

## —原著—

## トレハロースタブレットの口腔保湿に対する効果の分析

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## Analysis of Oral Moisturizing Effects of Trehalose Tablets

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**Abstract**

In recent years, the elderly population has been growing, and subsequently, the number of patients complaining of xerostomia symptoms has been increasing. In this study, we focused on the moisturizing effects of trehalose. We developed a trehalose tablet as a novel supplement for xerostomia and compared it to a sucrose tablet for verification of its moisturizing effects. A total of 72 healthy female subjects were randomly assigned to receive either the sucrose or trehalose tablet, followed by the other. Subjects received a 14-question survey soon after each tablet had dissolved completely in the mouth. For objective evaluation of the tablets, compression and solubility tests were performed. Based on sensory (subjective) evaluation, the trehalose tablets provided significantly more moisturization in the mouth and a sense of salivation than the sucrose tablets. Therefore, trehalose may confer a greater effect on moisturizing the mouth than sucrose. In addition, the trehalose tablets were about 2/3 as soft and dissolved 2 to 3 times more quickly than the sucrose tablets. These subjective and objective evaluations suggest that trehalose is a better supplement for maintaining oral moisture than sucrose, and may therefore be useful as a therapeutic supplement. The use of this convenient and safe therapeutic supplement could help improve the quality of life of elderly individuals who are suffering from xerostomia by allowing them to better enjoy meals and conversation.

**和文抄録**

近年、高齢者の急激な増加に伴い、口腔乾燥症の症状を訴える患者も増加の一途を辿っている。本研究では、トレハロースの保湿効果に着目し、口腔乾燥のための新しい治療補助食品として、トレハロースタブレットを開発した。保湿効果の比較の為に蔗糖のタブレットも同時に開発し、効果について主観的、客観的に検証を行った。実験には72名の健常な女性に参加して頂き、タブレットを試食して口腔内で完全に溶解して消失してから、その後14項目の

アンケート調査を実施した。また、タブレットの物性について客観的評価として、圧縮試験及び溶解度試験も合わせて実施した。主観的評価結果から、トレハロースが蔗糖と比較して、有意に口腔内の保湿に影響を与えている可能性が示された。また、客観的評価より、トレハロースタブレットは、蔗糖タブレットと比較して2/3程度軟らかく、口腔内で2~3倍早く溶解することが示された。これらの結果より、トレハロースタブレットは、口腔内の保湿に寄与することが示唆された。このことから、口腔内の乾燥で悩む高齢者に対しても効果が期待でき、食事や会話への楽しみ、QOLの向上へと繋げていく可能性が示唆された。

## Introduction

In recent years, the elderly population has been growing rapidly, and accordingly, the number of patients complaining of xerostomia symptoms has been increasing. Xerostomia is an oral disease in which decreased saliva production results in oral dryness, possibly as a result of adverse drug reactions, aging, decreased muscular strength, the effects of diseases such as Sjögren's syndrome, diabetes and stress.<sup>1)</sup>

Treatments for xerostomia have generally included mouthwashes, lozenges, oral ointments, artificial saliva and oral drugs. However, these treatments fail to alleviate xerostomia symptoms resulting from multiple factors in some elderly patients, causing much concern over the appropriate response.

Trehalose is a nonreducing sugar with two glucoses joined by an  $\alpha, \alpha$ -1,1-glucosidic bond that is not found in starch. Furthermore, trehalose has anti-cariogenic characteristics.<sup>2)</sup> With a sweetness 45% that of sugar, trehalose is more frequently being used in Japanese and Western confectionery and other types of cooking.<sup>3, 4)</sup> It can also be used to protect proteins and cells against drying and freezing by replacing water.<sup>5, 6)</sup> There has been a report that adding trehalose to bacteria<sup>7)</sup> or transferring trehalose synthase into human fibroblasts confers a tolerance to desiccation.<sup>8)</sup> Trehalose is therefore often used as a moisturizer in cosmetics and other products.<sup>9, 10)</sup>

In this study, we focused on the moisturizing effects of trehalose and developed a trehalose tablet as a new supplement for treatment of xerostomia. We concurrently developed a sucrose tablet for comparing and verifying various aspects of these moisturizing effects.

## Materials and Methods

### 1. Subjects

As xerostomia is a common ailment among women,

we enrolled 72 healthy female subjects with a mean age of  $30.28 \pm 7.87$  years. Informed consent was obtained from all subjects. Because this was a primary experiment, individuals who were currently suffering from dry mouth were excluded.

### 2. Methods

Subjects were randomly assigned with a number to determine the order of tablet sampling. The even-numbered subjects were first directed to take a sucrose tablet, followed by a trehalose tablet, while the odd-numbered subjects were first directed to have a trehalose tablet followed by a sucrose tablet. Subjects were blinded to the ingredients of the tablets and the order in which they were taken. The two samples were labeled "A" for sucrose and "B" for trehalose (Figure 1). Between sampling, subjects rested for 5 min and rinsed to exclude bias based on the order of sampling. To exclude the effects of differences in tablet dissolution time, subjects were asked to spit out the remaining portion of the tablets that had not dissolved in the mouth after 1 min. Subjects received a 14-question survey soon after the second tablet had dissolved completely in the mouth (Table 1). Questions 1-4 evaluated both tablets (sucrose or trehalose) with



A: Sucrose, B: Trehalose

Figure 1: Trehalose & Sucrose samples

Table1: Questionnaire

Evaluation of trehalose or sucrose alone		Response	
1. Did the inside or your mouth feel moisturized?	Yes	No change	No
2. Did the inside of your mouth feel sticky?	Yes	No change	No
3. Did you sense salivation in your mouth?	Yes	No change	No
4. Did the inside of your mouth feel dry?	Yes	No change	No
Evaluation of appearance			
5. Which had a better shape?	A	Neither	B
6. Which had a better color?	A	Neither	B
7. Which had a better appearance?	A	Neither	B
Comparison of trehalose and sucrose			
8. Which dissolved better in your mouth?	A	Neither	B
9. Which stickiness did you prefer?	A	Neither	B
10. Which sweetness did you prefer?	A	Neither	B
11. Which was more refreshing?	A	Neither	B
12. Which moistness did you prefer in your mouth?	A	Neither	B
13. Which conferred a moisturizing effect?	A	Neither	B
14. Which did you prefer overall?	A	Neither	B

A: Sucrose B: Trehalose

the following three responses: “yes”, “no”, or “no change”. Questions 5-14 evaluated subjects’ preferences for “sample A”, “sample B”, or “neither”. The response rate was 100% and the surveys were completed anonymously. This research was approved by the Niigata University Faculty of Dentistry Ethics Committee.

### 3. Samples

Each compound (0.997g) was mixed with 0.003 g calcium stearate and compressed into a tablet. All manufacturing of the test tablets was consigned to the Product Development Center, Bourbon Corporation (Niigata, Japan).

### 4. Physical Measurements

Compression testing is useful for determining the behavior of materials under crushing loads. The specimen was compressed and deformation at various loads was recorded. Compressive loads and deformation were calculated and plotted as load-deformation diagrams, which were used to determine elastic limit, proportional limit, yield point, yield strength and, for some materials, compressive strength. Both tablets underwent compression testing according to standard methods using a plunger with a surface diameter of 8 mm.

### 5. Solubility Test

In order to provide an environment similar to the inside of the mouth, 20 ml of phosphate buffer (pH 7, or 8) heated to 37° C was placed on a shaker set at a rate of 80 rpm assumed to mimic the activity of the tongue.

Samples were added, and the time to dissolution was measured.

### 6. Statistical Analysis

For Questions 1-4, subjects that indicated a strong sensation for trehalose were compared with those who indicated a strong sensation for sucrose. Our null hypothesis that “dryness after consumption of trehalose and sucrose does not differ” was accepted at  $P > 0.05$ . Data were statistically analyzed by McNemar’s test, a test for differences in ratios for paired data. For Questions 5-14, Pearson’s chi-square test, a non-parametric test, was used to test the null hypothesis that “the proportion of subjects that preferred trehalose to those who preferred sucrose is comparable” at  $P > 0.05$ . The correlation between the response to Question 13 (about moisturizing effects) and responses to other questions was verified using the  $k$  coefficient to investigate sensations similar to the sensation of the moisturizing effect. Statistical analysis was carried out using Excel Tokei (Esumi Co., Tokyo, Japan).

## Results and Discussion

### Subjective Evaluation

#### 1. Comparison of subjects with strong moisture sensation for trehalose and sucrose

For the results for Questions 1-4, subjects were divided into four groups based on their responses: Group 1, “strong sensation for both”; Group 2, “strong sensation for sucrose only”; Group 3, “strong sensation

for trehalose only”; and Group 4, “strong sensation for neither”. Analysis of Groups 2 and 3 with McNemar’s test revealed a significantly stronger sensation for trehalose with respect to Questions 1 and 3 (Table 2). Therefore, the null hypothesis was rejected, which suggests that trehalose tablets provide significantly more moisturization in the mouth and a greater sense of salivation than the sucrose tablets.

2. Comparison of trehalose and sucrose

Pearson’s chi-squared test was used to analyze responses to Questions 5 to7 regarding shape, color, and appearance preferences. No significant differences

in the impressions of trehalose and sucrose were noted. Significantly more subjects indicated a stronger sensation for trehalose than sucrose in the three questions regarding moisturization (Questions 8, 12 and 13). Therefore, trehalose exerts a moisturizing effect. Moreover, the test results revealed no significant differences in the preference for trehalose or sucrose in response to Question 10 (Figure 2), indicating no influence of taste differences on the evaluation of moisturizing effects, although it has been demonstrated that taste preferences and sourness induced salivation.<sup>11), 12), 13)</sup> These observations might be partially explained by the fact that the sole comparison was conducted between the sample which is not taste sour and those with the taste sweet in this study. There is a possibility that a difference of the salivation is hard to be over the comparison between sweet taste samples. Though a difference did not appear by the question about the taste sweet, the moisturizing effect of trehalose was shown. From these results, effect of factor except the sweet taste is suggested.

3. Correlation between each question and the question on moisturizing effects (Question 13)

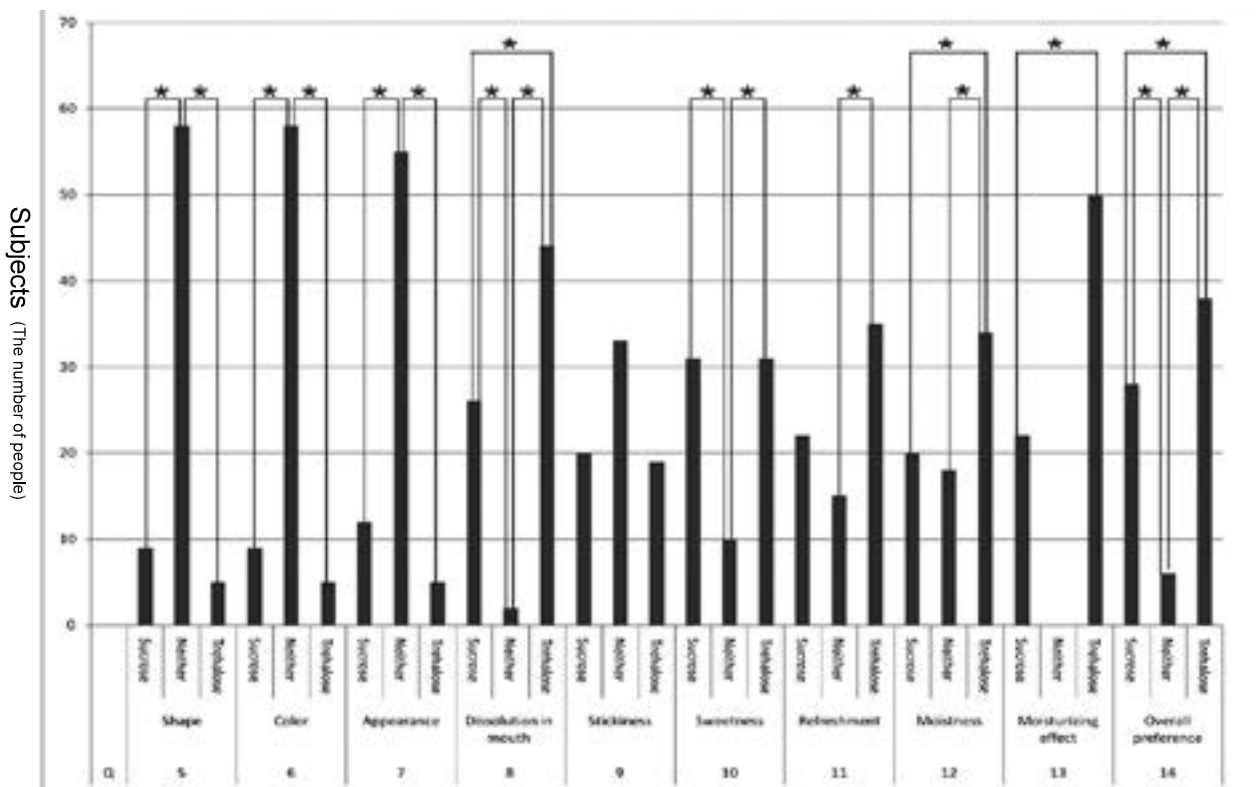
The survey in this study evaluated samples based

Table 2: Comparison of subjects with strong sensation for trehalose and sucrose

Question	P value
No. 1 Did the inside of your mouth feel moisturized?	p = 0.01*
2 Did the inside of your mouth feel sticky?	p = 0.70
3 Did you sense salivation in your mouth?	p = 0.03*
4 Did the inside of your mouth feel dry?	p = 0.16

\*P < 0.05 McNemar’s test

McNemar’s test	sucrose	
	Yes	No
trehalose	Yes	Group 1
	No	Group 2
		Group 3
		Group 4



\* P<0.05 Pearson'schi-square test

Figure 2: Comparison of trehalose and sucrose samples

Table 3: Correlation between each question and the question on the moisturizing effect(Question 13)

Question	kcoeff.
No. 5. Which had a better shape?	0.1
6. Which had a better color?	0.0
7. Which had a better appearance?	0.1
8. Which dissolved better in your mouth?	0.3*
9. Which stickiness did you prefer	0.1
10. Which sweetness did you prefer?	0.1
11. Which was more refreshing?	0.2
12. Which moistness did you prefer in your mouth?	0.5*
14. Which did you prefer overall?	0.4*

Question 13 is not included because these No. 5 to 14 questions were compared to Question 13.

on a variety of aspects, including dissolution, moistness, moisturization and refreshment. We thus investigated the correlation between the response to the question directly regarding the moisturizing effect (Question 13) and responses to other questions (Questions 8, 12 and 14). Analysis using the k coefficient revealed a close correlation for Questions 8, 12 and 14 with Question 13 (Table 3). These results indicated that subjects considered their mouths to be moisturized on sensing the dissolved tablet in the mouth or moistness.

#### 4. Age-based comparison

Next, to examine age differences in the sensory evaluation, results that were obtained from young women were compared with those of middle-aged women. The 72 subjects were divided into two groups: a group of 51 “young women” aged 20 to 39 years and a group of 21 “middle-aged women” aged 40 to 59 years. Generally, the disease rate of xerostomia was high in these age groups. Like the overall evaluation, analysis of Questions 1-4 using McNemar’s test revealed that young women had a significantly stronger sensation for trehalose in Question 1. Although no significant differences were noted, middle-aged women tended to have a strong sensation for trehalose in Question 3 (Table 4). From these results, “young women” tended to feel moisturizing effect than “middle-aged women”. It was suggested that it is strongly felt moisturizing effect when it is not mouth dryness. However, the elderly people can expect this effect because “middle-aged women” feels moisturizing. The study to determine the effect on elderly people is going to be performed in future.

Analysis of Questions 5-14 revealed no significant differences between the young and middle-aged women. The age difference in the responses was small, which was not statistically significant. It will be

Table 4: Comparison of results of Questions 1 to 4 based on age and order of sample consumption

	Age		Sample consumed first	
	20-30s	40-50s	Sucrose tablet	Trehalose tablet
Q1 Did the inside of your mouth feel moisturized?	0.02*	0.58	0.17	0.07
Q2 Did the inside of your mouth feel sticky?	0.65	1.00	0.39	1.00
Q3 Did you sense salivation in your mouth?	0.36	0.08	0.75	0.03*
Q4 Did the inside of your mouth feel dry?	0.77	0.13	0.29	0.55

\* P>0.05McNemar’s test

necessary to determine whether these small differences are due to aging, objective measurements on saliva volume and the state of oral dryness in further studies.

#### 5. Comparison based on order of sampling

We evaluated the difference in the responses to questions by comparison of the order of tablet sampling. Analysis of Questions 1-4 with McNemar’s test revealed that significantly more subjects who sampled trehalose first indicated a strong sensation regarding Question 3 (Table 4). These results might be occurred by sampling order. The time interval might be taken more. However, Pearson’s chi-square test, used to analyze Questions 5 to 14, indicated no effect of sampling order. Despite the slight difference in response to Question 3, the overall results were not substantially influenced.

The null hypothesis, “The proportion of subjects that preferred trehalose to those that preferred sucrose is comparable,” was thus rejected, and the results of subjective evaluation indicated that trehalose had a greater effect on moisturizing the mouth than sucrose.

#### Objective Evaluation

##### 1. Compression test

Measurement of the compression of the tablets at the same initial slope showed that the trehalose tablet had approximately 2/3 the hardness as the sucrose tablet. The initial slope appeared to be non-linear with a waveform, indicating some kind of compression deformation. As the plunger (tip) of the testing device had a surface diameter of 8 mm and the tablet surface

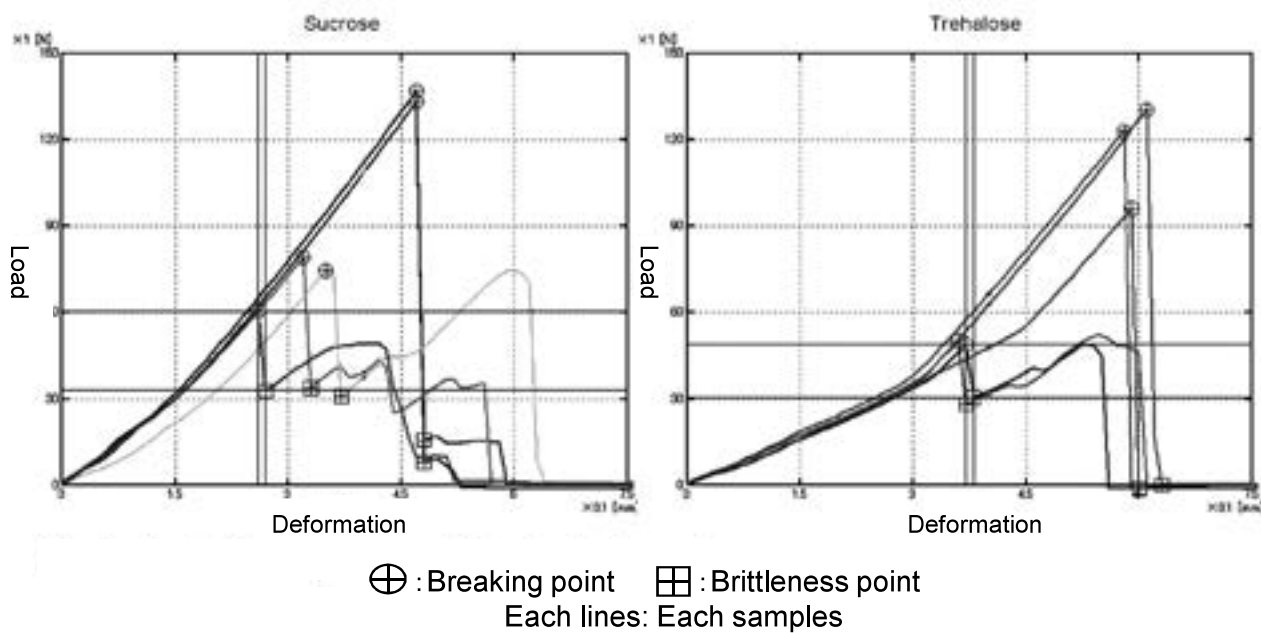


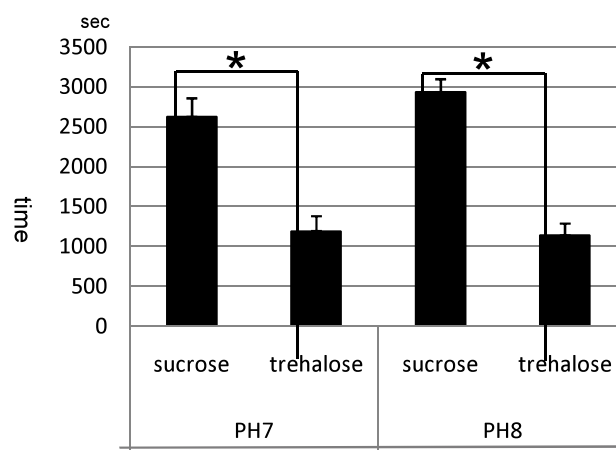
Figure 3: Compression test

had a gentle spheroid shape, they had an initial point contact that consequently increased as the tablet was crushed. This compression deformation could have occurred even without being divided into inner and outer layers. This phenomenon, however, ceased after the sucrose and trehalose tablets had been crushed by the spot where initial slope is over. The force against the tablets continued to rise sharply until breakage. Load at the breakage point was slightly smaller for the trehalose tablet; no differences were observed in their initial slopes (Figure 3). These results suggest that the trehalose samples break under a weaker force than the sucrose samples.

## 2. Solubility test

In the solubility test with phosphate buffers at pH 7, and 8, the trehalose tablets dissolved in 1,194sec, and 1,142sec, respectively, while the sucrose tablets dissolved in 2,627sec, and 2,938sec, respectively (Figure 4). At each pH level, the trehalose tablet dissolved 2 to 3 times more quickly than the sucrose tablet. The differences were significant between trehalose and sucrose.

These two objective findings reflect the subjective evaluation results of the stronger dissolution sensation in the mouth with trehalose. It was suggested that two properties of the softness and soluble influenced moisture retention. Although not shown in the results, oral moisture content following sampling of the same sample by 14 young women showed variability with



\* P&lt;0.05 Pearson's chi-square test

Figure 4: Solubility test

no differences between the sucrose and trehalose samples. As many reports on the methods for measuring the degree of oral moistness have provided few consistent results<sup>14,15)</sup>, and training is required for such measurements, establishment of a reliable and objective method for evaluating oral moisture is another future challenge.

The trehalose tablet developed in this study is intended as a supplement for xerostomia. It is appropriate as a therapeutic supplement, particularly in elderly individuals who have reduced physical strength, as the tablet is easily dissolved in the mouth. In addition, because trehalose has anti-cariogenic characteristics, it is not necessary to worry about oral

hygiene. The use of this convenient and safe therapeutic supplement can be expected to improve the quality of life of many elderly individuals who are suffering from xerostomia by helping them to better enjoy meals and conversation.

### Conclusions

These subjective and objective evaluations indicate that trehalose is a better supplement for maintaining oral moisture than sucrose, and may therefore be useful as a therapeutic supplement.

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