CHEMICAL STUDIES ON WASABI (Eutrema Wasabi, Maxim.)

I. On the Pungent Substances of Wasabi.*

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長沢 徹・曽我 治：ワサビの化学的研究
第1報 ワサビの辛味成分に就て

I. INTRODUCTION

Wasabi (*Eutrema Wasabi*, Maxim.) is an perennial plant belonging to Cruciferae, and have an acrid taste throughout the whole plant, especially have a strong pungent and flavoring tastes in the root-stock. It is an indispensable and characteristic spice for Japanese raw fish dishes.

The Wasabi plant grows in the valleys with clean streams in a cold climate. It is cultivated on the pebble-stone beds with water running through them. The water should preferably be neutral or faintly alkaline, with little organic matters and with the constant temperature of 14°C throughout the year. The Wasabi flourishes only on a pebbly and barren ground, abhorring conventional fertilizers.[1] The above-stated characteristics of "Wasabi" make its growing an especially profitable enterprise for farmers in mountainous districts.

The recent improvement in staple food supply has been responsible for the flourishing of the cultivation of this spice-plant. Shimane Prefecture ranks fourth in the production of Wasabi in Japan, the first three being Shizuoka, Nagano, and Yamaguchi Prefectures. The Wasabi is said to have been planted for the first time in Shimane Prefecture 180 years ago in Kano-ashi County. The main producing areas at present are Kano-ashi, Mi-no, and Na-ka Counties, the production in San-be areas rather decreasing (see Table 1.).

The Wasabi is believed to have different flavors according to its own places of production. "Shizuoka-products" are preferred in Tokyo area, while in Osaka "Shimane-products" are highly valued. The reason for Tokyoites' choice is the plain taste and stimulating pungency of the former, that for Osakans' being the mild sweetness of the latter.

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Table 1. The Situation of Wasabi in Shimane Prefecture (1956)*

Producing Regions (Name)			Cultivation area (Tan)	Crop in one year (Kan)
County or City	Village	Section		
Yatsuka	Yakumo	Iwasaka	4.0	75
〃	〃	Kumano	2.5	30
Nogi	Hirose	Yamasa	1.0	15
Nita	Yakawa	—	3.0	150
Ii-ishi	Kakeai	Hata	5.0	80
〃	Tonbara	—	0.8	30
〃	Shishi	—	1.0	10
〃	Yoshida	Tai	1.0	15
Oda	—	Sahime	20.0	2,000
〃	—	Yamaguchi	5.0	50
Nima	Yunotsu	Yunotsu	1.0	15
〃	〃	Yuzato	5.3	330
〃	Omori	Mizukami	5.6	380
O-chi	O-chi	Kasubuchi	1.0	15
〃	〃	Hamabara	1.6	30
〃	〃	Sawatani	1.0	48
〃	Tsugayuki	—	1.0	30
〃	Asuna	—	10.0	250
〃	Ichiki	—	4.0	200
〃	Iwami	Hinui	1.4	120
〃	Sakurae	Nagatani	1.0	30
〃		Ichiyama	1.0	30
〃		Kawato	1.3	25
〃		Tanijugo	5.0	350
〃	Kawamoto	Mitani	1.0	25
〃	Soshiki	—	12.0	400
Ii-ishi	Akana	Tani	5.0	150
Naka	Asahi	Imaichi	5.0	325
〃	Anjo	—	12.0	40
〃	Hasa	—	12.0	560
Mino	Mitsu	Tomo	40.0	1,050
〃	〃	Futakawa	35.0	1,000
〃	Hikimi	Michikawa	10.0	320
〃	〃	Hikimi-Kami	160.0	9,000
〃	〃	Hikimi-Shimo	47.0	6,000
Masuda	Masago	Masago	1.0	100
Kanoashi	Nichihara	—	180.0	5,000
〃	Kakinoki	—	57.0	1,500
〃	Nanukaichi	Nanukaichi	65.0	2,800
〃	〃	Asakura	89.0	4,400
〃	Muikaichi	Muikaichi	54.0	2,400
〃	〃	Kuraki	10.0	500
Total			878.5	39,878

N. B. * This table was investigated by Shimane Administration of Forestry in February, 1956.

Wasabi plants are apt to be attacked with diseases of *Bacillus alliariae* Omori or *Phoma wasabiae* Yokogi.[2] Several valuable researches on the diseases of Wasabi by Nozu and Yokogi,[2] both the engineers of Shimane Agricultural Experiment Station, are to be greatfully remembered. Chemical studies on this special product of Shimane Prefecture have hitherto been almost entirely neglected. The authors of this paper, therefore, have commenced the chemical studies on Wasabi for the future improvement of the local varieties. In the present issue, the authors have treated with "Shimane-Wasabi" on the quantitative methods of the pungent substances and their distribution in the plant and also their seasonal variations.

The writers wish to express many thanks to both Engineers K. Yokogi in Shimane Agricultural Experiment Station and T. Matsuo of Forestry Administration Section of Shimane Prefectural Government for their sincere guidance and cooperation. The authors are also greatly indebted to the Futagawa Agricultural Cooperative Association (Mino-County) and Mizukami Wasabi Production Union (Oda-City).

II. CRITICISM ON LITERATURE

1. Pungent substances and their distribution:—The pungent substances of Wasabi were found first by Dr. Ch. Nagai[1] to be allyl mustard oil. Nagashima[4] has, recently found, in his particular research, that the acrid substances of Wasabi are composed not only of *allylisothiocyanate* (allyl mustard oil), which exists in abundance, but also of a minute quantity of *sec-butylisothiocyanate*. Tanaka[5] reported on the chemical components and spicy substances of Yamaguchi-Wasabi two years age. But the writers cannot understand his report that the same amount (0.059% upon air dried basis) of mustard oil is evenly distributed through the different parts (root-stock, root-hair, leaf-stem) of the plant. It has generally been known that the top of the root-stock is richest in spicy matter. Kojima et al.[6),7)], contrary to Tanaka, announced the content of the mustard oil in the root-stock to be ten times as rich as that in the leaf-stem. The writers also have obtained similar results.

2. Quantitative analyses of the pungent substances:—There are many methods regarding to the analyses of mustard oil. The mustard oil R.NCS, in general, is reduced by the reaction of *ammonia*, to white crystals of *Thiourea* derivative H_2N-CS-NHR, which is analyzed or determined[4] by paper chromatography[8] or spectrophotometer.

For the quantitative analysis of Allyl mustard oil there are gravimetric and volumetric methods, of which the latter is relatively simple. In the Japanese Pharmacopoeia,[9] therefore, the following procedure has been adopted for analyzing mustard oils in mustard

powder.

The glucoside *Sinigrin*, the parent body of Allyl mustard oil, is hydrolyzed by the enzyme *Myrosinase* in the plant and the generated Allyl mustard oil (*Allyl isothiocyanate*) is subsequently distilled into *Ammoniacal-Ethanol*, and forms *Thiocyanamine*, to which certain quantities of *Silver nitrate* solution is poured and warmed on water bath. The residual (excess of) *Silver nitrate* is titrated, and then the quantities of Allyl mustard oil are calculated. The chemical reactions are as follows:—

$$C_3H_5NC\begin{matrix}\diagup S\text{-}C_6H_{11}O_5\\ \diagdown O\text{-}SO_3K\end{matrix} + H_2O \longrightarrow C_3H_5NC:S + C_6H_{12}O_6 + KHSO_4$$

Sinigrin — Allyl mustard oil

$$C_3H_5NC:S + NH_3 \longrightarrow C_3H_5\text{-}NH\text{-}CS\text{-}NH_2$$

Thiocyanamine

$$C_3H_5NH\text{-}CS\text{-}NH_2 + 2AgNO_3 + 2NH_3 \longrightarrow C_3H_5NH\text{-}CN + Ag_2S + 2NH_4NO_3$$

The points that require the greatest attention in the analysis of Allyl mustard oil are as the following:—

(1) to reveal the pungent matter completely,

(2) to catch perfectly the revealed pungent matter.

It is generally believed among Japanese cooks and housewives the best way to draw out full flavor and pungence from Wasabi is to crush it gently on the porcelain grater making clockwise motion repeatedly.

Their empirical preference of porcelain graters to brass or copper graters is not without a scientific basis, since the reaction of copper on Allyl mustard oil may produce *Allyl cyanide*[1] with evil odor.

$$CH_2:CH\text{-}CH_2\text{-}N:C:S + Cu \longrightarrow CH_2:CH\text{-}CH_2\text{-}CN + CuS$$

Allyl mustard oil — Allyl cyanide

There are several opinions on the optimum temperature for the action of decomposing enzyme *Mirosinase* upon *Sinigrin* is 37°C[10] or 70°C[11]. But Garbit[12] had found that the active power of the enzyme decreases at 50～60°C. On the other hand, Kojima[6),7)] has treated several portions of Wasabi at the temperatures between room to 80°C for 15 minutes, and showed the one treated at 50°C had the maximum yield of Allyl mustard oil. Thus we should pay ample attention to the pre-treating on the analysis of Wasabi. At the same time, it is important to estimate completely without loss of the generated Allyl mustard oil. Taking above-mentioned points into full account the writers have performed the following researches.

III. EXPERIMENTAL PART

1. Samples:—The samples taken for these experiments are both red-stem species, cultivated in Oda city, of which (A) is the "Shigaku-products" collected early in January, and (B) is the "Mizukami-products" gathered at the end of April this year (1957) (about two years of age). (see Fig. 1.). (A) were winter samples and relatively small in both leaves and stems, but (B) were in full bloom, so that the flower-stems expanded very long and the leaf-stems had grown above four times as long as those of (A). The results of the measurements are shown in the Table 2.

2. Reagents:—(1) *Ferric ammonium sulfate* Solution (*Iron alum* as an indicator):—8g. of *Ferric ammonium sulfate* is dissolved in distilled water to make 100cc. solution.

(2) 1/10N-*Silver nitrate* Solution:—17.5g. of *Silver nitrate* is dissolved in 1,000cc. of water, and the normality of the solution is estimated by titration with 1/10N-*Sodium chloride* Solution.

(3) 1/10N-*Ammonium thiocyanate* Solution:—Dissolve about 8g. of *Ammonium thiocyanate* in 1,000cc. of water and determine its normality by titration with 1/10N-*Silver nitrate* Solution.

3. New Method of Analysis:—After the fresh

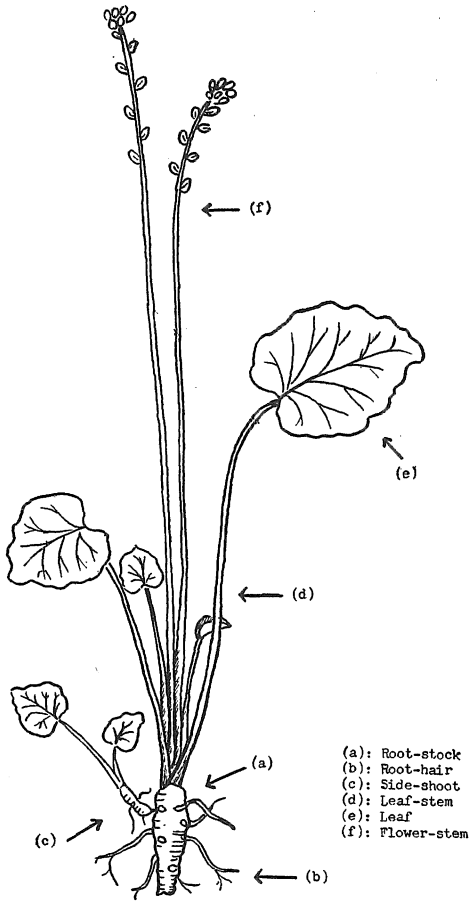


Fig. 1. Botanical Form of Wasabi

Table 2. States of the Growth of Wasabi

Sample	A (Winter Period)	B (Spring, Flowering Season)
Native Land	Shigaku	Mizukami
Date	(32. I. 6)	(32. IV. 29)
Species	Red-stem	Red-stem
Wt. of One Stump (g.)	39.2 (100%)	117.2 (100%)
Leaf	6.8 (27.2%)	19.0 (16.2%)
Leaf-stem	10.6 (27.2%)	43.0 (36.7%)
Flower-stem	—	20.2 (17.3%)
Root-stock	14.8 (37.8%)	17.1 (14.6%)
Root-hair	7.0 (17.8%)	4.6 (3.9%)
Side-shoot	—	13.3 (11.3%)
Nos. of Leaf-stems	29	12
* Length of Main Leaf-stem (cm.)	11.8	35.3
Nos. of Flower-stem	—	3
* Length of Main Flower-stem (cm.)	—	61.3
* Length of Root-stem (cm.)	6	9.5

N. B. * Means average value of 10 stumps of A, and 15 stumps of B, respectively.

Wasabi plant was washed with water to remove any soils attached, the whole plant was weighed and then separated into different portions, i. e. leaves, leaf-stems, root-stocks, and others and measured respectively.

The leaves were cut finely, root-stocks and others were cut into pieces one cm. long. Take, as samples, 5g. of root-stocks or root-hairs, of 20g. of leaves or leaf-stems respectively, and crush them, with 70cc.of water, using a mixer for two minutes at the speed of 13,000 revolutions per minute. Then, each crushed sample above was poured into a round-bottomed flask of 500cc. -capacity and kept standing for two hours at 25°C, being shaken a few times. Then it was distilled with Wagner's bulb and Liebig's condenser of 24cm. length and the tip of the condenser was dipped slightly into the reagents contained in a 100cc-messflask. (see Fig. 2.).

The reagents in the above receiver consisted of 20cc. of the mixture of concentrated *ammonia* solution and 95% *ethanol*, each of equal volume. The distillation was commenced carefully and slowly at first, and when the mustard oil appeared in the condenser, the reaction products were heated strongly for 15 minutes until 50cc. of the distillate was obtained. When the distillation was over, allyl mustard oils, adhering to the wall of the condenser and to the dipped tube, must be collected thoroughly into the receiver.

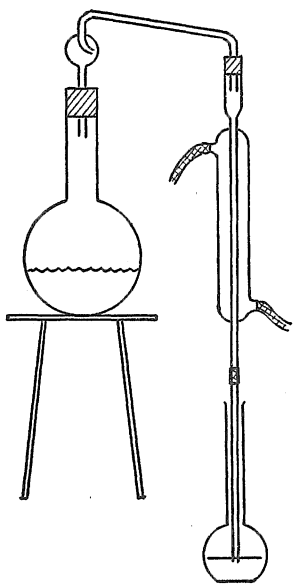


Fig. 2. Distillation Apparatus

Now, pour 20cc. of 1/10N-$AgNO_3$ solution into the receiver and warm the content on water bath for one hour, when black precipitates of *silver sulfide* are deposited. The content was then cooled to the room temperature and necessary amount of water was added so that the whole content attain the volume of 100cc. Take out 50cc. of the above liquid with a pipette into Erlenmeyer flask together with 5cc. of *iron alum* solution and 6cc. of concentrated *nitric acid*. And the mixture was titrated with 1/10N-*ammonium thiocyanate* solution until red color appeared. By determining the quantity (cc.) of the *silver nitrate* consumed, the allyl mustard oil can be estimated as the following descriptions—Each volume (cc.) of 1/10N-*silver nitrate* solution is equivalent to 4.955mg. of *allyl isothiocyanate* (allyl mustard oil).

When 5g. of the sample is taken and the volume of the consumed 1/10N-$AgNO_3$ is $x$cc., the quantities of allyl mustard oil are obtainable with the following relation:—

$$\text{Amount of Allyl mustard oil } (C_3H_5NCS) = \frac{4.955 \cdot x \cdot 100}{2500}$$

4. Results of the Experimentations:—(1) The amount of allyl mustard oils contained in each organ of fresh "Winter-Wasabi" (A) and "Spring-Wasabi" (B) is shown in the Table 3. The writers have found that the pungent substance is contained in higher percentage (0.17%) in the root-stocks and lower (0.02%) in leaf-stems. It is remarkable that the "Winter-Wasabi" contained twice (0.17%) as much spicy substance as the blooming (0.09%) "Spring-Wasabi".

Table 3. Content of Mustard oil in each Part of Wasabi (A, B)
(% upon fresh basis)

Sample	Leaf	Leaf-stem	Flower-stem	Root-stock	Root-hair
A	0.061	0.027	—	0.171	0.075
B	0.021	0.018	0.028	0.099	0.079

(2) The quantities of mustard oil in each portion of the root-stocks:— Each root of the "Spring-Wasabi" (8 stumps, mean length 9.2cm., total weight 105.7g.) was cut into three equal parts in length and each part was numbered I, II, III from top to bottom. (see Fig. 3).

The amounts of allyl mustard oils in each part (I, 32.7g.; II, 43.0g.; III, 30.0g.) were measured by above-stated methods, the results being shown in the Table 4. The writers have reason to believe the maximum amount of the spicy substance is to be found in the upper part of (II) (0.17%) and the minimum amount found in the lower extreme of (III) (0.02%).

Table 4. Content of Mustard oil in each Section of Wasabi (B) (% upon fresh basis)

Section	I	II	III	Mean
(i)	0.103	0.159	0.017	0.093
(ii)	0.110	0.183	0.020	0.104
Mean	0.107	0.171	0.019	0.099

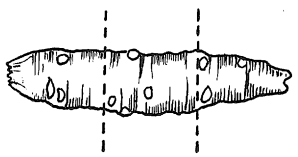


Fig. 3. Dividing Part of Root-stock

(3) Transformation of the mustard oil after the crushing of Wasabi:— Taking the "Spring-Wasabi" (B) II as the samples, the writers estimated the mustard oil in each sample in the next three ways: (i) immediately after crushing, (ii) after letting it stand for two hrs. at 25°C, (iii) after 12 hrs. enclosed at room temperature (about 15°C). The results are indicated in the Table 5. We have found that the method (i) shows the maximum value.

Table 5. Estimation of Mustard oil under various Treatment* of Wasabi (B)-II (% upon fresh basis)

Time standing	Mustard oil (%)
(i) Analyzed subsequently	0.194
(ii) At 25°C/2 hrs.	0.160
(iii) Room temp./12 hrs. enclosed, then at 25°C/2 hrs.	0.130

N. B. * Middle section of "Spring-Wasabi" as the sample.

IV. SUMMARY

The authors have made the above researches into the allyl mustard oil, the pungent substances of Wasabi (*Eutrema Wasabi*, Maxim.), with a view to contributing to the improvement of the cultivation method of the said plant in Shimane Prefecture, and they summarize their present views as follows:—

1. The amount of allyl mustard oil contained in each part of the whole Wasabi plant was noticed to exist in the following order:

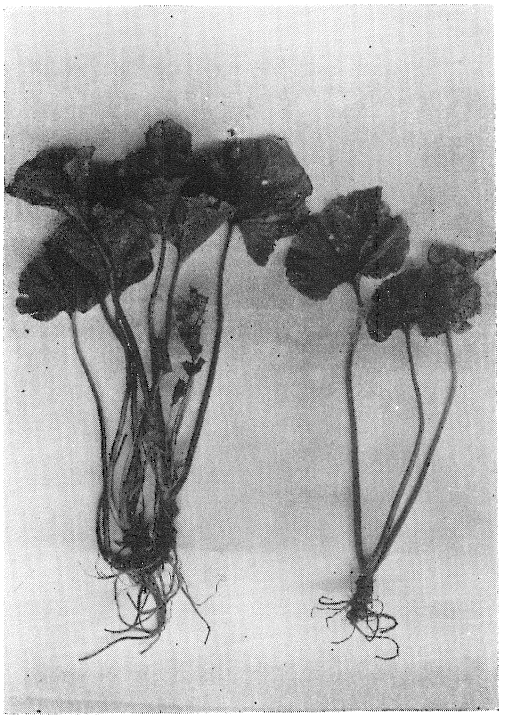
Root-stocks > Root-hairs > Leaves > Leaf-stems.

2. As regards the seasonal variation of the quantities of allyl mustard oil in the root, the writers have certified the "Winter-Wasabi" contains twice as rich allyl mustard oil

as the "Spring-Wasabi," and that the amount of the oil diminishes markedly with the blooming period. (as 0.17 : 0.09).

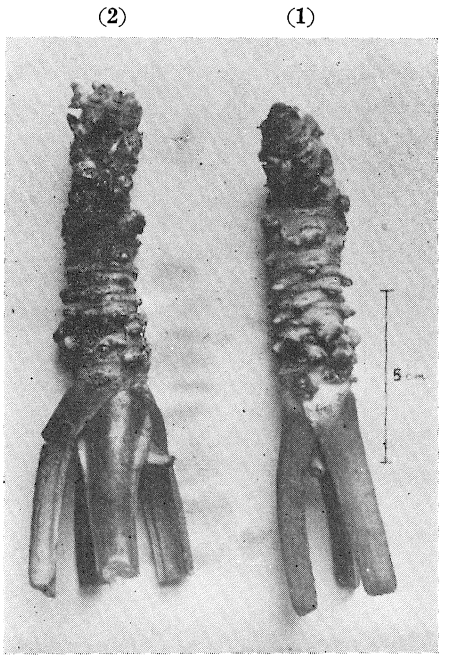
3. The authors have noticed that the allyl mustard oil is most richly concentrated at the upper-middle part of the root-stock and also that the lower end of the root contains the smallest amount. This fact seems to support the validity of the popular belief that the root-stock of Wasabi should be crushed on the grate from the top.

4. In the analyses of allyl mustard oil after crushing of the Wasabi-roots the writers have perceived that higher values are obtained in proportion to the shortness of time for which each sample is kept standing.[13] Since there exist no literature regarding this last mentioned point, the authors would like to make further researches in details in the future. (3. XI. 1957).



A B

Photograph of Wassabi
(*Eutrema Wasabi*, Maxim.)
(Produced in Nichihara, on June 28, 1956)
A : Diseased plant
(*Phoma Wasabiae*, Yokogi)
B : Normal plant (*Ao-Kuki* Species)



Wasabi-root
(1) "Shimane No. 3"
(2) Conventional species

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