On the Chemistry of Japanese Plants X. Utilisation of Plant Ash in Chemical Industries I.

By

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"Koji" which is made from "Tane-koji" or Moyashi, leaven and steamed rice, is mixed with steamed rice in order to make Sake, the common beverage of Japan. In the process the rice starch is converted into alcohol by the enzymatic action of Aspergillus oryzae grown in the Koji.[1]

Tane-koji, a yellowish green powder consisting of the spores of Aspergillus oryzae, is prepared both in Sake breweries and in special works, as it is used for various purposes in Japan besides Sake making.

In the preparation of Tane-koji,[2] the polished rice is boiled by steam; to it are added 1 m. of rice and 0.2 m. of a plant ash, and the whole is packed in straw matting and placed on stands in the kiln. After the lapse of ten days, mould developes.

The writers have interested themselves in the process of the preparation of Tane-koji and especially in the use of plant ash. The Koji works in Kyoto use for making Koji plant ash prepared by burning in air the young twigs of the Hoso-tree, Quercus senata Thumberg, grown at Ohara, near Kyoto City, gathered in summer and dried in the shade, but the ash from soy bean stems is usually employed for making an inferior kind of Koji. We also learn from "The History of Sake-making at Nada[3]" that Tane-koji is prepared there from steamed rice and plant ash obtained from the young leaves of the Nara or Tsubaki which are gathered in summer and dried in the shade.

1. K. Yabe & S. Sugiyama: The Brewing Industry of Japan; Industrial Japan 530-547 (1929); R. W. Atkinson: Studies on Sake. (1880-1881).
2. J. J. Hoffman: Sake Brewing in Japan (1872).
3. In Japanese, in referring the writers' thanks to Mr. Y. Tatsuma.

According to R. W. Atkinson[1] who has made a comparative study of polished rice and Koji by analysis, the formation of Koji is an illustration of the growth of the mycelium of a fungus which uses the starch of the rice grain as food.

Table I

	Whitened rice dried at 100°	Koji dried at 100°
Starch	82.27 %	56.00 %
Cellulose	4.79 „	4.20 „
Dextrose & dextrin	1.91 „	28.90 „
Fat	0.49 „	0.43 „
Albumenoides	9.45 „	9.84 „
Ash	1.09 „	0.61 „

However, the writers' opinion is that some of the ash constitutents in Koji have an intimate connection with the growth of the organism; in other words, they play an important rôle in the conversion of the starch molecule of the rice into CO_2 and water and other organic compounds of lower molecular weight, and the energy released by the process is used for the growth of organism.

Referring to the literature,[2] we noticed that the ash prepared from Sake-yeast and spores of Koji was mostly composed, as may be seen in Table I, of P, K & Mg.

The results[3] of analysis of the ash from yeasts of various sources also show that the elements K, P, Ca and Mg are its chief constituents.

Table II

	Ash of yeast	Ash	
		Sake-yeast	Spores of Koji
K_2O	23.33 — 39.5 %	31.9	45.9
Na_2O	0.5 — 2.26 „	17.6	4.1
CaO	1.0 — 7.58 „	trace	1.1
MgO	3.77 — 6.34 „	5.9	4.4
Fe_2O_3	0.06 — 0.7 „	—	4.9
P_2O_5	44.8 — 59.4 „	44.5	39.6
SO_3	0.57 — 6.35 „	0.4	2.0
SiO_2	0.92 — 1.88 „	—	0.4

1. Loc. cit.
2. K. Kurono: Handbook of Physiology of Fermentation (Japanese) (1927) p. 420.
3. E. Duclaux: Traité de microbiologie III, 135 (1900); H. Euler & P. Lindner: Chemie der Hefe (1915) p. 72.

From these reports, it may be supposed that such inorganic elements as K, P, Ca & Mg, which constitute the greater part of the ash of yeast and Koji have an intimate connection with the development of Aspergillus oryzea which uses the starch of the rice as food, and accordingly these inorganic materials are supplied to accomplish the metabolism of the starch in the living cells from the plant ash employed in the preparation of Tane-koji. This idea is partly illustrated by reference to the analysis of the ash from soy bean stems and the Nara-tree.

Table III

	K_2O	Na_2O	CaO	MgO	P_2O_5	SO_3	SiO_2
Soy bean stems	15.3	2.1	44.6	15.3	10.0	6.4	5.5
Nara-tree	44.6	2.2	40.0	7.9	4.3	0.2	3.3

The ash of the Hoso-tree is, therefore, used simply as a source of potassium, calcium, magnesium and phosphorus, which are important inorganic materials in the metabolism of starch in the Aspergillus oryzae, and it may be assumed that the reason why the plants are gathered in summer is that there is an abundance of these elements in the plant tissues in this season. The present study of the seasonal variation of ash constituents, was therefore undertaken by the writers to confirm this supposition.

The samples used for the experiment, twigs one or two years old, were gathered once a month from 10 to 11 o'clock on fine mornings from 18 trees growing on a mountain slope about 120 m^2 in area in the Gosho-tani forest, Hanazono, Iwakura, Kyoto, located about 4 miles from the laboratory.

Each sample was divided into two parts, leaves and twigs, and weighed and dried to constant weight in an air bath at 105°, and the loss of weight was designated moisture-content.

The samples, dried as above, were incinerated in the usual way; the crude ash, prepared by burning in a platinum crucible with an alcohol lamp, was treated with water to extract the soluble constituents, and the insoluble residue was again heated in an electric oven at 600°.

The results given in the table show the mean value obtained in three experiments.

Table IV

		Wt. of dried sample	T/L (ratio of twig (T) to leaf (L))
17/IV	(T)	176 gms.	—
	(L)	—	—
15/V	(T)	144	0.9
	(L)	166	
15/VI	(T)	142	1.0
	(L)	142	
16/VII	(T)	166	1.0
	(L)	159	
31/VII	(T)	250	1.5
	(L)	162	
15/VIII	(T)	140	1.2
	(L)	118	
16/IX	(T)	182	1.4
	(L)	130	
15/X	(T)	210	1.7
	(L)	123	
15/XI	(T)	180	0.9
	(L)	195	
19/XII	(T)	139	—
27/I	(T)	140	—

The ratio of dry weight of twigs to that of leaves, as may be seen in the table, increased gradually from 0.9 to 1.7 and then decreased to 0.9, depending mostly upon their water-content.

The percentage of water and ash in twigs and leaves changes with the months in the following manner:—

Table V

	Twig		Leaf		Twig+Leaf
	H_2O %	Ash %	H_2O %	Ash %	Ash %
IV	43.51	2.93	64.60	4.10	—
V	68.17	4.72	68.71	4.49	4.37
VI	53.86	4.12	57.0	5.18	4.65
VII (16)	48.14	3.04	54.07	5.24	4.14
VII (31)	51.67	2.99	59.37	5.26	4.87
VIII	52.11	3.12	58.85	5.31	4.53
IX	48.05	2.39	56.10	5.24	4.29
X	48.16	3.31	54.54	4.94	3.88
XI	52.87	3.49	45.00	4.14	3.63
XII	46.07	4.05	fallen	—	4.03
I	50.79	4.15	fallen	—	4.15

The monthly change in the ash content of the twigs runs parallel with that of the water content, showing a maximum in May and a minimum in September, but in the case of the leaf, the monthly change in these constituents is different, the water content diminishing gradually with time from 65 % to 45 % though the ash content shows a maximum in August. However, the average ash content of twigs and leaves shows a maximum in summer.

The chemical composition of the ash both of twigs and leaves was studied by analysis with the following results (Table VI).

As may be seen in Table VI, the content of elements in both twigs and leaves changes with the month, and P_2O_5 and K_2O in the twigs show the highest percentage in August and July respectively (15 % and 30 %), but in leaf-ash these compounds show a high percentage at the end of spring and then their content decreases gradually with time. The content of both Mg and Mn, however, is the maximum in summer.

From the seasonal variation of the content of the ash constituents expressed on the dry bases of twigs and leaves, shown in Table VII, we learned that such elements as P, K and Mg are accumulated abundantly in the plant tissues in summer.

As a matter of fact, the essential elements of the ash of Sake-yeast and Koji-spores are found as the chief constituents of the ash of Hoso-trees in the stage of vigorous growth and also of soy bean stems and the Nara-tree, and thus the biochemical significance of the use of plant ash in Koji-making is explained; it supplies the inorganic elements which facilitate the development of the organism.

"Moyashi," as mentioned above, is prepared in Japan by the method handed down from ancient times, a method unknown to western peoples. The essential part of this method is here explained biochemically; it being supposed that the inorganic elements which occur in the active parts of plant tissues play an essential part when they enter the body of a micro organism, in the process of the metabolism of organic material, this view was confirmed by the study of the seasonal variation of the ash constituents.

It is the desire of one of the writers (S. K.) to devote himself to the biochemical study of oriental customs or phases of oriental civilization which are based on the utilization of indigenous natural substances as a means of supporting or enriching life.

Table VI

	SiO_2	SO_3	P_2O_5	Al_2O_3	Fe_2O_3	Mn_3O_4	CaO	MgO	K_2O	Na_2O	Cl
IV	42	15	33	—	10	8	85	17	64	10	1
V	36 (twigs)	28	54	4	12	10	151	13	125	21	4
	93 (leaves)	25	36	1	9	4	117	26	123	10	4
VI	12	13	49	7	10	14	161	17	95	8	3
	122	26	37	1	7	5	146	34	128	10	
VII	13	11	43	5	6	10	80	17	92	11	3
	148	26	33	8	4	12	118	33	113	11	4
	11	10	42	5	4	9	102	15	84	9	2
	150	22	33	7	10	13	109	32	111	5	4
VIII	10	9	47	2	5	9	104	14	91	9	3
	156	22	34	5	9	12	110	31	114	7	3
IX	7	7	31	6	2	9	86	15	60	6	1
	161	24	31	5	6	18	119	30	107	14	4
X	26	17	27	2	5	19	113	17	71	12	2
	192	22	27	2	4	5	125	17	62	15	1
XI	17	11	31	3	2	12	133	19	76	19	2
	151	9	20	3	6	11	118	18	54	8	1
XII	16	18	15	4	4	11	179	19	87	27	5
I	11	14	31	5	4	11	174	26	90	27	5

The ash content of leaves, as may be seen in Table V, drops suddenly before the autumn leaf-fall, while that of twigs increases after that season, and this fact is explained by assuming that some of the inorganic elements migrate from the former to the latter before autumn comes. The assumption that the loss of ash in leaves is balanced by an accumulation in the twigs may be confirmed by the fact that the average ash content, both of twigs and leaves, as is seen in Table V, remains almost constant after spring has passed. However, the percentage of ash constituents expressed on water free bases shows that when autumn comes the content of P, Al, Mn, Ca, Na and Si is decreased in both leaf and twig. This is probably due to the fact that they are given off from the plant by the fallen-leaf in the form of mineral matter and of organic constituents, a view put

forward in the "Biochemical Study on the Autumn Fall of the Leaf" by one of the writers (S. K.) and Ozawa[1]. There it is stated that one of the most important functions of the autumn leaf-fall is to enable the plant to get rid of inorganic materials—ash—so that the accumulated ash constituents are prevented from controlling the metabolic changes of the tissue materials and the plant is able to live a normal life in winter.

The inorganic elements in the Hoso-tree are divided biochemically into three classes: (1) elements like Si, the content of which increases with time: (2) those like S, P & K which decrease as the season progresses, and (3) such elements as Al, Mn, Mg and Ca which show a maximum or minimum in their seasonal change. The characteristics of the elements in their seasonal variation are probably intimately connected with the functions of these elements in the vital processes of plant tissues. The functions of the elements in the life of the plant are supposed to be different from each other; the elements which belong to the first class probably give rigidity and comparative permanense to the plant skeleton, and those belonging to the second and the third class are regarded as taking part in metabolism or photosynthesis.

It is to be supposed that the inorganic elements which function in the metabolic process of the organic matter in plants—the germination of seeds and budding etc.—will be found abundantly in the ash obtained from plants in the stages of vigorous growth. Such elements as Ca, Mg, P & K which constitute the major part of the ash of leaves of the Hoso-tree gathered in summer will partly migrate from leaf to twig before the autumn leaf-fall in order to prepare for the budding or vital processes in the plant tissues in the following spring.

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1. These Memoirs, **15**, 57 (1932).

Fig. 1

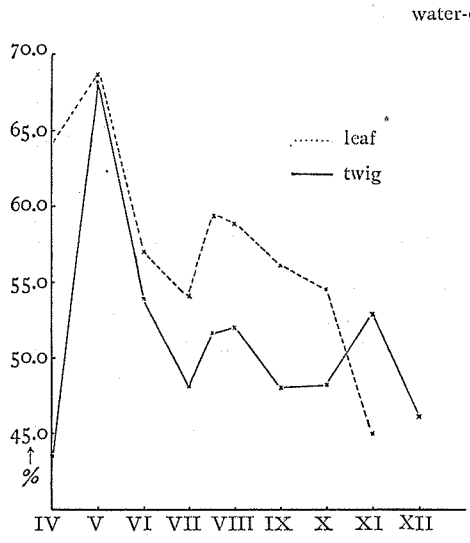

Fig. 3

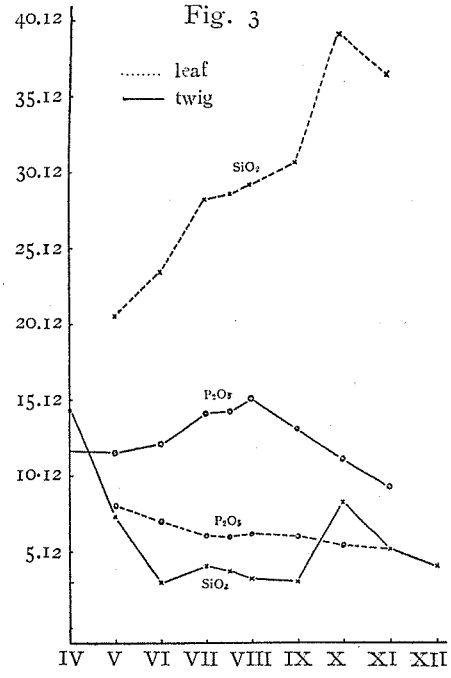

Fig. 2

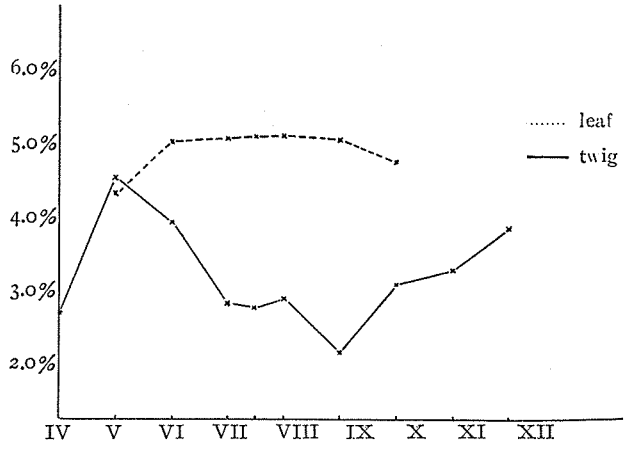

Fig. 4

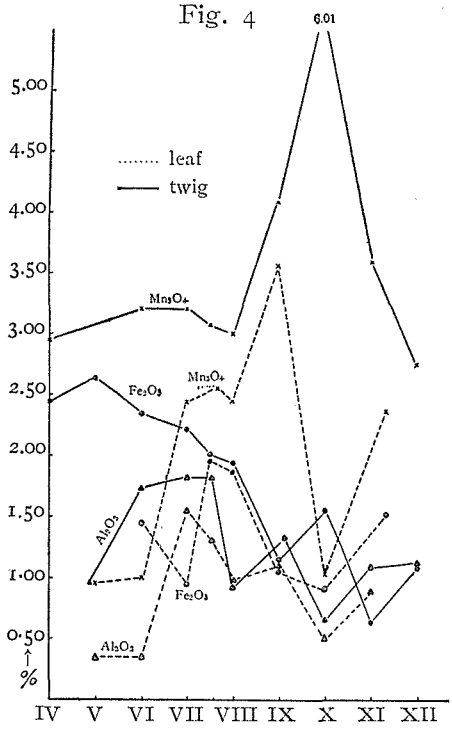

Fig. 5

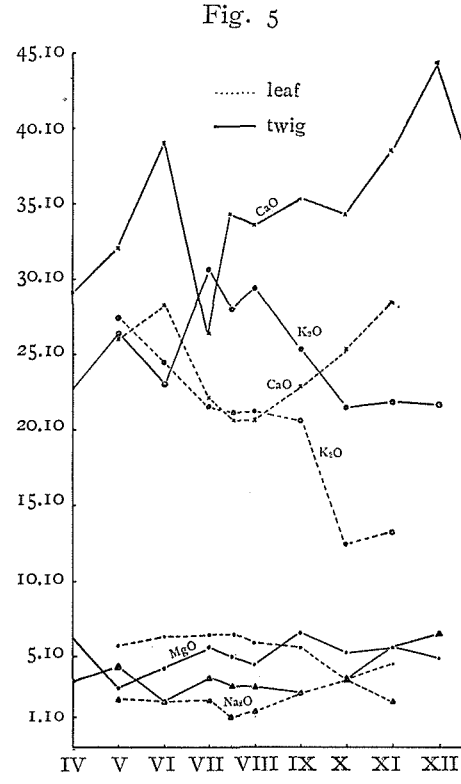

Fig. 6