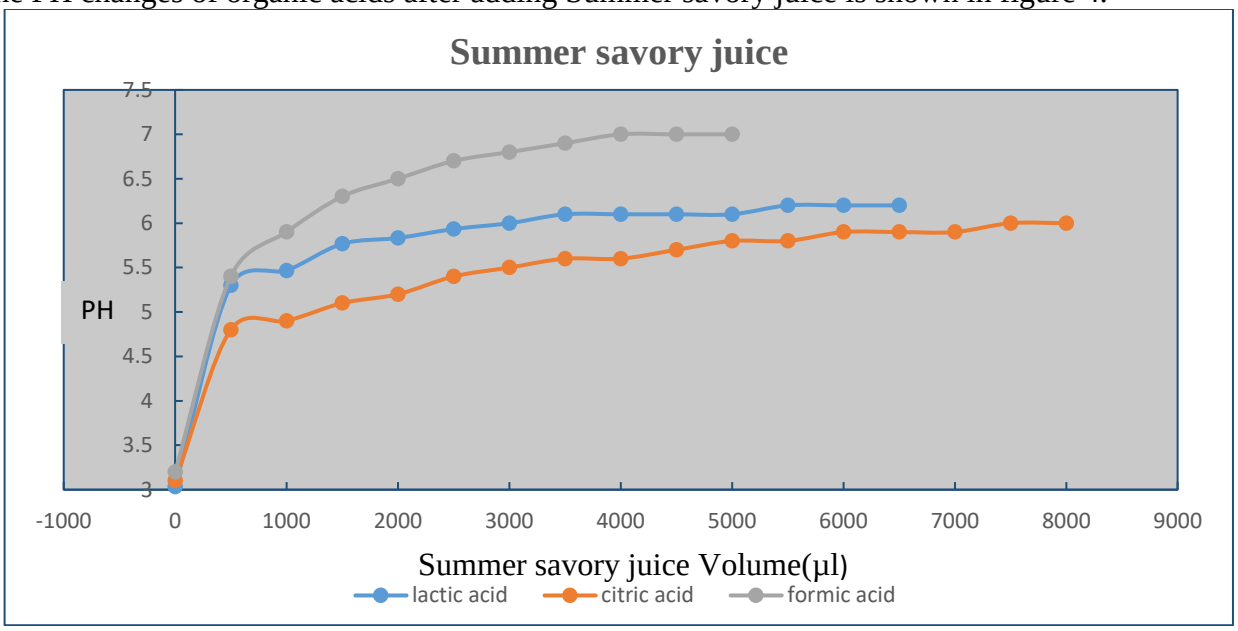

Figure 4. Mean PH changes of cariogenic organic acids against different volumes of Summer savory juice

The PH of formic acid changed faster than the other two acids with Summer savory juice and was fixed at nearly neutral value after adding 5000 µl of the juice. However the PH of lactic acid after adding 6500 µl and of citric acid, after adding 8500 µl Summer savory juice were then stabled.

The PH changes of organic acids after adding Mint juice is shown in figure 5.

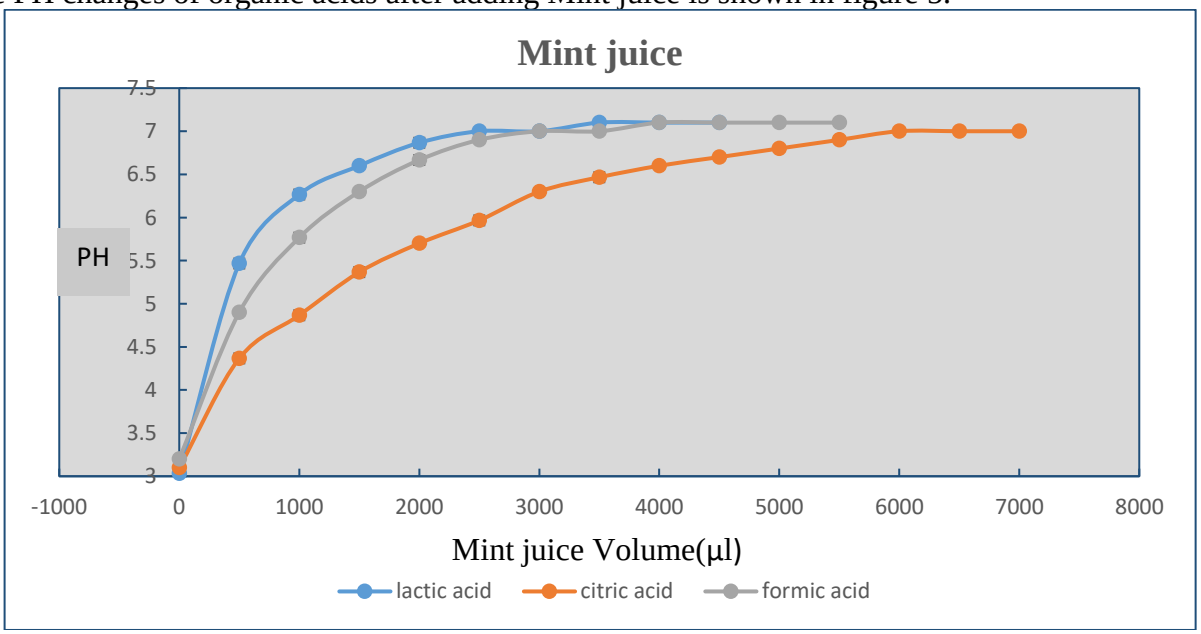

Figure 5. Mean PH changes of cariogenic organic acids against different volumes of Mint juice

PH changes of formic acid and lactic acid were close to each other and far from that of citric acid. Lactic acid showed PH changes by Mint juice earlier than the other two acids, the PH reached to nearly neutral value after adding only 2500 µl of Mint juice.

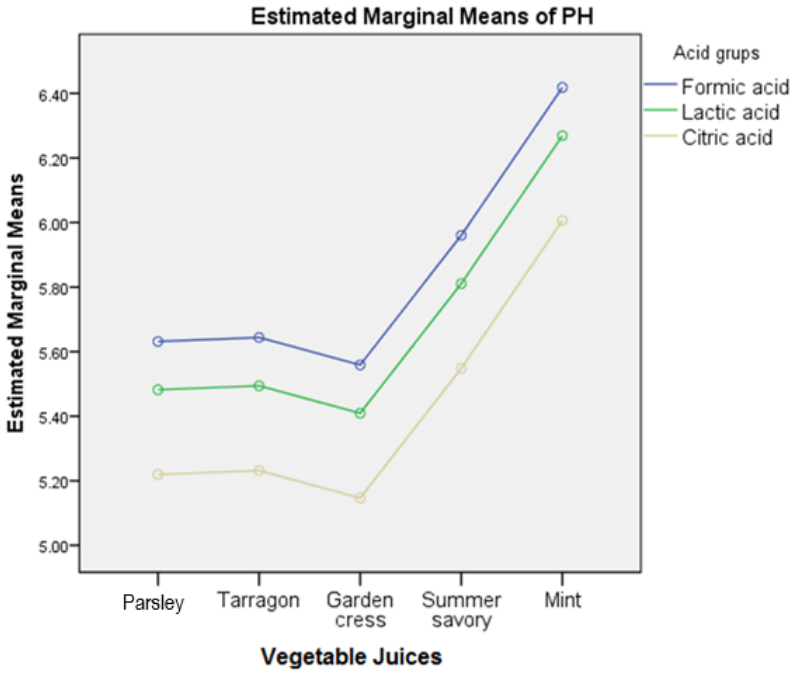

Figure 6. Simultaneous marginal PH changes of cariogenic organic acids in contact with vegetable juices

Simultaneous PH changes (marginal PH values) of lactic, formic, and citric acids in contact with five edible vegetable juices is shown here. As it is demonstrated, the Mint, and after it, the Summer savory juices increased the PH of all three acids more than other three vegetable juices and brought it to near neutral zones. Citric acid was the most resistant acid compound against buffering action of vegetable juices.

Discussion:

In the present study, the effect of five edible vegetables including Parsley, Tarragon, Garden cress , Summer savory and Mint on the PH of cariogenic organic acids (formic acid, lactic acid and citric acid) was investigated in vitro environment. The choice of these vegetables was due to their antimicrobial and antioxidant properties, which was found in previous studies. Some vegetable juices like Thyme juice, when added to Zinc oxide Eugenol as an effective routine root canal filling material in primary teeth may also be used to elevate it's antimicrobial properties. The antimicrobial effect of Thyme in the form of herbal extract is proven in some other studies (19, 20)

In the present study, we found that Parsley and Garden cress and Summer savory can increase the PH of formic acid earlier than lactic and citric acids to reach a stable level. Also it has been known that Summer savory and Mint have more buffer capability to increase PH of acid solutions. Mint can neutralize all three acids while Summer savory can bring only formic acid to nearly neutral PH zone. This study was in line with the study by Gayathri Ramesh et al. (2012). They evaluate saliva and tongue coating PH and also to assess the degree of tongue coating in healthy subjects before and after chewing Tulsi, Mint, and Curry leaf. The results showed that the mean saliva PH was increased after 30 minutes of herbal leaves chewing (21)and it was significant difference between Mint and Curry leaf groups immediately after chewing and between Tulsi and Curry leaf groups 30 min after chewing the leaves. Results of both studies confirmed that herbs and vegetables can enhance oral acid PH level increment, although our study was conducted in the in vitro environment and the study of Gayathri Ramesh was done in the in vivo.

Ahmed et al. (2017) investigated the effects of green tea and Chamomile tea on the salivary PH in 30 healthy peoples, and reported that these two types of tea inhibit salivary Streptococcus mutans count and cause reduction of PH in saliva, however did not differ significantly in terms of PH (22).

Also, in other studies on different tea types, Pallepati et al. (2017) reported that PH levels of saliva after consumption of tea containing Stevia was significantly higher (tending to neutral PH) in comparison with tea containing Sucrose and Jaggery (23). Srinidhi et al. (2014) also found that both green tea and black tea could increase saliva PH in caries-free and caries containing groups, but green tea had more effect in increasing PH (24).

It was observed that PH of formic acid was increased earlier than two other acids when most of five types of vegetable juice were used. This property appears to be related to the chemical structure of formic acid. As the formic acid is a simple carboxylic acid with the chemical formula HCOOH and PK = 3/75. Therefore it is a strong acid with high H+ releasing power, which quickly releases H+ in the environment and, neutralized by the hydroxyl functional group of phenolic and terpene compounds of the vegetables. Thus the number of protons or ions of the hydronium are reduced and the PH was increased. These changes were significant specially at the time of the addition of Mint and Summer savory and reached a neutral PH point. In the study of Vani and Rajashree Mashru (2018) in India, it was found that many plants and vegetables, such as Mint, had acid neutralization capacity, the examinations were done in the in vitro environment and on hydrochloric acid (25).These remarkable and significant effect in relation to Mint juice was due to the presence of phenolic and polyphenolic antioxidants in its structure. As in various studies, including Antolak et al. (2018), the extract of Mint contains many phenolic compounds such as gallic, chlorogenic, neo-chlorogenic, p-coumaric, ferrolic, rosemary acid, epicatechin, quercetin (26). In other studies, rosemary acid, luteolin 7 glucoside, salvianolic acid and pyridine also reported as non-volatile compounds in Mint(27)(26)(25)(Santoro, et al. 2011)(25)(26). These compounds give strong antioxidant properties to Mint. Hydroxyl group is one of the main functional groups in antioxidants, therefore with the release of these functional groups, the ability to neutralize protons increases and hence the PH also increases. Similar results of the significant releiving effect of Mint extracts in the duodenal ulcers resulting from the acid excess in duodenum were observed(28).

In addition, the use of some edible vegetable compounds rich in Flavonoids, Trepans, and Terpenoids give antimicrobial properties(29) and may have antimicrobial effects in cariogenic microorganisms. The effect needs to be investigated in further studies. Also the findings of this study emphasize that the mentioned vegetables have anti-acidic properties and, regarding that low PH is one of the main factors involved in dental caries, consumption of edible herbs can be regarded as a simple, inexpensive method (without complications rather than drugs and chemicals) to reduce dental caries(21).

Conclusion:

The vegetable juices including Parsley, Garden cress and Summer savory could bring the PH of formic acid to a stable level before other cariogenic organic acids. Tarragon and Mint could bring the PH of lactic acid to a stable level before the two other acids. Mint could change the PH of all three organic acids to almost neutral level. Summer savory could change the PH of formic acid only to nearly neutral value. In total, Mint and Summer savory have the highest buffering capacity to neutralize the cariogenic organic acids and bringing their PH to almost neutral level.

Suggestions:

Exposure to edible vegetables, especially Mint and Summer savory, have special anti-acid properties in the mouth environment. It is recommended to use edible vegetables along with meals to reduce the incidence of caries in the oral environment by increasing the PH of fermented carbohydrates. Further studies are required to confirm the exact buffering mechanisms of these vegetable compounds.

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