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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL COMMITTEE

Twenty-First Session

Geneva, November 12 and 13, 1985

JAPANESE STUDY ON THE USE OF GAS-CHROMATOGRAPHY
IN THE TESTING OF VARIETIESprepared by the Ministry of Agriculture,
Forestry and Fisheries of Japan

The Annex to this document contains the results of a study made by the Ministry of Agriculture, Forestry and Fisheries of Japan on the use of gas-chromatography in the testing of varieties.

[Annex follows]

November 1, 1985

DEVELOPMENT OF EXAMINATION TECHNIQUE

1. From 1983, Ministry of Agriculture, Forestry and Fisheries(MAFF) has been developing a new examination technique for the granting of plant variety protection, i.e. an objective evaluation system of the characteristics relating to aroma or pungency in the products of the varieties. We have already finalized the applicability study of this technique to melon, pepper, tea, rose, grape and garlic, and got the successful result. In 1985, the study for the aroma of stock and the pungency of onion is being carried out.

2. To facilitate the understanding of this study, an example i.e. the aroma of rose is taken and the process and result of this study are explained below.

(1) This study focused on the correlation between the result of the machinery analysis and the sensory evaluation in order to establish the numerical evaluation system of the intensity and quality of aroma.

(2) Method of Analysis

a. Analyzer:

Continuous distillation-extraction method suggested by Dean and Stark is used for the analysis of 30 rose varieties. 40 grams of petal, 200 grams of pure water, 0.5 ml of internal standard (2 mg/ml of n-Hexadecane solution) and 10 ml of diethyl ether:hexane=1:1 (v/v) as extract solvent have been boiled together for 90 minutes. After cooling it down, the water phase is quietly removed and the solvent phase is dehydrated by 5 g of sodium sulfate and filtrated by glassfilter. This material is concentrated by Nitrogen gas for the analysis. 2 μ l of it is injected in the gas-chromatograph.

b. Sensory Evaluation

Intensity of aroma: Ninety of 120 ml flasks containing 4 grams of petal are placed at random. 10 observers are asked to do sensory evaluation of these samples on their intensity by five grades. 30 varieties are three times evaluated.

Quality of aroma: 9 obserbers (different from the previous tests) are asked to evaluate the same samples on their quality by three grades (good, ordinary, bad).

(3) Applicability to the examination for plant variety protection

It was appeared that the intensity of aroma can be measured by gas-chromatograph instead of sensory evaluation, because the result of the analysis, after the estimation by multiple linear regression, almost corresponded with the result of sensory evaluation.

With regard to the quality of the aroma, adaptability and repeatability of the test result were lower than the intensity of aroma, but the gas chromatograph method might still be applicable to the measurement of the quality of aroma.

(4) Problems

a. Because it is expected that some of the small gradients which have not been detected through the test may play the important role to control the quality of aroma, further study is needed to assess the influence of these small gradients to the quality of aroma.

b. As the judgements of the quality of aroma i.e. its smell is good or not, sometimes vary depending on the individuals' subjective feeling, it is difficult to establish a completely objective evaluation system.

3. The result of the analysis of other crops can be briefly summarized as follows:

(1) As far as tea, grape and garlic are concerned, it became clear that gas chromatography analysis method is applicable as a measure of intensity or quality of aroma.

(2) As far as melon is concerned, gas chromatograph method is not applicable. This method is useful only for the grouping of varieties (oriental variety group, winter melon variety group, netted melon variety group and cantaloup variety group), but not for distincting varieties themselves. Because the aroma of melon is variable even in the series of fruit produced under the same growing condition depending on its maturity stage.

(3) As far as pepper is concerned, its pungency is successfully measured by photospectrometer. This method is hopeful.

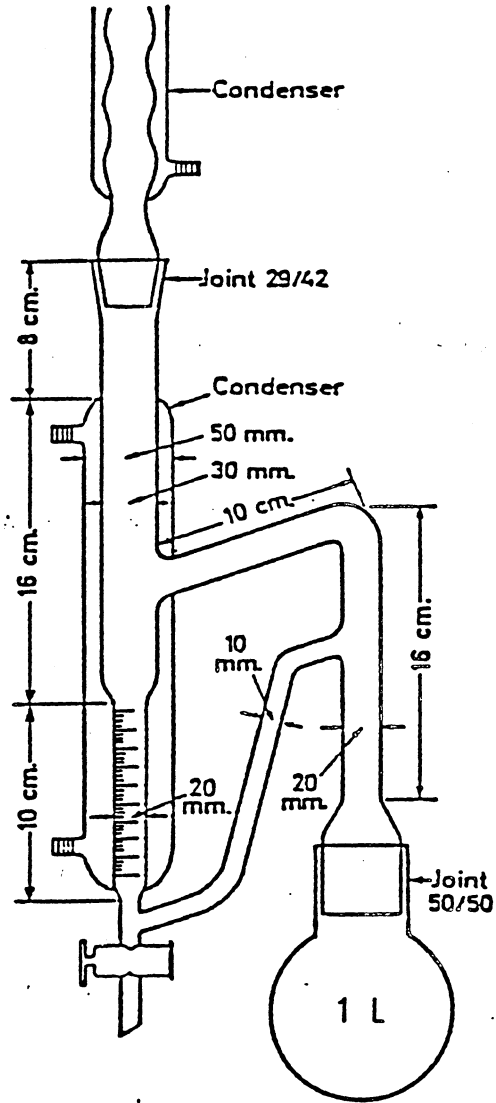
(4) In all cases mentioned above, the influence of different growing conditions and climatic conditions to the result of the analysis is not clear. Further study may be needed at this point.

List of rose varieties tested

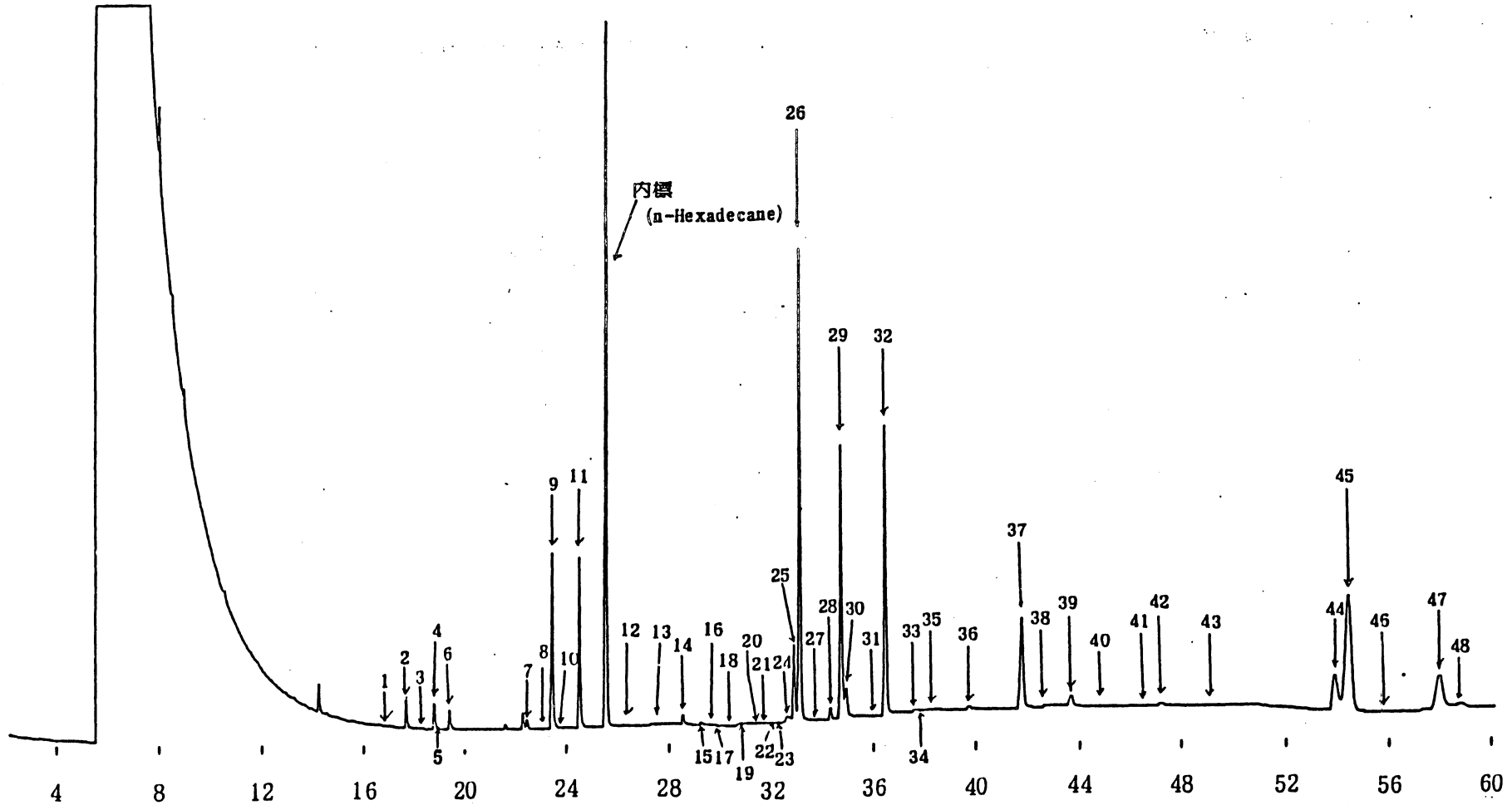
No.	name of variety	Type of flower
1	White Christmas	white large flower
2	White Masterpiece	"
3	Shugetsu	yellow large flower
4	Peace	"
5	Wiener Charme	orange large flower
6	Ocho	"
7	Hojun	pink large flower
8	Yuzen	"
9	Carina	"
10	Queen Elizabeth	"
11	Sonia	"
12	Harmonie	brilliant red large flower
13	Maderon	"
14	Super Star	"
15	Atoll	"
16	American Pride	scarlet large flower
17	Papa Meilland	dark red large flower
18	Brilliant Light	variegated large flower
19	Las Vegas	"
20	Olympic Toach	marginal variegated large flower
21	Frohsinn 82	"
22	Shiun	mauve large flower
23	Blue Moon	"
24	Charles De Gaulle	"
25	Friesi	yellow middle flower
26	Monalisa	orange middle flower
27	Bridal Pink	pink middle flower
28	Cherish	"
29	Tchin Tchin	Brilliant red middle flower
30	Mimi Rose	pink cluster-flowering middle flower
31	* Rosa damascena	wild species
32	* <u>Rosa rugosa</u>	wild species

GRADE OF SENSORY EVALUATION

grade	intensity of aroma
9	very strong
7	strong
5	medium
3	weak
1	very weak



Improved distillatory apparatus of
Dean and Stark



An example of gas-chromatograph by continuous distillation-extraction method
as to rose variety (Qu Elizabeth)

IDENTIFICATION OF MAJOR PEAK

Peak (estimated molecular weight)			Peak (estimated molecular weight)		
No.	M.W.	SUBSTANCE	No.	M.W.	SUBSTANCE
1	130	Hexyl formate	26	152	1,3-Dimethoxy-5-methyl benzene
2	102	Hexanol	27	108	Benzyl alcohol
3	154	Rose oxide	28	268	Nonadecane
4	100	cis-3-Hexenol	29	122	Phenylethyl alcohol
5	154	Rose oxide	30	266	Nonadecene
6	142	Nonanal	(31	220	2,6-Di-tert-butyl-4-methyl) ^{*1}
7	212	Pentadecane			phenol
8	204	Copaene	32		Unknown ^{*2}
9	194	Theaspirane	33	282	Eicosane
10	154	Linalool	34	178	Methyl eugenol
11	194	Theaspirane	35	280	Eicosane
12	204	Farnesene	36	264	Nonadecadiene
13	198	Citronellyl acetate	37	296	Heneicosane
14	240	Heptadecane	38	294	Hydrocarbon (C ₂₁ H ₄₂)
15	204	Caryophyllene	39	294	Hydrocarbon (C ₂₁ H ₄₂)
16	204	γ -Cadinene (or Muurolene)	40	164	Eugenol
17	152	Neral	41	222	Cadinol T
18	156	Citoronellol	42	310	Docosane
19	204	δ -Cadinene	43	324	Tricosane
20	154	Nerol	(44	206	2,6-Di-tert-butyl-phenol) ^{*1}
21	164	Phenylethyl acetate	45	352	Pentacosane
22	154	Isogeraniol	46	168	trans-Geranic acid
23	222	Ledol	47	380	Heptacosane
24	154	Geraniol	48	322	Tricosene
25	194	7,8-Dihydro- β -ionone			

* 1 excluded from evaluation

* 2 several substances are mixed

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the estimate formula of intensity of aroma

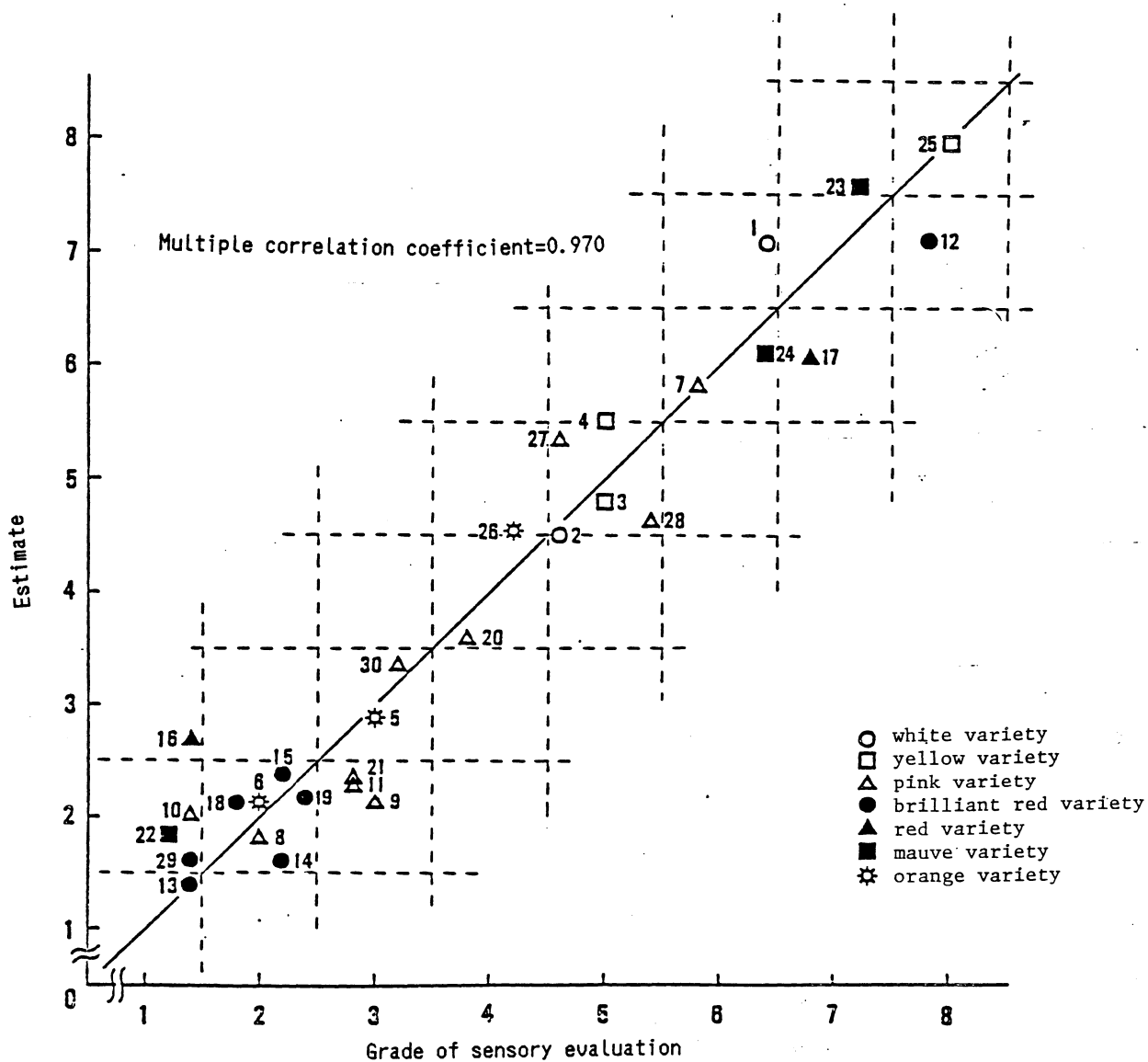
$$\hat{Y}_a = 2.413 + 0.1734X_{p1} - 0.1707X_{p9} + 0.4023X_{p13} + 0.0556X_{p14} \\ + 0.0974X_{p19} + 0.3146X_{p25} + 0.0129X_{p29} - 0.0127X_{p45}$$

the estimate formula of quality of aroma

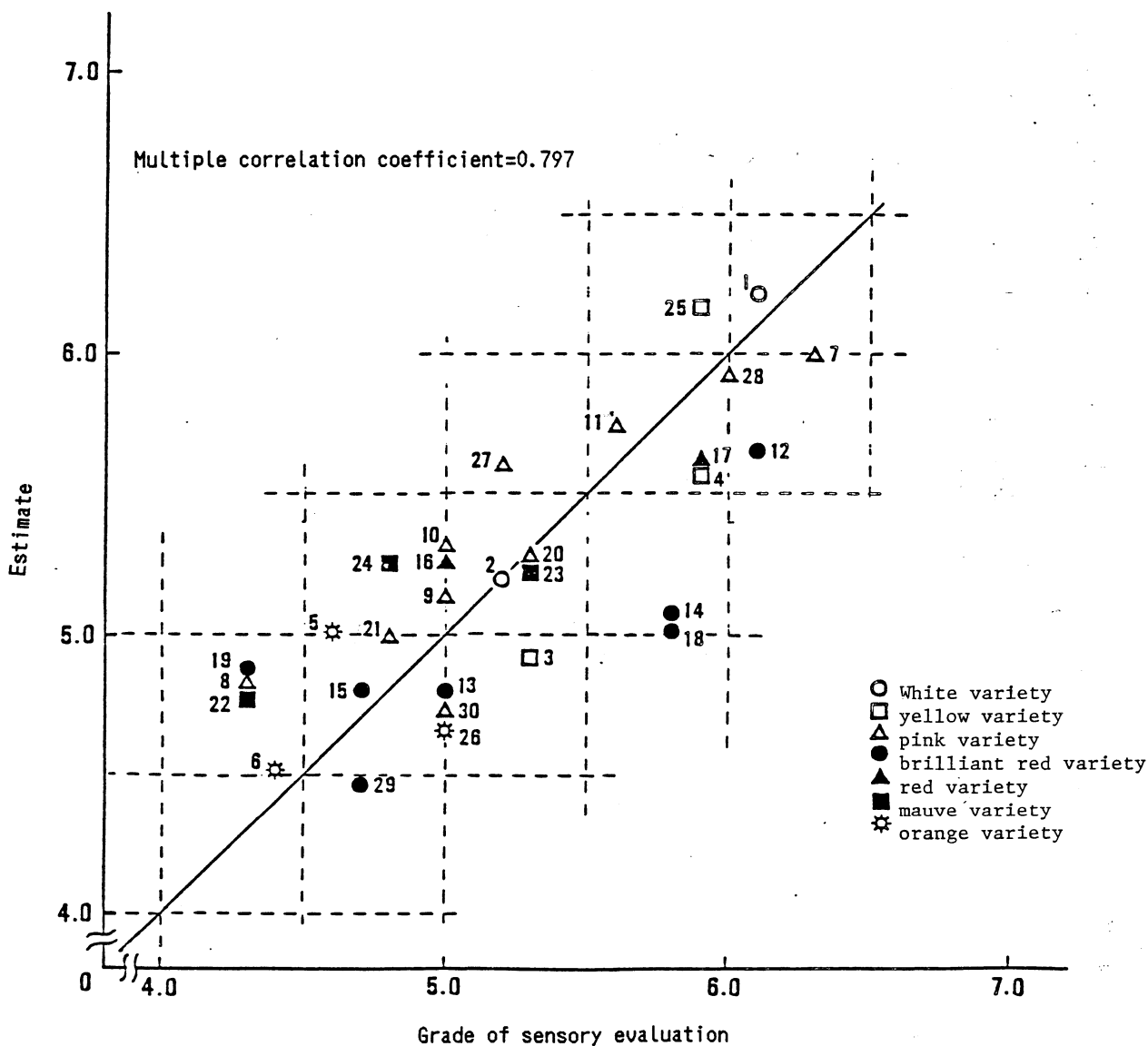
$$\hat{Y}_b = 5.430 + 0.1090X_{p8} + 0.0240X_{p15} + 0.0083X_{p23} + 0.0085X_{p32} \\ - 0.1924X_{p42}$$

the result of multiple linear regression analysis

aroma	Peak No.	Canonical partial coefficient	partial regression coefficient	standard error	t-value
	1	0.160	0.1734	0.0628	2.75
	9	-0.427	-0.1707	0.0355	-4.80
	13	0.467	0.4023	0.0542	7.41
	14	0.260	0.0556	0.0158	3.52
	19	0.441	0.0974	0.0155	6.24
	25	0.519	0.3146	0.0558	5.63
	29	0.476	0.0129	0.0021	5.96
	45	-0.180	-0.0127	0.0059	-2.14
constnt		2.413			
	8	0.509	0.1090	0.0289	3.76
	15	0.312	0.0240	0.0099	2.42
	23	0.409	0.0083	0.0026	3.19
	32	0.275	0.0085	0.0041	2.05
	42	-0.567	-0.1924	0.0481	-3.99
constnt		5.430			



Relationship between grades of sensory evaluation and estimates by multiple linear regression analysis in intensity of aroma.



Relationship between grades of sensory evaluation and estimates by multiple linear regression analysis in quality of aroma.

RELATIONSHIP BETWEEN GRADL. OF SENSORY EVALUATION AND ESTIMATE
BY MULTIPLE LINEAR REGRESSION ANALYSIS IN INTENSITY OF AROMA

No.	grede of sensoty evaluation	estimate	residue	standard error
1	6.4	7.07	-0.67	0.352
2	4.6	4.50	0.10	0.495
3	5.0	4.79	0.21	0.499
4	5.0	5.50	-0.50	0.522
5	3.0	2.88	0.12	0.569
6	2.0	2.13	-0.13	0.570
7	5.8	5.81	-0.01	0.474
8	2.0	1.80	0.20	0.567
9	3.0	2.12	0.88	0.573
10	1.4	2.02	-0.62	0.319
11	2.8	2.31	0.49	0.405
12	7.8	7.11	0.69	0.460
13	1.4	1.38	0.02	0.565
14	2.2	1.60	0.60	0.578
15	2.2	2.37	-0.17	0.545
16	1.4	2.68	-1.28	0.561
17	6.8	6.05	0.75	0.506
18	1.8	2.12	-0.32	0.566
19	2.4	2.17	0.23	0.550
20	3.8	3.58	0.22	0.514
21	2.8	2.32	0.48	0.564
22	1.2	1.82	-0.62	0.574
23	7.2	7.56	-0.36	0.408
24	6.4	6.10	0.30	0.438
25	8.0	7.96	0.04	0.314
26	4.2	4.54	-0.34	0.302
27	4.6	5.33	-0.73	0.500
28	5.4	4.61	0.79	0.559
29	1.4	1.62	-0.22	0.574
30	3.2	3.35	-0.15	0.515

RELATIONSHIP BETWEEN GRADL. OF SENSORY EVALUATION AND ESTIMATE
BY MULTIPLE LINEAR REGRESSION ANALYSIS IN QUALITY OF AROMA

No.	name of variety	grade of sensory evaluation	estimate	residue	standard error
1	White Christmas	6.1	6.22	-0.12	0.202
2	White Masterpiece	5.2	5.20	0.00	0.362
3	Shugetsu	5.3	4.92	0.38	0.366
4	Peace	5.9	5.58	0.32	0.294
5	Wiener Charme	4.6	5.01	-0.41	0.377
6	Ocho	4.4	4.52	-0.12	0.359
7	Hojun	6.3	5.99	0.31	0.294
8	Yuzen	4.3	4.84	-0.54	0.383
9	Carina	5.0	5.13	-0.13	0.379
10	Queen Elizabeth	5.0	5.31	-0.31	0.336
11	Sonia	5.6	5.74	-0.14	0.357
12	Harmonie	6.1	5.65	0.45	0.351
13	Maderon	5.0	4.80	0.20	0.381
14	Super Star	5.8	5.08	0.72	0.381
15	Atoll	4.7	4.80	-0.10	0.381
16	American Pride	5.0	5.26	-0.26	0.383
17	Papa Meilland	5.9	5.61	0.29	0.354
18	Brilliant Light	5.8	5.02	0.78	0.385
19	Las Vegas	4.3	4.85	-0.55	0.381
20	Olympic Toach	5.3	5.27	0.03	0.363
21	Frohsinn 82	4.8	4.99	-0.19	0.382
22	Shiun	4.3	4.79	-0.49	0.381
23	Blue Moon	5.3	5.25	0.05	0.210
24	Charles De Gaulle	4.8	5.25	-0.45	0.383
25	Friesi	5.9	6.17	-0.27	0.304
26	Monalisa	5.0	4.66	0.34	0.377
27	Bridal Pink	5.2	5.60	-0.40	0.353
28	Cherish	6.0	5.92	0.08	0.333
29	Tchin Tchin	4.7	4.47	0.23	0.366
30	Mimi Rose	5.0	4.73	0.27	0.377