Sichuan Pepper Oil

(SalivaMoist™)

Stimulate salivary secretion,
reduce oral bacteria, and prevent foul breath

■SalivaMoist™-5
(Oil, Food Grade)

■SalivaMoist™-P5
(Powder, Food Grade)

■SalivaMoist™-P10
(Powder, Food Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver.1NY

Food ingredient for stimulating salivary secretion, reducing oral bacteria and preventing halitosis

Sichuan pepper Oil

1. Introduction

Sichuan pepper (*Zanthoxylum bungeanum*) is a plant belonging to Zanthoxylum, Rutaceae. Its skin has a characteristic pungent taste and flavor. It has been used in China as a traditional ingredient for its effects to improve stomach functions as well as its anti-inflammatory, anti-parasite, and perspiration effects. The fruit is red and approximately 3 mm in diameter. Usually, it is dried, and the inner black fruit is removed, and only the red skin is used. In China, people grind it into a powder to use it for cooking. They also mix it with roasted salt and add it to deep-fried food as Sichuan pepper-salt. Recently, this food ingredient has been attracting people's attention in Japan as a seasoning with a "biting spiciness" used in Chinese dishes such as the spicy Sichuan dish of tofu and minced meat. Sichuan pepper skin contains various components. One of them, hydroxy-α-sanshool, has an effect to stimulate salivary secretion and it has been reported to be effective in the improvement of dry mouth based on a study of AveDes K.K.[1)]

Oryza Oil & Fat Chemical introduces Sichuan Pepper Oil (SalivaMoist^{TM}) as an ingredient for oral care. Ingestion of oil containing hydroxy- α -sanshool extracted from the skin of Sichuan pepper fruit improves dry mouth and bad breath.

Figure 1 Sichuan pepper fruit skin

2. The Role of Saliva

Saliva is a fluid secreted in the mouth that plays an essential role in oral care. Each day, about 1,000 to 1,500 mL (equivalent to two or three 500 mL plastic bottles) is secreted. Saliva has three major functions.

The first are physical functions: lubricating function to moisten the surface of the mucous membrane and facilitate chewing, swallowing, and speaking; mucous membrane protection function to protect the mucous membrane from various stimuli with components contained in saliva; chewing assistance function to make food settle without sticking to the teeth and mucous membrane during chewing; and cleaning function to prevent food from sticking to the teeth and mucous membrane during chewing by covering the surface of the mucous membrane and teeth.

The second are chemical functions: digestive function to assist in the digestion of starchy substances by amylase, and dissolution function to dissolve taste components in food and help with the perception of taste, as well as buffering function to maintain a neutral environment in the mouth.

The third are biochemical functions: antibacterial function by enzymes and antibacterial substances such as lysozyme, peroxidase, and lactoferrin contained in saliva, and antibacterial factors such as antibodies known as IgA, as well as excretion function to reduce the concentration of some antibiotics and other drugs in the blood by excreting them into saliva.[2)]

3. Dry Mouth

Dry mouth is often associated with symptoms such as a dried oral cavity and sticky saliva, and in fact, these are deeply related to a decrease in the amount of saliva produced and changes in its quality.

Dry mouth can be classified into three types depending on the cause: cases caused by the dysfunction of the salivary glands themselves, such as Sjogren's syndrome or radiation xerostomia; cases caused by neurological or pharmaceutical causes; and cases caused by a systemic disease or metabolism problem.[3)]

The number of patients with subjective symptoms of dry mouth is increasing every year. It is reported that the majority of patients are women, especially those in their 50s to 70s. Figure 2 shows the results of a survey on 83 patients who visited the outpatient clinic for dry mouth at Chiba Hospital, Tokyo Medical and Dental University from August 2004 to May 2008. As for the number of patients by gender, 14 were males and 69 were females, about five times as many as males.[4)]

Dry mouth is caused not only by aging, but also by stress, breathing through the mouth, autonomic nervous system disorders, etc. In recent years, it has been reported that as fast food has become more prevalent, people eat without chewing that much, hindering salivary secretion and resulting in dry mouth.

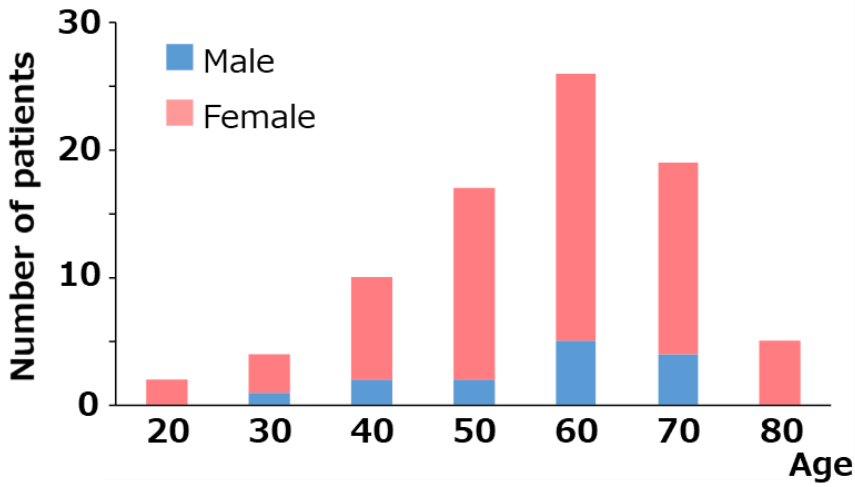

Figure 2 Number of patients visiting the outpatient clinic for dry mouth by age and gender at Chiba Hospital, Tokyo Medical and Dental University[3)]

4. Hydroxy Sanshool

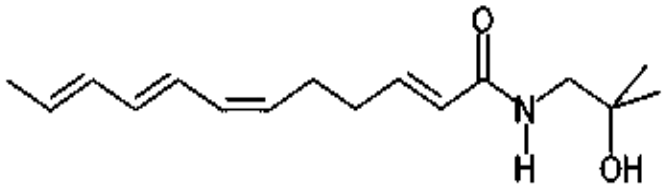

Figure 3 Structural formula of hydroxy- α -sanshool

Sichuan pepper contains various components. For example, Sichuan pepper fruit skin contains approximately 10% oil. It contains essential oil components such as geraniol and limonene as well as fat and oil components such as palmitic acid and palmitoleic acid.

The pungent components, characteristic to Sichuan pepper that people are attracted to, are hydroxy- α -sanshool, sanshoamide, etc.

For developing Sichuan Pepper Oil, Oryza Oil & Fat Chemical focused on hydroxy- α -sanshool.

Hydroxy-alpha-sanshool is a pungent component with a strong biting sensation which is characteristic to Zanthoxylum plants. Structurally, it is alkenyl amide where the hydroxy group is bonded to the tertiary carbon of the isobutyl group. The quality of the pungent taste of hydroxy sanshool is expressed as “fiery, biting” for hydroxy- α -sanshool and “biting, astringent, bitter” for hydroxy- β -sanshool.[5)]

5. TRP Channels and Fluid Secretion Mechanism

A transient receptor potential (TRP) channel works as a “sensor” protein in cells directly or indirectly via signals from receptors by getting activated by various physiological active substances. It has been clarified that the TRP channel interacts with various types of proteins. It is currently believed to work as a “base” for the formation of a signal complex to effectively transmit its own ion influx downstream as an intracellular signal in addition to working as a signal transmission element.[6)]

The TRP vanilloid receptor (TRPV) family was discovered when the vanilloid receptor (TRPV1), which is activated by capsaicin, a pungency of hot pepper, was first found. The TRPV family is activated by physical and chemical stimulations such as chemical substances like nitric oxide (NO), increase in temperature, changes in pH, mechanical irritations, and changes in the osmotic pressure.[7, 8, 9)]

TRP ankyrin (TRPA) has many ankyrin repeat (ANKR) structures at N terminal and has been reported as gene p120 involved in signal transmission and control of growth. [10)]

In mammals, only one type of TRPA1 has currently been identified and it is activated by changes in pH or temperature, oxidation stress, osmotic pressure, mechanical stimulus, etc.

TRPV1 and TRPA1 are called "nociceptive stimulus receptors" just like TRPV2 and TRPM8. These intensively express in sensory nerve cells and are believed to be involved in the reception of nociceptive stimulus or reduction of pain.[11)]

TRPV1 is high Ca^{2+} transmission, non-selective cation channel. It is activated by heat or acid (proton) that evokes pain to living organisms similar to capsaicin. Compounds that have the vanillyl group are generally called vanilloid and examples are piperine (pungency of pepper), capsiate (sweetness of the new type of hot pepper CH19), gingerol (pungency of ginger), shogaol (pungency of ginger), sanshool (pungency of Japanese pepper), allicin (pungency of onion and raw garlic). It has been clarified that the target of the action of these compounds is TRPV1 after TRPV1 was discovered.

TRPA1 was, in 2003, reported as a new temperature-sensitive TRP channel that is activated by nociceptive cold stimulus at 17℃ or lower.[12)] Also, it has been reported that TRPA1 is activated by allyl isothiocyanate (AITC), cinnamaldehyde, and allicin that are components of mustard, cinnamon, and raw garlic respectively. It has been reported that it is activated by an extremely large number of substances including aldehyde, methylparaben, and Ca^{2+} ion. Because many of them are known as substances that evoke pain, TRPA1 is believed to be clearly involved with the reception of nociceptive stimulus.

Based on these, TRPV1 and TRPA1 are believed to have an ability to transmit cation (Ca^{2+}, Na^{+}) influx downstream as intracellular signals as described above by getting activated using the substances above as ligand.

Three salivary glands, the parotid gland under the ears, the submandibular gland under the chin, and the sublingual gland under the tongue, are involved in salivary secretion.

Salivary glands consist of duct and acinar portions as shown in Figure 4 and saliva is secreted in the acinar portion.

The salivary secretion mechanism is believed as shown below.

The autonomous nervous system is in charge of information to evoke salivary secretion. When the parasympathetic nerves are stimulated, large amounts of salivary fluid with high fluid content are secreted and the neurotransmitter acetylcholine is secreted from the parasympathetic nerve endings. When acetylcholine binds to muscarinic receptor (M3 receptor) in the membrane on the base side of acinar cells, fluid in plasma components is secreted as saliva. This requires an increase in the intracellular Ca^{2+} concentration.[13)]

Figure 4 shows the mechanism of salivary secretion.

When hydroxy-α-sanshool activates TRPV1, Ca^{2+} flows into cells (❶). Increased intracellular Ca^{2+} opens the Ca^{2+} dependent K^+ channel existing in the membrane on the base side(❷). This induces intracellular K^+ to flow out from the cell. Being conjugated by that, $Na^+/K^+/2Cl^-$ cotransporter existing in the basal membrane is activated. As K^+ is returned into the cell because of this, extracellular Na^+ flows into the cell and then Cl^- also flows into the cell. There is a Na^+-K^+ pump (Na^+-K^+-ATPase) in the membrane on the base side and Na^+ is discharged from the cell by this pump (❸).

Increased intracellular Ca^{2+} opens the Ca^{2+} dependent Cl^- channel that exists in the membrane on the glandular cavity side simultaneously and intracellular Cl^- flows into the glandular cavity side (❹). As Cl^- creates an electrically negative state, Na^+ in plasma is attracted via the paracellular transport pathway. As a result, the osmotic pressure increases on the glandular cavity side. It is believed that the increase in the osmotic pressure moves the intracellular fluid as well as the fluid from plasma via the paracellular transport pathway (❺).[14)]

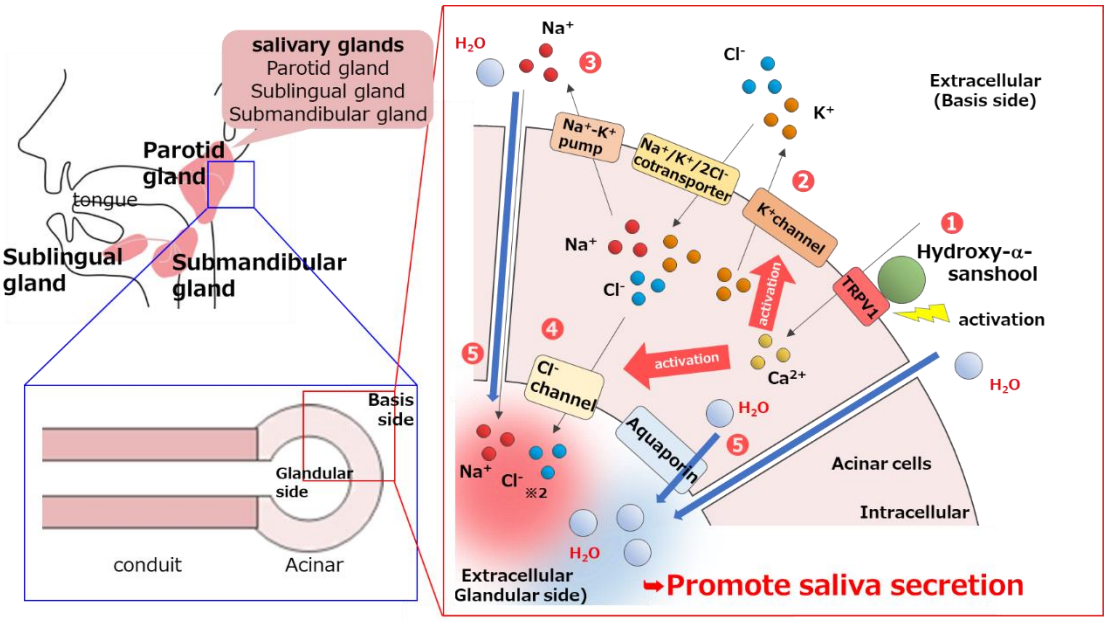

Figure 4 Position and structure of salivary glands as well as the salivary secretion mechanism

6. Activation of TRP Channel by Sichuan Pepper Oil

Based on the report above, it is believed that hydroxy sanshool binds to nociceptive stimulus receptors called TRPV1 and TRPA1 and applies stimulus to induce Ca^{2+} to enter cells, causing salivary secretion.

Therefore, we conducted an *in vitro* test to see if Sichuan Pepper Oil activates the TRP channel. As introduced on the previous page, TRPV1 has been confirmed to be the initial flow of the biological reaction when saliva is secreted. When it is activated by a substance like hydroxy sanshool, the salivary secretion mechanism will act. Therefore, it is assumed that carrying out a test to confirm that Sichuan Pepper Oil activates the TRP channel can check if Sichuan Pepper Oil is involved in the salivary secretion function.

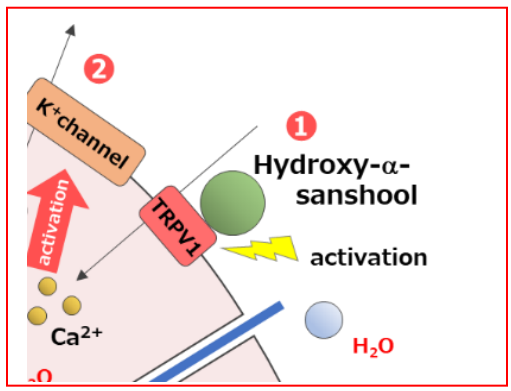

Figure 5 Extracted from the initial flow part of the figure of the salivary secretion mechanism in Figure 4

In the test, we used oil extracted from Sichuan pepper as the evaluation sample and capsaicin as the positive control and then we added them to human-derived cell strains in which we let the TRPV1 channel express. We checked the concentration of each sample when they activated the TRP channel by fluorescence assay using FLIPR assay.

Table 1 shows the results.

Oil extracted from Sichuan pepper produced by Oryza Oil & Fat Chemical was confirmed to have a tendency to activate the TRPV1 channel when it is added at a concentration of 100 μg/mL or more. However, positive results could not be obtained from a different company's product which had also been reported to act on the TRPV1 channel.

Table 1 The concentrations that each sample activates TRPV1 channel

Sample	Oil extracted from Sichuan pepper (Production areas A)	Oil extracted from Sichuan pepper (Production areas B)	Other companie's products	Positive control
Concentration to activate TRPV1	>100μg/mL	>100μg/mL	N.D.	1.0×10^{-7}M (30.5×10^{-3}μg/mL)

7. Effects of Sichuan Pepper Oil on Salivary Secretion (1)

We carried out a sensory test on our company employees to see if oil extracted from Sichuan pepper produced by Oryza Oil & Fat Chemical has any effect on salivary secretion using different forms of products and then aggregated the questionnaire results.

Test subjects were 16 to 20 males or females in their 20s to 70s and we evaluated three samples: SalivaMoist ™-5, SalivaMoist ™-P5, as well as SalivaMoist ™-P10. Test subjects ingested the recommended amount (50 mg, 100 mg) of each product, evaluated "stimulus", "refreshing feeling", as well as "the sensation of salivary secretion" in five degrees, and wrote down how long salivary secretion continued for.

Figure 6 shows the results.

The graph shows the questionnaire results regarding stimulus, refreshing feeling, as well as the sensation of salivary secretion. SalivaMoist™-P5 and -P10 in powder form were evaluated to provide a milder refreshing feeling and stimulus than the product in oil form due to the influence of cyclodextrin used as a filler.

Table 2 shows the results of the salivary secretion duration. Although the powder provided less refreshing feeling and stimulus, it was confirmed that it created the sensation of salivary secretion. It was also confirmed that the degree that test subjects felt about the effects of three samples increased concentration or dose-dependently.

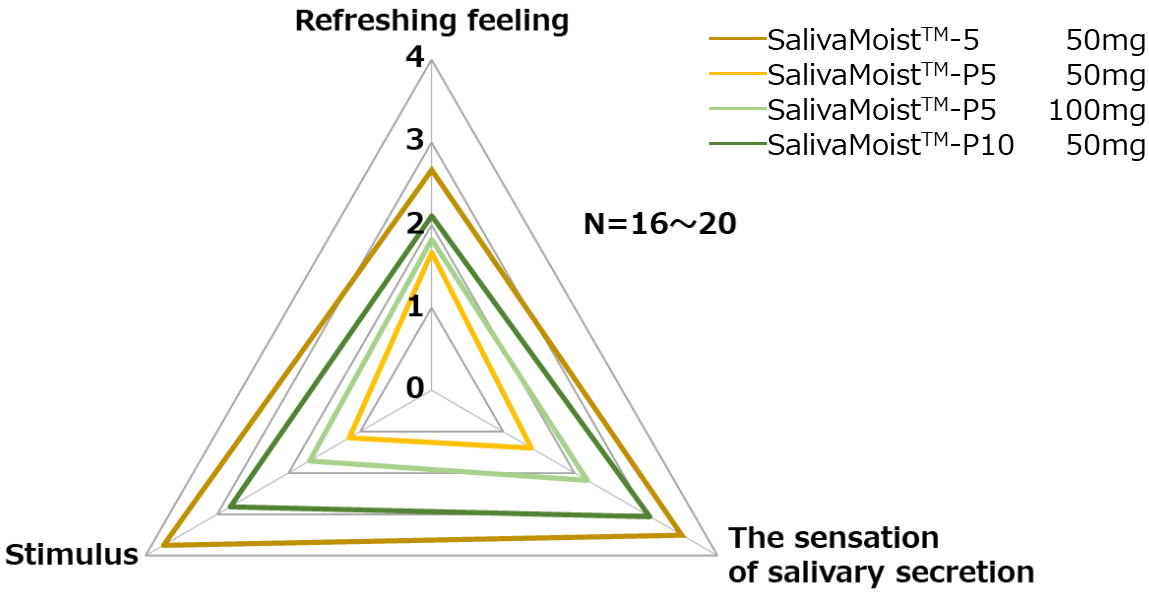

Figure 6 Results of sensory test on SalivaMoist™ in different product forms

Table 2 Salivary secretion duration of SalivaMoist™ in different product forms

Sample	SalivaMoist™-5 50mg	SalivaMoist™-P5 50mg	SalivaMoist™-P5 100mg	SalivaMoist™-P10 50mg
Salivary secretion duration	10min	4min	7min	8min

8. Effects of Sichuan Pepper Oil on Salivary Secretion (2)

We recruited carried out tests where in-house volunteers ingested Sichuan Pepper Oil to check its effect to promote salivary secretion.

In the first test, subjects ingested oil-form SalivaMoist™-5 or rice oil by putting two drops of the sample directly into their mouth using a dropper.

Test subjects were five males or females in their 20s to 30s. We conducted a Saxon test to measure the salivary secretion amount. In the Saxon test, test subjects chew a gauze before and after ingesting the sample and the weight of the gauze before and after chewing is compared to check the amount of saliva secreted. In order to eliminate any influence of individual differences in the speed of chewing gauze, we used a metronome and instructed the subjects to chew at a speed of once per second continuously for two minutes.

In clinical testing, we measured the amount of saliva in a resting state before ingestion of the sample, subjects rested for 10 minutes, and then they ingested the sample SalivaMoist™-5 or rice oil. Then, 30 seconds later, we measured the salivary secretion amount again.

As a result, although no increase or decrease of salivary secretion amount was observed before and after the ingestion of rice oil, ingestion of SalivaMoist™-5 was confirmed to increase salivary section by approximately 36% as compared to before ingestion (Figure 7).

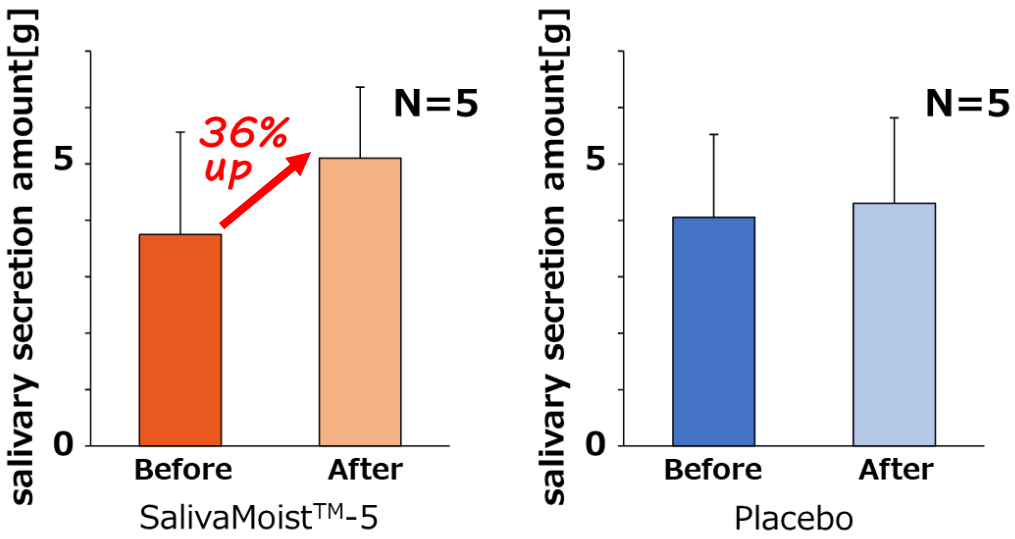

Figure 7 Changes in salivary secretion amount before and after direct ingestion of SalivaMoist™-5 or rice oil

Following the test, we conducted a long-term ingestion test. We filled hard capsules with powder containing 50 mg of powder form SalivaMoist™-P10 or 200 mg of a starch decomposition product as the placebo and had test subjects ingest them by swallowing for two weeks.

Test subjects were six males or females in their 20s to 60s. We checked their normal salivary secretion amount using a Saxon test for three days and selected people with stable values. To measure the salivary secretion amount, we conducted a Saxon test, same as the first test.

We measured the salivary secretion amount of test subjects in a resting state before starting the test. Test subjects ingested one hard capsule by swallowing it after breakfast every morning. We measured their salivary secretion amount weekly for two weeks.

As a result, no increase or decrease in the amount of salivary secretion was observed before ingestion as well as one and two weeks later in the group that only ingested the starch decomposition product. However, in the group that continuously ingested SalivaMoist™-10 for two weeks, the salivary secretion amount increased by approximately 34% one week later as compared to before ingestion, which was a significant increase. In addition, it was confirmed that the salivary secretion amount remained at the same level one week and two weeks after ingestion (Figure 8).

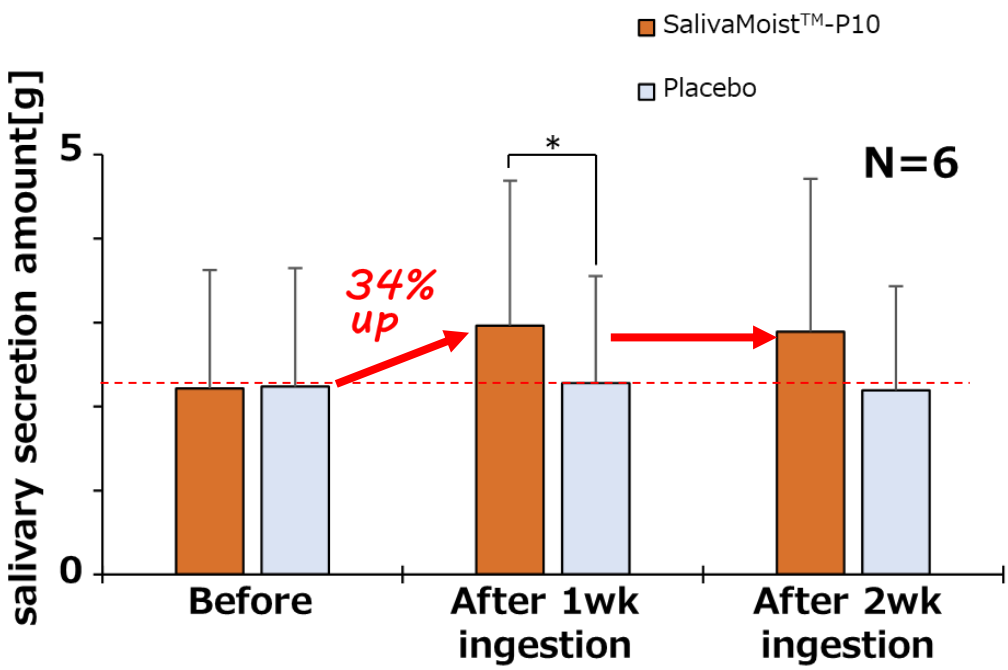

Figure 8 Changes in the salivary secretion amount after two weeks of continuous ingestion of SalivaMoist™-P10 or the starch decomposition product by swallowing

Next test data shown below was obtained via a test AveDes K.K. carried out with Tsurumi University.

Test subjects were nine non-smoking females (average age: 68.3±9.5) with dry mouth symptoms. They ingested three seamless capsules containing Sichuan Pepper Oil before every meal by chewing them in their mouth for four weeks.

Figure 9 shows the results of calculation of the amount of secreted saliva in the Saxon test.

As a result, a significant increase in salivary secretion amount was confirmed after the first ingestion of Sichuan Pepper Oil as compared to before ingestion.

Although the salivary secretion amount decreased after continuous ingestion for two and four weeks as compared to the first ingestion, the salivary secretion amount tended to increase as compared to before ingestion.

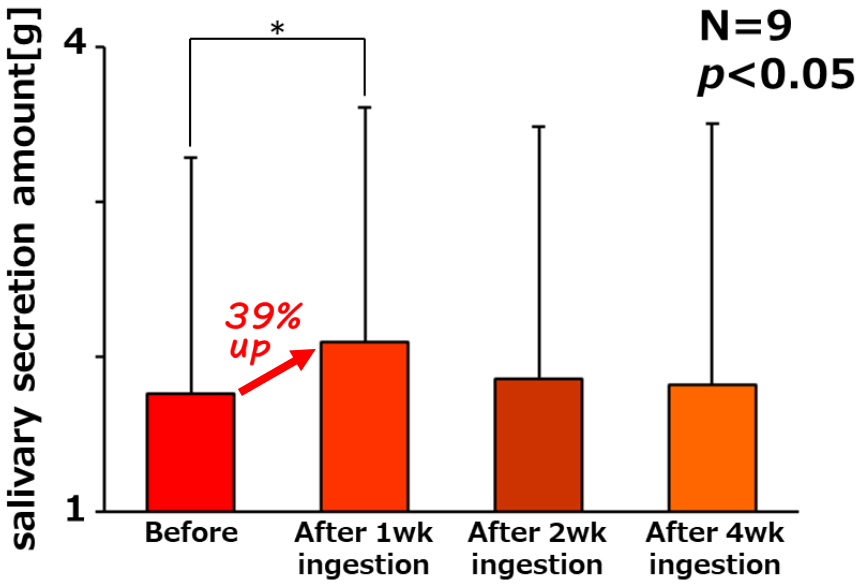

Figure 9 Results of the Saxon test AveDes K.K. carried out with Tsurumi University

We also evaluated changes in subjective symptoms of dry mouth in five levels regarding the four weeks of continuous ingestion of seamless capsules containing Sichuan Pepper Oil via a medical interview with a doctor.

The medical interview regarded the 30 items as shown in Table 3.

Among these items, an improvement tendency was observed in the eight items below. "1. Drying of the mouth", "15. feeling uneasiness about the inside of the mouth and teeth ", "16. Worried about poor conditions of the mouth and teeth", "17. Worrying about eyes to the mouth ", "22. Breathing with mouth ", "23. Worrying about halitosis ", "27. Rough lips ", "28. Worrying about inferiority of the corners of the mouth "

Especially, a significant improvement tendency was confirmed for No. 22 Breathing with mouth.

Table 3 Items discussed in the medical interview with a doctor

No. 1	**Drying of the mouth**
2	Pain and discomfort in the mouth
3	Feeling of insufficient salivary secretion
4	Difficulty swallowing saliva
5	Feeling of stickiness in the mouth
6	Saliva without appear sustainably
7	Feeling of stimulation in the mouth
8	Pain in the tongue
9	Difficulty conversing
10	Food without being eaten
11	Difficult to chew any food
12	Difficult to swallow any food
13	Difficulty in eating
14	Cannot get along with a person because of constantly being anxious about the mouth
15	**feeling uneasiness about the inside of the mouth and teeth**
16	**Worrying about something wrong with the mouth and teeth**
17	**Worrying about eyes to the mouth**
18	Worry about the mouth and not relaxed when eating in public
19	Have sensitive teeth and mouth
20	Worrying about water at the time of a meal
21	Strange taste
22	**Breathing with mouth**
23	**Worrying about halitosis**
24	Food stuck between the teeth
25	Shaking of teeth
26	Swollen gums
27	**Rough lips**
28	**Worrying about inferiority of the corners of the mouth**
29	Worrying about wrinkles of the whole face
30	Worrying about wrinkles around the mouth

9. Effect of Sichuan Pepper Oil to Inhibit Bacterial Growth

Next, we evaluated the effect of Sichuan Pepper Oil to inhibit the growth of bacteria that cause dental cavities (*S.mutans*) and periodontal disease bacteria (*P.gingivalis*).

Bacteria that cause dental cavities are related to dental caries. When food containing sugar is ingested, sticky polysaccharide (glucan) is formed by enzymes generated by the bacteria using sucrose. When glucan is formed, a lump is formed on the tooth surface with other oral bacteria. This is called plaque and is the greatest cause of dental cavity and its progression. [3)]

As Sichuan Pepper Oil has an effect to induce salivary secretion, secreted saliva removes bacteria that cause dental cavities and periodontal disease bacteria from the mouth. In addition, it can be considered as an ingredient for more effective oral care if it has an effect to inhibit the growth of such bacteria.

As a result of evaluation, Sichuan Pepper Oil was confirmed to have an effect to inhibit the growth of bacteria that cause dental cavities and periodontal disease bacteria.

In our experiment, we prepared four evaluation samples; A to D based on the district where the skin of Sichuan pepper fruit was produced as well as benzylpenicillin sodium as a positive control.

The growth inhibition rate was calculated using the formula as below.

$$\text{Growth suppression rate}[\%] = \frac{(\text{Sample's absorbance}) - (\text{Normal's absorbance})}{(\text{Control's absorbance}) - (\text{Normal's absorbance})}$$

When Sichuan pepper extract oil was added, each sample's rate to inhibit the growth of bacteria that cause dental cavities was confirmed to increase concentration-dependently as the final concentration increased from 0, 12.5, 25, 50, to 100 μg/mL.

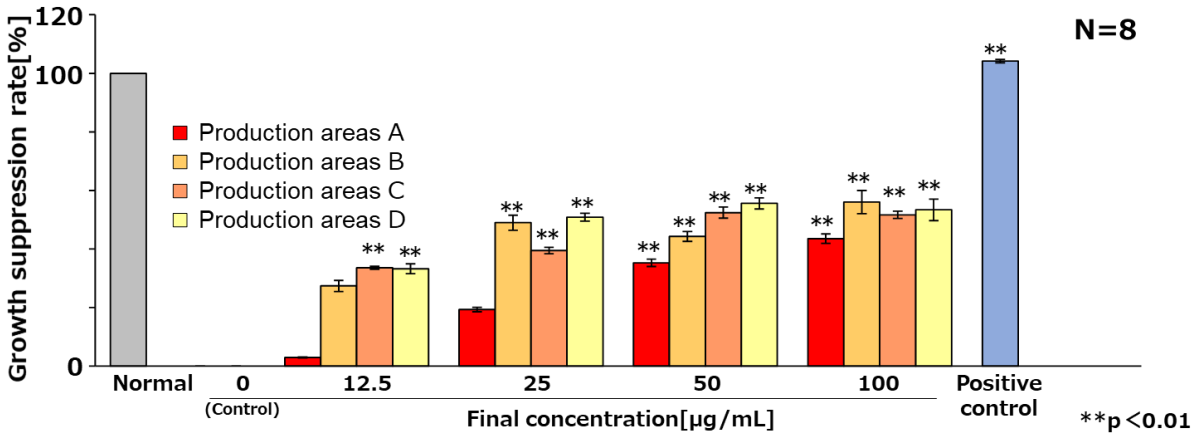

Figure 10 Effect to inhibit growth of bacteria that caused dental cavities

Regarding periodontal disease bacteria, although the effect value of adding Sichuan pepper extract oil was smaller than the effect on bacteria that cause dental cavities, the growth inhibitory rate was confirmed to increase depending on the concentration of the samples.

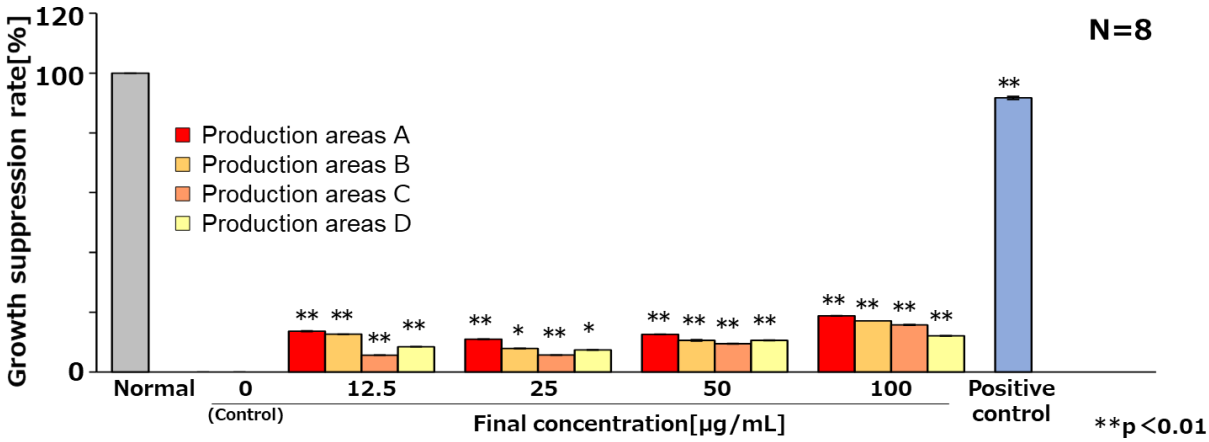

Figure 11 Effect to inhibit growth of periodontal disease bacteria

According to the results above, the ingestion of Sichuan Pepper Oil could inhibit bacteria that caused dental cavities and periodontal disease bacteria.

10. References

1) Study on the salivation effect of encapsulated food products containing Sichuan pepper oil Koufuchi Ryo, et al. (2019) Clin Exp Dent Res. 5:7-13

2)唾液の働き/唾液腺/唾液の分泌量について　ドライマウス | 歯科通信 | 中通歯科診療所 http://www.meiwakai.or.jp/n_sika/archive/sika/1493　(2020 年 10 月 12 日)

3)口腔乾燥症（ドライマウス）の分類―日本口腔粘膜学会雑誌. 14; 2（2008 年 12 月）: 86-88.

4)口腔乾燥症（ドライマウス）の臨床統計的検討-東京医科歯科大学千葉病院におけるドライマウス外来について-―日本口腔検査学会雑誌,1(1):40-43.

5) Hydroxy-a-sanshool activates TRPV1 and TRPA1 in sensory neurons　Jae Yeon Koo, et al. (2007) European Journal of Neuroscience, Vol. 26, pp. 1139‑1147

6)TRP チャネルの構造と多様な機能―生化学 第 81 巻 第 11 号，pp.962-983，2009

7) TRP ion channels in the nervous system　Moran, M.M. et al.（2004）Curr. Opin. in Neurobiol., 14,362-369.

8)TRPV channels as temperature sensors　Benham, C.D. et al.（2003）Cell Calcium,33,479-487.

9)TRPV4 calcium entry channel: a paradigm for gating diversity　Nilius, B. et al.（2004）Am. J. Physiol. Cell Physiol., 286,C195-C205.

10)An Ankyrin-like Protein with Transmembrane Domains Is Specifically Lost after Oncogenic Transformation of Human Fibroblasts　Jaquemar, D. et al.（1999）J. Biol. Chem.,274,7325-7333.

11)侵害刺激受容に係わる transient receptor potential vanilloid 1(TRPV1)及び transient receptor potential ankyrin 1(TRPA1)の活性化，制御メカニズム―YAKUGAKU ZASSHI,130(3),289-294

12) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures　Story G. M. et al. (2003) Cell,112,819-829

13)唾液分泌のメカニズム―杉谷博士 2009 年度口腔四学会合同研修会

14)Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Melvin, J.E., Yule, D., et al., Annu Rev Physiol 67: 445-469 2005.

11. Heat stability of SalivaMoist™

（1） Heat stability of SalivaMoist™

As a result of examining the thermal stability of the Sichuan pepper oil (rice bran oil additive-free product), the total hydroxy sanshool content was reduced by about 30% by continuous heating (100 ° C, 120 ° C) for 1 hour.

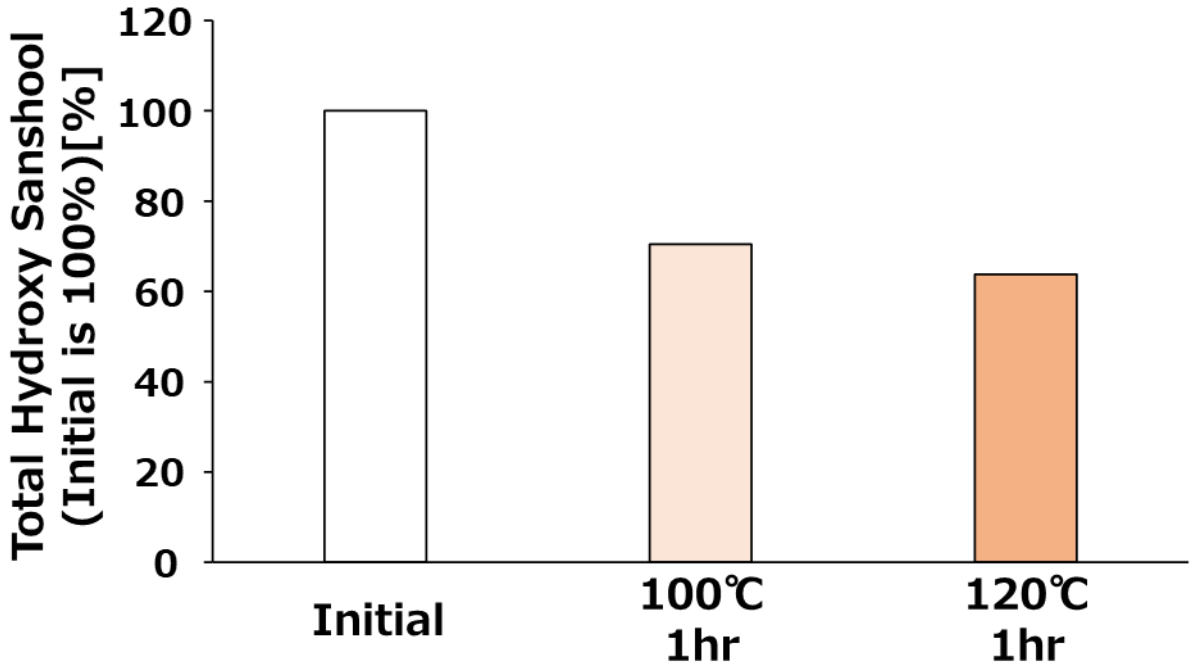

Figure12 Heat stability of Sichuan pepper oil（rice bran oil additive-free product）

12. Nutrition Information (SalivaMoist™)

Description	5(Oil)	P5	Analytical Method
Moisture	＜0.1g/100g	6.0g/100g	Karl Fischer Reagent Heat-drying at atmospheric pressure
Protein	0.2g/100g	0.4g/100g	Combustion Method *1）
Fat	99.4g/100g	4.0g/100g	Soxhlet extraction method Acid degradation
Ash	＜0.1g/100g	0.4g/100g	Direct Incineration
Carbohydrate －Sugar －Dietary fiber	0.4g/100g	89.2g/100g 79.9g/100g 9.3g/100g	*2） *3） Prosky Method
Energy	897kcal/100g	376kcal/100g	*4）
Sodium	0.3mg/100g	14.5mg/100g	Atomic absorption spectrophotometory
NaCl equiv.	＜0.01g/100g	0.04g/100g	

*1) Nitrogen, protein conversion factor：6.25
*2) Calculation：100 －(Moisture + Protein + Fat + Ash)
*3) Calculation：100 －(Moisture + Protein + Fat + Dietary fiber + Ash)
*4) Conversion factor：Protein 4; Fat 9; Dietary fiber; 4
Test trustee：SUNATEC
Date of analysis SalivaMoist™-5：September 2, 2020
SalivaMoist™-P5：SalivaMoist™-5：October 23, 2020
Test No.: SalivaMoist™-5：200821621-001-01
SalivaMoist™-P5：201009631-004-01

13. SalivaMoist™-Product Safety Profile

(1) Residual Agricultural Chemicals

SalivaMoist™-5 (without binder) is conformed to regulation stipulated for 504 residual agricultural chemical compounds. No residual agricultural chemicals detected as confirm by test trustee.

Test trustee：SUNATEC

Date of analysis：September 2, 2020

Request number：200821621-001-02

(2) Acute Toxicity（LD_{50}）

Acute Toxicity test was conducted accordingly to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products. Sichuan pepper oil（rice bran oil additive-free product）was orally administered to male and female ddY mice (aged 7 weeks old) at 1,000 mg/kg and kept for 14 days. No abnormalities and fatal event observed at 1,000 mg/kg. Upon autopsy performance, no abnormalities observed under macroscopic examinations. Thus, LD50 of Sichuan pepper oil（rice bran oil additive-free product） is deduced to be >1,000 mg/kg in both male and female mice.

(3) Mutagenicity test

Ames test was conducted to evaluate the mutagenicity of Sichuan pepper oil（rice bran oil additive-free product） using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and E.coli WP2 at concentration 4.88 – 5,000 μg/plate, no mutagenicity was observed.

14. Recommended Daily Dosage

The recommended daily dosage for SalivaMoist™-5 is 50 mg/time.

The recommended daily dosage for SalivaMoist™-P5 is 50～100 mg/time.

The recommended daily dosage for SalivaMoist™-P10 is 25～50 mg/time.

15. Packaging

SalivaMoist™-5 （Oil, Food Grade）

1kg、5kg Interior packaging：Coated can
Exterior packaging：Cardboard

SalivaMoist™-P5 （Powder, Food Grade）

1kg、5kg Interior packaging：Aluminumbag
Exterior packaging：Cardboard

SalivaMoist™-P10 （Powder, Food Grade）

1kg、5kg Interior packaging：Aluminumbag
Exterior packaging：Cardboard

16. Storage

Store at room temperature, avoiding high temperatures and direct sunlight.
Please use it immediately after opening.

17. Example

＜Food＞

SalivaMoist™-5
Expression：Edible Rice Oil、Pepper extract oil

SalivaMoist™-P5
Expression：Pepper extract oil / cyclodextrin、Arabian gum

SalivaMoist™-P10
Expression：Pepper extract oil / cyclodextrin、Arabian gum

※For food labeling, please check with your local health center or the Regional Agricultural Administration Bureau.

PRODUCT STANDARD

PRODUCT NAME : **SalivaMoist™-5** (FOOD)

This product is extracted with hexane from Sichuan pepper *Zanthoxylum bungeanum*.
It guarantees minimum of 0.1 % of Hydroxy sanshools.

Appearance	Yellow-brown to dark green liquid oil with unique pungent odor.	
Hydroxy sanshools	Min. 0.1 %	(HPLC)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content (Values are just a guide)
Rice Bran Oil	95%
Sichuan pepper Oil	5%
Total	100%

Expiry date	2 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high tempe rature and sun light.

PRODUCT STANDARD

PRODUCT NAME : **SalivaMoist^{TM}-P5** (FOOD)

This product is extracted with hexane from Sichuan pepper *Zanthoxylum bungeanum*.
It guarantees minimum of 0.1 % of Hydroxy sanshools.

Appearance	Greenish white to pale green white powder with slightly peculiar pungent odor.	
Hydroxy sanshools	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content (Values are just a guide)
Cycrodextrin	85%
Gum arabic	10%
Sichuan pepper Oil	5%
Total	100%

Expiry date	1 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high tempe rature and sun light.

PRODUCT STANDARD

PRODUCT NAME : **SalivaMoist^{TM}-P10** (FOOD)

This product is extracted with hexane from Sichuan pepper *Zanthoxylum bungeanum*.
It guarantees minimum of 0.2 % of Hydroxy sanshools.

Appearance	Greenish white to pale green white powder with slightly Peculiar pungent odor.	
Hydroxy sanshools	Min. 0.2 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content (Values are just a guide)
Cycrodextrin	70%
Gum arabic	20%
Sichuan pepper Oil	10%
Total	100%

Expiry date	1 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high tempe rature and sun light.

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact:

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Newly added data: 4-6. Effect of β-sitosterol 3-*O*-glucoside (BSG) Existing in Glucosylcermaide Fraction on Enzyme Expressions Involved in Ceramide Production in Human Keratinocytes, page 27.

Established Date: March 4, 2022
Revised Date: -

ORYZA OIL & FAT CHEMICAL CO., LTD.