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STUDIES ON THE BITING TASTE OF BURLEY TOBACCO LEAVES (NUTRITIONAL PHYSIOLOGY OF JAPANESE TOBACCO) I. RELATIONS BETWEEN THE BITING TASTE AND CHEMICAL COMPONENTS

By

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Introduction

Burley tobacco is cultivated widely in the Unite States of America, and is raised commonly throughout the northern part of the main island of Japan, because of its high yield from the heavy dressing of fertilizers which enables its vigorous growth even under cold climate.

Owing to its inferiority in the quality of cured leaves, the area of cultivation has exceedingly decreased in recent years. One important reason of its inferiority in quality is the severe biting taste, which is peculiar to the leaves produced in the cold climate.

It is expected that if this biting taste could be removed, the demand of burley tobacco might increase in the future and be a good enterprise for the farmers in northern Japan.

Therefore the authors have started to investigate the problem of removal of this biting taste from the stand point of plant nutrition and soils. At first studies were carried on the relation between the formation of this biting taste and environment as the true cause of this taste is unknown.

The authors have investigated the status of nitrogenous material and carbohydrate as it is generally known that protein and nicotine are unsatisfactry to smoking taste and carbohydrate is able to neutralize this effect (1), (2).

The effect of phosphorus on Japanese tobacco was considered for a long time, since the growth promoting and carbohydrate accumulating effect of phosphorus on rice and other plants as the authors have already confirmed, was conspicuous.

Therefore the forms and content of phosphorus compounds were fully investigated.

Considering the environmental factors, the authors tried to analyse the factors affecting the quality of burley tobacco with the prupose to improve the tobacco harvest in Japan.

Materils and Methods

Many samples of burley tobacco leaves, grown in the same or adjacent field, including different kinds of qualities relating to the leaf position in phyllotaxis and date of transplanting, were collected to investigate the status of constituents which may affect the quality of the harvested leaves.

Samples were raised at Sannohe, Aomori Prefecture in 1950. Details of cultivation records and qualities are shown in Table 1.

Table 1. Sample grade and culture method

Cultivating season	Position of leaf on stalk	Grade of quality	Date of Sowing	Date of transplanting	Date of Harvest	Manure	Rapeseed lees	Human faeces	Ammcnium sulfate	Super-phosphate	Plant ash	Urea
						Kan/Tan						
Early crop	Granulators	4	25/III	21/V	10/VIII	400	10	25	5	5	40	0
	〃	6	25/III	21/V	5/VIII	350	10	20	5	5	35	0
	Cutters	Ex.	24/III	20/V	15/VIII	500	20	20	7	10	60	0
	〃	5	25/III	20/V	15/VIII	400	10	25	7	5	40	0
	Red leaf	1	24/III	20/V	23/VIII	400	30	30	6	10	60	0
	〃	5	24/III	21/V	30/VIII	450	15	35	3	7	50	0
	Tips	4	25/III	20/V	30/VIII	400	20	30	6	7	60	0
	〃	6	25/III	20/V	30/VIII	400	20	30	6	7	60	0
Middle crop	Granulators	4	25/III	25/V	12/VIII	450	10		6	6	50	0
	〃	6	25/III	25/V	7/VIII	400	10		6	6	50	0
	Cutters	Ex.	25/III	25/V	20/VIII							
	〃	5	25/III	25/V	15/VIII	400	10	25	7	5	40	0
	Red leaf	1	25/III	25/V	25/VIII	350	20	30	6	7	60	0
	〃	6	25/III	25/V	2/IX	350	20	30	6	7	60	0
	Tips	4	25/III	25/V	5/IX	400	17	35	7	7	50	0
	〃	6	25/III	25/V	5/IX	400	17	35	7	7	50	0
Late crop	Granulators	4	29/III	5/VI	20/VIII	500	10	30	8	3	35	0
	〃	6	29/III	5/VI	15/VIII	500	10	30	8	3	35	0
	Cutters	1	28/III	5/VI	25/VIII	500	10	30	9	5	35	0
	〃	5	28/III	5/VI	20/VIII	400	10	25	8	5	35	0
	Red leaf	1	25/III	2/VI	28/VIII	500	15	50	8	7	50	2
	〃	6	25/III	2/VI	15/IX	500	15	50	8	7	50	2
	Tips	4	28/III	5/VI	20/IX	450	10	40	8	5	35	2
	〃	6	28/III	5/VI	20/IX	450	10	40	8	5	35	2

Ex. excellent grade. Empty space is unknown quantity.

The position of each sampled leaf in phyllotaxis of the whole plant is represented by four classes, namely tips, red leaf, cutters and granulators for each leaf position, also those of the higher and the inferior grades were selected. For the cultivation season or in other words the date of transplanting and harvesting, three classes namely, early, middle and late crops, were set up.

Relating to qualities, seven grades are adopted, namely, excellent, 1, 2, 3, 4, 5 and 6. Samples are pulverized and analysed as follows.

I. Nitrogenous Compounds

1. Total nitrogen: determined according to Kjeldahl's method and added nitrate nitrogen.
2. Soluble nitrogen: Sample is extracted for 5 minutes with 0.5 per cent acetic acid and filtered. Nicotine, in filtrate and washings, is precipitated by silicotungstate method. Soluble nitrogen, in the filtrate from nicotine silicotungstate, is measured according to Kjeldahl's method.
3. Nicotine nitrogen: precipitated as silicotungstate (3).
4. Ammonia nitrogen: An adequate part of the filtrate from nicotine silicotungstate was added with bicarbonate buffer solution and distilled by the aeration method. The volatiled ammonia was caught and determined colorimetrically by use of Nessler's reagent.
5. Amide nitrogen: 10 ml of filtrate from nicotine silicotungstate was hydrolysed by adding with 30 per cent sulphuric acid and heating for two hours on the boiling water-bath. Then it was neutralyzed and the amount of total ammonia nitrogen was determined as above. From this substracted ammonia nitrogen in 4, and the resulting value was doubled for amide nitrogen.
6. Nitrate nitrogen: measured according to Harper's method (4).
7. Protein nitrogen: Calculated from the difference between total nitrogen and soluble plus nicotine nitrogen.

II. Sugars

1. Reducing sugar: An adequate part of the solution, for the determination of ammonia nitrogen, is measured colorimetrically by the method of Fohlin and Wu (5).
2. Non-reducing sugar: Solution for amide nitrogen is determined by colorimetrically as total sugar, and calculated from the difference between this and 1.

III. Phosphorus Compounds

1. Phosphatide phosphorus: 20 g of the sample is extratced with 250 ml of boiling ethanol for six hours, filtered and washed with hot ethanol. Phosphorus in the ethanol solution is measured for phosphatide phosphorus.
2. Phytine phosphorus: Extracted residue of phosphatide is air dried and treated 400 ml of 1 per cent acetic acid solution buffered by ammonium acetate for 24 hours, and then washed with the same solution. Filtrate is filled up to 450 ml, pH adjusted to 2.7–2.8, added with 50 ml of 2.5 per cent copper acetate solution. Phytine is precipitated as copper salt.

Precipitated phytine is separated and phosphorus is measured.

3. Inorganic phosphorus : Filtrate from copper salt of phytine contains inorganic phosphorus.
4. Nuclein phosphorus : Extracted residue with 1 per cent acetic acid retains principally nuclein and protein bound phosphorus. Each phosphorus fraction is estimated colorimetrically.

Results and Discussion

Results of experiments are shown in Table 2 to 11.

Tabel 2. Nitrogen compounds : Early crop (%of dry matter)

Position of leaf on stalk	Grade of quality	Total. N	Prot. N	Nicot. N	Sol. N	Amide. N	NH_3-N	NO_3-N	Resid. N
Granulators	4	2.908	1.522	0.330	1.046	0.094	0.488	0.095	0.369
〃	6	1.951	0.642	0.338	0.971	0.185	0.630	0.124	0.032
Cutters	Ex.	3.774	1.373	0.396	2.005	0.701	0.633	0.172	0.499
〃	5	3.047	1.212	0.295	1.540	0.060	0.515	0.308	0.657
Red leaf	1	3.183	1.337	0.334	1.512	0.337	0.605	0.065	0.505
〃	5	5.228	2.367	0.527	2.394	0.908	0.922	0.070	0.494
Tips	4	3.678	0.952	0.358	2.368	1.016	0.708	0.026	0.618
〃	6	5.559	2.948	0.432	2.179	0.893	0.954	0.037	0.295

Ex. excellent grade.

Table 3. Nitrogen compouds : Middle crop (%of dry matter)

Position of leaf on stalk	Grade of quality	Total. N	Prot. N	Nicot. N	Sol. N	Amide. N	NH_3-N.	NO_3-N.	Resid. N
Granulators	4	3.252	1.372	0.264	1.616	0.153	0.790	0.269	0.404
〃	6	4.085	1.358	0.354	2.373	0.085	0.354	0.654	1.280
Cutters	Ex.	3.280	1.226	0.352	1.702	0.279	0.896	0.046	0.481
〃	5	3.323	0.811	0.314	2.198	0.226	0.572	0.470	0.930
Red leaf	1	3.696	1.937	0.362	1.397	0.594	0.426	0.026	0.351
〃	6	4.060	2.290	0.345	1.425	0.630	0.479	0.035	0.281
Tips	4	4.569	1.895	0.468	2.206	0.967	0.541	0.058	0.640
〃	6	5:112	2.055	0.456	2.601	1.021	0.683	0.035	0.862

Table 4. Nitrogen compounds : Late crop (% of dry matter)

Position of leaf on stalk	Grade of quality	Total. N	Prot. N	Nicot. N	Sol. N	Amide. N	NH_3-N	NO_3-N	Resid. N
Granulators	4	4.040	1.700	0.352	1.988	0.370	0.328	0.532	0.758
〃	6	4.628	2.496	0.252	1.880	0.413	0.334	0.248	0.885
Cutters	1	3.875	1.920	0.316	1.639	0.255	0.569	0.542	0.273
〃	1	2.948	1.211	0.295	1.440	0.323	0.201	0.273	0.643
Red leaf	5	4.065	1.823	0.399	1.843	0.097	0.802	0.279	0.665
〃	1	5.949	3.319	0.397	2.233	0.149	1.319	0.262	0.503
Tips	6	4.758	2.427	0.445	1.886	0.314	1.081	0.098	0.393
〃	4	6.700	3.664	0.464	2.572	0.588	0.959	0.134	0.891

1. *Nitrogenous Compounds*

The total nitrogen content, as shown in Table 2, 3 and 4, is highest in the tips, and decreases in regular order to the lower leaves. The low grade leaves have in general higher nitrogen content than the high grade leaves. These results are quite in accord with the former investigations (6), (7), (8). Late crop has generally higher nitrogen content as compared with the middle and early crop, being especially remarkable in the lower situated leaves or those of low grade. In this case also, the content of protein and nicotine nitrogen is almost parallel to that of total nitrogen. As to qualities, the nicotine content in the red leaf is higher in better ones, and in cutters, the results are the reverse. Generally speaking it seems that the nicotine content of each leaves in the early crops are slightly lower than that of the middle and early crops.

Ammonia nitrogen is apt to be higher in the younger leaves but not remarkably. Amide nitrogen has almost the same tendency as ammonia nitrogen. Nitrate nitrogen seems to be richer in older leaves.

The accumulation of nitrogenous material especially protein nitrogen has unsatisfactory effect for maintaining better qualities of cured leaves.

The accumulation and distribution of nitrogen solely depends upon the nutritive physiological phenomena of nitrogen absorption and carbohydrate synthesis.

2. *Sugars*

In the granulators the total sugar content is conspicuously low as compared with other positioned leaves. Relating to qualities, better leaves have distinct higher content of total sugar than bad leaves.

Especially the highest class cutters are remarkably rich in total sugar. Also

Table 5. Carbohydrates : Early crop (% of dry matter)

Position of leaf on stalk	Grade of quality	Sol. sugar	Reducing sugar	Non-red. sugar
Granulators	4	1.137	0.714	0.423
〃	6	1.038	0.533	0.505
Cutters	Ex.	2.078	1.310	0.768
〃	5	1.173	0.680	0.493
Red leaf	1	1.697	1.627	0.070
〃	5	1.833	1.319	0.514
Tips	4	2.185	1.260	0.925
〃	6	2.165	1.272	0.893

Table 6. Carbohydrates : Middle crop (% of dry matter)

Position of leaf on stalk	Grade of quality	Sol. sugar	Reducing sugar	Non-red. sugar
Granulators	4	1.026	0.805	0.221
〃	6	1.272	0.541	0.731
Cutters	Ex.	2.010	1.141	0.869
〃	5	1.498	0.577	0.921
Red leaf	1	1.347	1.243	0.104
〃	6	1.218	1.025	0.193
Tips	4	1.503	1.141	0.362
〃	6	1.736	0.988	0.748

Table 7. Carbohydrates : Late crop (%of dry matter)

Position of leaf on stalk	Grade of quality	Sol. sugar	Reducing sugar	Non-red sugar
Granu lators	4	1.127	0.985	0.142
〃	6	1.027	0.750	0.277
Cutters	1	1.952	1.371	0.581
〃	5	1.373	1.007	0.366
Red leaf	1	1.838	1.395	0.443
〃	6	1.845	1.613	0.232
Tips	4	1.806	1.028	0.778
〃	6	1.452	0.968	0.484

the tips of the early crop has a high sugar content. Reducing sugar has almost the same tendency as total sugar. Non-reducing sugar seems to be higher in cutters and tips.

3. *Relation between sugar and nitrogen*

Contents of each component are explained as above, however, it is well known that the taste of the tobacco rather depends upon the quantitative relation between these components. The ratio of soluble carbohydrate to protein of Schmuck Quotient has been considered as the indicator of tobacce qualities for a long time.

The authors tried to calculate this quotient and other numerical values concerning these contents of nitrogen and sugars. For our burley leaves, the ratio of reducing sugar to total nitrogen, instead of soluble carbohydrate to protein, is far more suitable than the Schmuck Quotient for qualifying leaves. In case of tobacco curing, some parts of protein and carbohydrates are hydrolysed or consumed, so the authors would propose to use the ratio of reducing sugar to the total nitrogen. Reducing sugar seems to be the main residual carbohydrates after curing, and in burley leaves nicotine content can not be ignored.

The data for this newly proposed ratio is shown in Table 8.

Table 8. Reducing sugar/Total nitrogen

Position of leaf on stalk	Early crop		Middle crop		Late crop	
	Grade of quality	Sugar/N	Grade of quality	Sugar/N	Grade of quality	Sugar/N
Granulators	4	0.25	4	0.25	4	0.24
〃	6	0.27	6	0.13	6	0.16
Cutters	Ex.	0.35	Ex.	0.32	1	0.35
〃	5	0.22	5	0.17	5	0.34
Redleaf	1	0.51	1	0.34	1	0.34
	5	0.25	6	0.25	6	0.27
Tips 〃	4	0.34	4	0.25	4	0.22
	6	0.23	6	0.19	6	0.14

Generally speaking, granulators and tips have lower figures for this ratio which shows inferior quality.

In the red leaf and cutters these ratio are remarkably wide, especially in better qualified leaves. Relating the period of cultivation, this ratio is markedly wider in tips of the early crop than in that of the late crop. Granulators of the late crop has the narrowest ratio. From these facts, in the late crop the accumulation of carbohydrate by photosynthesis is quite insufficient as compared with nitrogen accumulation which continues as the usual crop.

For burley leaves, this condition is unsatisfactory, so late sowing and transplanting should be avoided in northern Japan. On smoking test, the higher content of total nitrogen gives an irritating bitter taste, especally when the total nitrogen content exceeds 4.5 per conet, then a strong biting taste occurs. This biting taste is remarkable in low grade tips and granulators especially on late crop. In burley leaves, nicotine content could not be ignored, nicotine content has close relation to this biting taste, namely when the nicotine content exceeds 2 per cent or nitotine nitrogen rises above 0.35 per cent, the taste becomes extraordinarily bitter. In low grade leaves, even though the content of protein and nicotine is high, the taste tends to be mild. On the contrary in these low grade leaves, if the sugar content is low, the biting taste prevent its use for smoking.

The authors have tried to improve the qualities of high grade leaves by soaking in reducing sugar solution, which lead to a great success, that is to say, we obtained excellent leaves as produced in the U.S.A. However, when the low grade leaves are soaked similarly, a considerable quality improvement was observed, but still the result was insufficient to attain the best Japanese qualities.

4. *Phosphorus*

Effect of phosphorus on the quality of tobacoc is still obscure, and unless due to a strongly phosphorus deficient soil, we could not find remarkable effects on the yield of burley tobacco, but in U.S.A. commonly 3 : 9 : 9 is recommended for tobacco.

Results of analysis obtained in our experiments are shown in Table 9, 10 and 11. Among the four forms of phosphorus, the inorganic state is most abundant. The content of inorganic phosphorus is low in granulators. Comparing equally positioned leaves, the higher grade leaves are richer in in-

Table 9. Phosphorous compouns : Early crop (% of dry matter)

Position of leaf on stalk	Grade of quality	Total $-P_2O_5$	Inorg. $-P_2O_5$	Nucl. $-P_2O_5$	Phosphatide $-P_2O_5$	Phytin $-P_2O_5$
Granulators	4	12.64	8.78	3.43	0.29	0.14
〃	6	12.33	8.49	2.86	0.58	0.40
Cutters	Ex.	13.98	10.92	2.24	0.47	0.35
〃	5	14.48	11.10	2.85	0.25	0.28
Red leaf	1	16.41	12.79	2.97	0.45	0.20
〃	5	14.60	11.28	2.73	0.35	0.24
Tips	4	17.96	14.22	2.71	0.30	0.73
〃	6	15.84	12.96	2.45	0.28	0.15

Table 10. Phosphorous compouds : Middle crop (‰of dry matter)

Position of leaf on stalk	Grade of quality	Total. $-P_2O_5$	Inorg. $-P_2O_5$	Nucl. $-P_2O_5$	Phosphatide $-P_2O_5$	Phytin $-P_2O_5$
Granulator	4	15.27	11.86	2.89	0.22	0.30
〃	6	13.09	9.27	3.28	0.38	0.16
Cutters	Ex.	13.69	10.62	2.27	0.34	0.46
〃	5	15.21	11.84	2.92	0.14	0.31
Red leaf	1	13.17	11.23	1.43	0.25	0.26
〃	5	13.58	10.65	2.32	0.26	0.35
Tips	4	12.72	10.05	1.91	0.42	0.34
〃	6	12.87	10.01	2.39	0.23	0.24

Table 11. Phosphrous compounds : Late crop (‰of dry matter)

Position of leaf on stalk	Grade of quality	Total $-P_2O_5$	Inorg. $-P_2O_5$	Nucl. $-P_2O_5$	Phosphatide $-P_2O_5$	Phytin $-P_2O_5$
Granulators	4	9.72	7.32	2.12	0.07	0.15
〃	6	8.13	5.86	2.07	0.09	0.11
Cutters	1	13.84	11.84	1.65	0.11	0.25
〃	5	10.85	8.28	2.10	0.11	0.36
Red leaf	1	11.45	9.52	1.55	0.16	0.22
〃	6	11.99	10.00	1.67	0.17	0.15
Tips	4	12.86	10.49	1.94	0.17	0.26
〃	6	11.77	9.10	2.33	0.21	0.13

organic phosphorus than in lower grade leaves.

It might be possible that soluble carbohydrate has some relation with inorganic phophorus, as these two components have the same tendency in their content relating to the grade of leaves.

The content of nuclein phosphorus has no definit tendency as to the situation of leaves, but the content is slightly poorer in the late crop and richer in low grade leaves of the middle and late crops.

Phytine phosphorus has no definit trends in its content, so as to phosphatide phosphorus.

Fujiwara, one of the authers, has found formerly in graminaceous plant leaves, that phosphatide and nuclein state have some definit value, regardless of the status of phosphorus dressing and the contents of the phytine and inorganic phosphorus which fructuate severely (9).

The authers have no clear explanations of the phosphorus trend in burley tobacco leaves.

Burley tobacco is a peculiar plant which grows vigorously within a short period, absorbs much amount of nutrients and synthesizes a heavy amount of organic matter. As after harvest it is cured, special attentions must be paid. in treating the results of chemical analysis. Vigorous growth brings on heavy absorption and accumulation of nitrogen in the plant, but retaining this condition is unsatisfactory for quality. At this time nitrogen should accerelate the photosynthesis and accumulating crabohydrate for diluting nitrogen. Accumulation of reducing and non-reducing sugars are desirable for maintaining sufficient qualities of the leaves.

For the tobacco cultivation, two opposite physiology, namely vegetative growth caused by increasing leaf area depending upon the rapid absorption of nitrogen and reproductive growth reigned by the accumulation of carboydrate depending upon the accerelation of photosynthesis, especially peculiar conditions which retain carbohydrate as soluble sugars and not as starch, should be maintained in the sametime or after a rather short interval, so the forced harmony between these two reverse physiology is most important. From this point phosphorus supply and factors which may affect the metabolism of nitrogen and sugar are expected to be reported.

Summary

1. Investigations were carried on the origin and nature of the biting taste of burley tobacco leaves. Many samples of leaves, cultivated in the same place, were examined.

The relations between this biting taste and some important components were very remarkable.

2. The lower grade leaves are rich in total and protein nitrogen but poor in carbohydrates. Therefore the quotient of reducing sugar to total nitrogen was narrow.

These leaves have severe biting taste.

3. The leaves obtained from plants which were sowed and transplanted early, have wider quotient of reducing sugar to total nitrogen. These leaves are generally sufficient in their qualities.

4. The influence of the phosphorus content to the quality was uncertain.

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