Improvement of Halitosis and Changes in Saliva Components after Using Dentifrices with Menthapiperita Extract

[1]Jung-Eun Park, Jong-Hwa Jang

Abstract: Background/Objectives: The objective of the present study was to evaluate the efficacy of dentifrices containing Menthapiperita extract on the changes in oral condition, halitosis, and saliva components. Methods/Statistical analysis:After recruiting the subjects, they were examined using the Turesky-Quigley-Hein Plaque index and Löe-Silness gingival index. To analyze the volatile sulfur compounds, which are halitosis inducing substances, H2S, CH3SH and (CH3)2S were quantitatively analyzed using Sensor Gas Chromatograph ODSA-P2 (FIS Inc., Hyogo, Japan). For HPLC analysis of organic acids and inorganic ions in saliva, Dionex ion chromatography system (gradient pump, ED 50 conductivity detector) was used.

Findings:In the evaluation of plaque index and gingival index, the values decreased from baseline of 1.87±0.66 and 0.57±0.45 to 1.63±0.88 and 0.17±0.12 after 4 weeks, respectively. Measurement of halitosis also showed a total reduction effect of 41.03% after 4 weeks. In the saliva analysis, total organic acid content decreased from baseline of 10.79±9.39 to 3.41±3.18 after 4 weeks, whereas the concentrations of chloride and phosphate increased after 4 weeks. Improvements/Applications: Based on the findings in the present study, it is determined that the dentifrices containing Menthapiperita (MP) extract used in the present study could improve the oral environment.

Keywords: Dentifrice, Halitosis, HPLC, Menthapiperita, Saliva

I. INTRODUCTION

*Menthapiperita (*Peppermint) is a natural extract obtained from the leaves of a plant called *Menthapiperita* L. by a distillation process. It is used as flavoring in teas, spices, foods, and dentifrices. This *Menthapiperita (MP)* contains various polyphenol compounds associated with protection against toxicity and ultraviolet rays [1, 2]. In addition, it can inhibit autoxidation of the while it may also have a positive effect on gingiva that can come in direct contact with the oral cavity [3]. Polyphenols, which are abundant in leaves and fruits, are known to possess various potentials based on their radical scavenging and chelating actions. In addition, polyphenols have antioxidant action through their biological activity and are known to have various efficacies, such as antiallergic, antithrombotic, and anticancer actions. As a result, they are being used in various foods and products [4, 5]. In some countries, MP extract is used to alleviate mild inflammation, pain, and congestion. It is also used in preservatives and anti-parasitic agents, as well as an additive in oral malodor agents owing to its unique cool scent [6]. Meanwhile, studies have reported that MP extract has anticariogenic effect on oral acidogenic bacteria that cause dental caries [7] and antimicrobial effect on *C. albicans, A. actinomycetemcomitans,* and *S. mutans*[8]. Although MP extract has been used steadily as an additive in foods and cosmetics, and dentifrices, there are insufficient amount of study results on its efficacy in dentifrices. In this respect, dentifrices containing MP extract may be able to exhibit antioxidant effect on gingiva and oral mucosa that can come in direct contact with the oral cavity and also contribute to prevention of halitosis and dental caries through its antimicrobial activity against various microorganisms present in the oral cavity.

Accordingly, the objective of the present study was to examine the oral health conditions with dental plaque index and gingivitis status with gingival index in the subjects after using dentifrices containing MP extract. In addition, to evaluate its efficacy against halitosis, volatile sulfur compounds were isolated into its components hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide $(CH_3)_2S$ and quantitatively analyzed. Lastly, to observe changes in saliva components, organic acids (lactate, acetate, propionate, formate) and inorganic anions (fluoride, chloride, phosphate) were quantitatively analyzed to evaluate the multi-faceted efficacy.

II. MATERIALS AND METHODS

2.1. Selection of subjects

Based on the inclusion criteria of healthy male and female adults residing in Korea who understood the purposes of study and voluntarily consented to participate in the study, a total of 10 subjects were enrolled. The study population consisted of a total of 10 adults who do not have any dental implants, dental caries, periodontal disease, or metabolic diseases. Five subjects each were assigned to the control and experimental groups. The present study was conducted after being reviewed and approved by the Institutional Review Board (DKU No. 2017-03-033). After being selected as the final subjects, the subjects received the study schedule from the researcher and made visits in accordance with the schedule. The subjects brushed their teeth within 5 minutes after regular meals (3 times a day), and at 1 hour after teeth brushing, the subjects underwent anhalitosis test, saliva tests, and oral observation. For collection of saliva, the subjects were instructed to visit at the designated times and days.

Revised Manuscript Received on January 03, 2019.

Jung-Eun Park, Research Scholar, Dept. of Preventive and Social Dentistry, School of Dentistry, Kyung Hee University, Republic of Korea

Jong-Hwa Jang, Corresponding author, Associate Professor,Dept. of Dental Hygiene, College of Health Science, Dankook University, Republic of Korea

2.2. Synthesis of dentifrices

The dentifrice used in the experimental group contained MP extract. Based on 500g of the main ingredient, the dentifrice for the experimental group was prepared by adding 25% MP extract and peppermint flavoring to a base dentifrice containing 50% glycerine, 25% xylitol, and 10% plum extract to delay hydrolysis. The dentifrice for the control group contained only the base with no additives. The dentifrices were used as follows: at 60 minutes after regular meals (3 times a day) and subsequent teeth brushing, the dentifrice was sprayed 3 times into the oral cavity, toward the right buccal, left buccal, and lingual sides.

2.3. Dental plaque index evaluation

The Turesky-Quingley-Hein Plaque index was applied to all teeth in the mouth of each subject to observe the condition of the supragingival plaque. After applying a coloring agent (Erythrosin, Sultan, USA) for 20 seconds to the labial and lingual surfaces of each tooth for staining, 6 sites (labial, distal buccal, mesial buccal, lingual, distal lingual, and mesial lingual surfaces) of each tooth were scored. The overall tooth surface scores of each subject were calculated as mean values.

2.4. Gingival index evaluation

The Löe-Silness gingival index was used to score gingivitis status in 6 sites (buccal, distobuccal, mesiobuccal, lingual, distolingual, and mesiolingual surfaces) of each tooth. The overall tooth surface scores of each subject were calculated as mean values.

2.5. Halitosis evaluation

Concentrations of volatile sulfur compounds were measured for halitosis evaluation. Sensor Gas Chromatograph ODSA- P2 (FIS Inc., Hyogo, Japan) was used as the measurement device to analyze halitosis inducing substances; H_2s, CH_3SH, and $(CH_3)_2S$.

2.6. Saliva collection and pre-treatment

1 mL of unstimulated saliva was collected from each subject and placed in an Eppendorf tube. The collected saliva was immediately centrifuged at 1200 rpm for 5 minutes, after which, the supernatant was diluted 10-fold using distilled water. The final analysis on the saliva sample was performed after filtering (hydrophobic polytetrafluorethylene (PTFE) membrane; pore size: 0.20 mm; Advantec MFS, Inc., Tokyo, Japan) to remove various enzymes and suspended solids in saliva.

2.7. HPLC analysis

The unstimulated saliva samples collected from the subjects were pretreated immediately upon collection and analyzed. However, when this could not be done, the samples were freeze stored and analyzed within 24 h. Required for preparation of the mobile phase used in the present study were purchased from Sigma Aldrich (St. Louis, MO, USA). The equipment used to analyze these materials was Dionex ion chromatography system (gradient pump, ED 50 conductivity detector). The column used in the analysis was DionexIonPac AG11-HG column (50mm×4mm). The analysis was performed using 100 mMNaOH for the mobile phase and with a flow rate of 1.0 mL/min, injection volume of 10 $\mu\ell$, and temperature of 20°C. Here, the mobile phase was gradiated to perform gradient elution.

III. RESULTS AND DISCUSSION

The objective of the present study was to evaluate changes in oral health environment, degree of halitosis improvement, and various saliva components after using dentifrices containing MP extract. In general, MP extract is added to many dentifrices to add flavoring, but the authors believed that the efficacy of MP may not only enhance the chemical stability of dentifrices, but also improve the oral environment and conditions. Oral examinations using the dental plaque index and gingival index were used as the methods for evaluating oral environment, the results of which are shown in Table 1. The plaque index decreased by approximately 12.83% after 4 weeks in the experimental group, indicating oral environment than the control group. The control group also showed better oral environment as compared to the baseline. The dentifrice used in the present study cannot physically remove biofilm on its own, but because plaque was evaluated at 1 hour after brushing, it may have affected the plaque reformation rate.

Oral examinations using the gingival index were performed to evaluate gingivitis status. The results showed 31.75% and 70.18% improvement in gingivitis in the control and experimental groups, respectively, after 1 week. Such findings suggested that since the dentifrice used for the control group also contained some natural extracts, components in MP extract in the dentifrice used for the experimental group may have contributed to the prevention of inflammation and oxidation.

TABLE 1. Changes in Dental Plaque Index and Gingival Index in the Oral Cavity from Before and After Using Each Dentifrice (n=10)

			Base line	1week	2weeks	4weeks
Control	Plaque index	mean±SD	1.84±0.57	1.87±0.74	1.53±0.27	1.70±0.48
		Δ^a(%)		-1.63	16.85	7.61
	Gingival index	mean±SD	0.63±0.55	0.67±0.59	0.37±0.56	0.43±0.53
		Δ^a(%)		-6.35	41.27	31.75
Experimental	Plaque	mean±SD	1.87±0.66	2.03±0.69	1.77±0.71	1.63±0.88

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

	index	Δ^a(%)		-8.56	5.35	12.83
	Gingival index	mean±SD	0.57±0.45	0.43±0.40	0.33±0.24	0.17±0.12
		Δ^a(%)		24.56	42.11	70.18

[a]Increase or decrease per week relative to the baseline

When volatile sulfur compounds H2S, CH3SH, and (CH3)2S, were measured separately to halitosis, the concentration of total VSCs, the sum of concentrations of all three components, was also evaluated. The results were as shown in Table 2.

In the experimental group, the concentrations of H2S and CH3SH decreased after 4 weeks, but the concentration of (CH3)2S increased after 1, 2, and 4 weeks, as compared to the baseline. However, the total VSCs decreased by 41.03%, indicating an overall decrease in halitosis-inducing substances. In the control group, the concentrations of CH3SH and (CH3)2S decreased, but total VSCs increased by approximately 6.14%. Measurement of halitosis involves analysis of gases inside the oral cavity, which can be very complicated.

TABLE 2. Changes in the Concentration of Volatile Sulfur Compounds from Before and After Using Each Dentifrice (ppb, n=10)

		Compound	Base line	1week	2weeks	4weeks
Control	H_2S	mean±SD	24.76±24.79	19.64±27.65	72.34±86.61	37.56±40.04
		Δ^a(%)		20.68	-192.16	-51.70
	CH_3SH	mean±SD	16.14±24.06	3.92±8.77	11.36±11.22	10.28±12.26
		Δ^a(%)		75.71	29.62	36.31
	$(CH_3)_2S$	mean±SD	8.94±9.52	1.50±1.40	7.90±8.10	5.06±6.11
		Δ^a(%)		83.22	11.63	43.40
	Total VSCs[b]	mean±SD	49.84±49.46	25.06±35.37	91.60±100.89	52.9±52.93
		Δ^a(%)		49.72	-83.79	-6.14
Experimental	H_2S	mean±SD	45.38±59.03	14.04±12.75	29.84±52.54	14.48±12.74
		Δ^a(%)		69.06	34.24	68.09
	CH_3SH	mean±SD	1.70±3.03	2.30±3.21	8.04±10.26	0.78±1.74
		Δ^a(%)		-35.29	-372.94	54.12
	$(CH_3)_2S$	mean±SD	1.28±1.86	2.82±3.02	21.14±41.32	13.26±21.99
		Δ^a(%)		-120.31	-1551.56	-935.94
	Total VSCs[b]	mean±SD	48.36±32.67	19.16±13.17	59.02±53.48	28.52±21.85
		Δ^a(%)		60.38	-22.04	41.03

a Increment and decrement concentration of volatile sulfur compounds compared to base line

bSum of H2S, CH3SH, and (CH3)2S

The results of analyzing the concentration of organic acids in saliva are shown in Table 3. In the experimental group, the concentration of all four organic acids decreased after 4 weeks, confirming decrease in the concentration of total organic acids after 4 weeks. In the control group, the concentration of all organic acids, except lactate, decreased after 4 weeks, showing a decrease in total organic acids relative to the baseline. However, both the control and experimental groups showed a rapid increase in the concentration of organic acids after 2 weeks. Considering that saliva components may be affected not only by the environment in the oral cavity, but also by systemic effects, it is expected that the concentration may have been affected by biological factors of the subjects.

As a result of processes that take place after sugar intake, fermentation into with various organic acids by acidogenic bacteria occurs [9-11]. The organic acids fermented by such process may appear differently depending on the type of microorganisms, and in particular, lactate, which is highly associated with causing dental caries, is known to be Actinomyces, Streptococcus, and lactobacillus[12, 13]. Therefore, higher concentration of organic acids means higher dental caries causing ability, which would also indicate higher acid production by bacteria.

TABLE 3. Changes in the Concentration of Organic Acids in Saliva from Before and After Using Each Dentifrice (mM, n=10)

	Compound	Base line	1week	2weeks	4weeks
Control (mean±SD)	Lactate	0.77±1.01	0.31±0.11	0.05±0.05	1.08±1.10
	Acetate	8.90±3.76	6.99±3.25	6.51±3.40	4.32±5.01
	Propionate	0.83±1.02	1.10±1.49	1.55±1.63	0.51±0.43
	Formate	0.50±0.28	0.35±0.36	1.21±0.92	0.30±0.18
	Total organic acids[a]	11.00±3.72	8.74±5.18	9.33±4.73	6.21±4.89
Experimental (mean±SD)	Lactate	0.69±1.16	0.60±0.52	0.03±0.03	0.62±0.40
	Acetate	7.82±5.79	1.87±1.76	12.40±2.92	2.35±3.37
	Propionate	1.30±1.93	0.04±0.10	4.86±3.00	0.26±0.49
	Formate	0.97±0.96	0.16±0.22	2.31±1.97	0.17±0.05
	Total organic acids[a]	10.79±9.39	2.67±1.96	19.60±4.78	3.41±3.18

aSum of lactate, acetate, propionate, and formate

Fluoride, chloride, and phosphate are inorganic anion components in saliva, which were compared for changes from before and after using each dentifrice. The results are shown in Table 4. In the experimental group, concentration of fluoride increased appreciably at 1 week and 2 weeks after using dentifrice, but increased slightly at after 4 weeks. The concentrations of chloride and phosphate increased after 4 weeks relative to the baseline. In contrast, in the control group, the concentrations of both chloride and phosphate decreased after 4 weeks, whereas the concentration of fluoride increased.

Inorganic ions in saliva are essential components in evaluating and determining the oral environment and predicting dental caries. Among them, fluoride is known to prevent dental caries by increasing the resistance of teeth structure against demineralization caused by acids generated by bacteria, facilitating remineralization process and inhibiting microbial enzymatic response [14, 15]. In addition, components such as chloride and phosphate are also known to play an important role in teeth and oral environment. Since they can contribute as a buffer to reduce solubility by acids and stability pH of saliva, while the actions and concentration of inorganic anions and organic acids in saliva are needed as indicators of oral bacteria and dental caries [16-18] Particularly, fluoride may originate from dental plaque and be released by saliva, while various stimulus in the oral cavity may cause the concentration of chloride to fluctuate.

TABLE 4. Changes in the Concentration of Inorganic Ions in Saliva from Before and After Using Each Dentifrice (mM, n=10)

	Compound	Base line	1week	2weeks	4weeks
Control (mean±SD)	Fluoride	0.02±0.01	0.14±0.25	0.06±0.02	0.14±0.09
	Chloride	13.99±2.72	10.27±5.93	13.28±3.53	9.67±3.30
	Phosphate	5.40±3.01	2.28±0.91	3.03±1.68	3.20±1.17
Experimental (mean±SD)	Fluoride	0.06±0.14	0.10±0.11	0.37±0.64	0.09±0.05
	Chloride	9.63±4.93	5.34±4.41	12.28±3.29	15.16±7.82
	Phosphate	1.18±1.36	1.27±0.70	3.47±1.36	5.47±2.73

The findings in the present study showed improved oral conditions and halitosis, along with decreased concentrations of organic acids in the control group as well. Therefore, such effects may be attributable to some natural ingredients used as the base. In addition, the dentifrices containing MP extract showed greater efficacy than the control group, confirming that MP contributed to improved oral environment of the study subjects.

The present study used plaque index and gingival index to evaluate oral health status from before and after using liquid dentifrices containing MP extract. The results showed that the experimental group achieved greater decrease in dental biofilm level and gingival index than the control group. In the measurement of halitosis, the experimental group showed reducing effect on all volatile sulfur compounds, except (CH3)2S, was found, while total VSCs decreased by 41.03% relative to the baseline. With respect to the changes in the concentration of dental caries-causing organic acids in saliva, the experimental group showed decrease in concentrations of all organic acids, while also showing increase in the concentrations of inorganic ions chloride and phosphate relative to the baseline. Based on these results, it is believed that dentifrices containing MP extract used in the present study can contribute to improving oral condition and preventing oral diseases by reducing halitosis and causing changes in saliva components.

IV. CONCLUSION

The experimental results showed that plaque index

and gingival index in the experimental group decreased from baseline values of 1.87±0.66 and 0.57±0.45 to 1.63±0.88 and 0.17±0.12 at 4 weeks after using the liquid dentifrices containing MP extract. In the measurement of halitosis, total VSCs, excluding (CH3)2S, decreased by 41.03%. In the analysis of organic acids in saliva, total organic acids decreased from baseline of 10.79±9.39 3.41±3.18 after 4 weeks, while the concentrations of inorganic ions chloride and phosphate increased after 4 weeks. The results demonstrated that the dentifrices containing MP extract used in the present study had a positive contribution to the oral health status of the study subjects.

ACKNOWLEDGMENT

This work was supported the Ministry of Science and ICT of the Republic of Korea and the National Research Foundation of Korea (NRF-2017R1A2B4012865).

REFERENCES

1. Shoji T. Polyphenols as natural food pigmants: Changes during food processing. Am J Food Technol. 2007;2(7):570-81. doi: 10.3923/ajft.2007.570.581
2. Kim J, Choe E. Effects of selected herb extracts on iron-catalyzed lipid oxidation in soybean oil-in-water emulsion. Food SciBiotechnol. 2016;25(4):1017-22. doi: 10.1007/s10068-016-0164-x.
3. Choe EO, Kim JH. Effect of Tocopherols Present in Soybean Oil on the Antioxidant Activity of Peppermint Extract During Autoxidation of Oil-in-Water Emulsion. J Food Cook Sci. 2018;34(2):172-7. doi:10.9724/kfcs.2018.34.2.172
4. Seal T. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchusarvensis*and *Oenanthelinearis*of North-Eastern region in India. J Appl Pharm Sci 2016;6(02):157-66. doi: 10.7324/JAPS.2016.60225
5. Maheshkumar SK, Kirti SL. Determination of total flavonoids content and quantification of rutin in Momordicatuberosa (Roxb) Cogn. fruits by RP-HPLC. Asian J Trad Med. 2012;7(5): 220-25.
6. Mathur A, Prasad GB, Rao N, Babu P, Dua VK. Isolation and identification of antimicrobial compound from *Menthapiperita L.* Rasayan J Chem 2011;4(1):36-42.
7. Wiwattanarattanabut K, Choonharuangdej S, Srithavaj T. In vitro anti-cariogenic plaque effects of essential oils extracted from culinary herbs. J ClinDiagn Res. 2017;11(9): DC30-5.doi: 10.7860/JCDR/2017/28327.10668.
8. Raghavan R, Devi MPS, Varghese M, Joseph A, Madhavan SS, Sreedevi PV. Effectiveness of Menthapiperita Leaf Extracts against Oral Pathogens: An in vitro Study. J Contemp Dent Preact. 2018;19(9): 1042-6.
9. Takahashi N, Yamada T. Glucose and lactate metabolism by actinomycesnaeslundii. Crit Rev Oral Biol Med 1999;10(4):487-503. doi:10.1177/10454411990100040501
10. Sommer P, Klein JP, Schöller M, Frank RM. Lactate dehydrogenase from streptococcus mutans: purification, characterization, and crossed antigenicity with lactate dehydrogenases from lactobacillus casei, actinomycesviscosus, and streptococcus sanguis. Infect Immun. 1985;47(2):489-95.
11. Gent-Ruijters ML, Meijere FA, Vries W, Stouthamer AH. Lactate metabolism in propionibacteriumpentosaceum growing with nitrate or oxygen as hydrogen acceptor. Antonie Van Leeuwenhoek. 1976;42(3):217-28. doi:10.1007/bf00394118
12. Oude Elferink SJ, Krooneman J, Gottschal JC, Spoelstra SF, Faber F, Driehuis F. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. Appl Environ Microbiol. 2001;67(1):125-32. doi: 10.1128/aem.67.1.125-132.2001
13. Schweiger G, Buckel W. On the dehydration of (R)-lactate in the fermentation of alanine to propionate by clostridium propionocum. FEBS Lett 1984;171(1):79-84. https://doi.org/10.1016/0014-5793(84)80463-9
14. Horowitz HS, Heifetz SB. The current status of topical fluorides in preventive dentistry. J Am Dent Assoc. 1970;81(1):166-77.
15. Kirkegaard E. In vitro fluoride uptake in human dental enamel from various fluoride solutions. Caries Res. 1977;11(1):16-23.
16. Odajima T, Onishi M. A study on the promotion and suppression of demineralization of human dental hard tissues and hydroxyapatite. Connect Tissue Res. 1998;38(1–4):119–27.
17. Leung VW, Darvell BW. Artificial salivas for in vitro studies of dental materials. Journal of Dentistry 1997;25:475–84.
18. Park YD, Jang JH, Oh YJ, Kwon HJ. Analyses of organic acids and inorganic anions and their relationship in human saliva before and after glucose intake. Arch Oral Biol. 2014;59(1):1-11. doi: 10.1016/j.archoralbio.2013.10.006.