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PEPPER ANALYSIS AND THE OCCURRENCE OF PIPERIDINE THEREIN.

By WILLIAM JOHNSTONE, PH.D., F.I.C., F.C.S., ETC.

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MR. PRESIDENT AND GENTLEMEN,

The subject of the paper I have the honour of reading before you this evening is 'Pepper Analysis and the Occurrence of Piperidine therein.'

What is known as black pepper is the dried immaturated fruit of *Piper Nigrum*, belonging to the *Natural Order Piperaceae*.

The shrub or plant is a native of tropical regions, and is cultivated in various parts of the Indian Archipelago and in the West Indies, and that imported into this country comes principally from the Islands of Malacca, Java, Borneo, and Sumatra.

The plants of the order of Piperaceæ contain an acid resin, a volatile oil, a volatile alkaloid, and a crystalline substance called piperine. They possess pungent aromatic, astringent, and narcotic properties.

Botany. Perennial, *Stem.*—Its stem is 8 to 12 ft. long, trailing or climbing, shrubby, flexuose, and dichotomously branched, jointed, swelling at the joints and often throwing out radicles there, which adhere to bodies like the roots of ivy, or become roots, striking into the ground.

Leaves.—From 4 to 5 in. long, alternate, distichous, broadly ovate, acuminate, of a full green and glossy colour, paler beneath, five to seven-nerved, the nerves connected by lesser transverse ones or veins, and prominent beneath.

Inflorescence.—Spikes opposite the leaves, chiefly near the upper ends of the branches; shortly stalked, pendulous, 3 to 6 in. long; petioles rounded, from $\frac{1}{2}$ to nearly 1 in. long.

Flowers unisexual or hermaphrodite, small, whitish.

Fruit.—Distinct, braccate, ripening irregularly all the year round, about the size of a pea, at first green, then red, afterwards black, covered with pulp.

The black pepper of commerce consists of the entire berries, with the pulp adhering, gathered before they are quite ripe and dried in the sun. The commercial varieties are at least ten in number, viz., Acheen, Alleppy, Kampoot, Lampong, Penang, Siam, Singapore, Tellicherry, Trang, and Long pepper, the names indicating the localities from whence they are obtained.

Blyth remarks that "the differences which these different varieties of pepper present to the eye are evident enough when the several samples are at hand for comparison," a statement I by no means agree with, "but it takes a very practised observer to identify a solitary sample; and if samples of each of the kinds were mixed together, it is doubtful whether an adept even could separate the berries again, identifying each sort with any correctness." Very doubtful, I should say, indeed.

The merchant, however, relies more upon the weight than the appearance. Taking a handful of peppercorns, he can tell in a moment whether it is a light or a heavy sample, and thereby judges the quality of the pepper.

That there is a difference in the weight of the various varieties of the different peppercorns is evident from the results obtained by the author, who carefully three times weighed 100 berries of each kind, which gave the following average results:—

Average of Three Weighings of 100 Peppercorns.				
Acheen	..	..	..	5.1976 grms.
Alleppy	..	..	..	3.8438 "
Kampoot	..	..	..	4.4540 "
Lampong	..	..	..	3.5410 "
Penang	..	..	..	3.9028 "
Siam	..	..	..	4.2776 "
Singapore	..	..	..	4.5338 "
Tellicherry	..	..	..	4.4421 "
Trang	..	..	..	4.8101 "
Penang, white	..	..	..	4.9360 "
Siam, "	..	..	..	5.1441 "
Singapore "	..	..	..	4.6936 "

The pepper of commerce, whole or ground, is therefore a mixture of the different kinds of peppers, and is a condiment which has had a good deal of attention paid it

lately, owing to some successful prosecutions which took place at Liverpool some time ago, when a large quantity of ground pepper was condemned as being adulterated with a material known by the name of *Poiverette*, a hard, tasteless, woody substance, absolutely worthless as a condiment, imported expressly into this country from Italy, for no other purpose than of fraudulently increasing the weight of ground pepper.

It was not, however, wholly owing to the addition of *Poiverette* to pepper that I was induced to undertake the following analysis, although it tended greatly in that direction, but to the great divergency of opinion that seemed to exist amongst various authorities as to the amount of piperine contained in pepper.

The standard works of reference are also at variance with one another, Watts' Dictionary stating that Penang white pepper gave a mean of 5.24 per cent. and Singapore white 9.15 per cent., whilst Singapore black gave a mean of 7.15 per cent.; Sumatra 8.10 per cent. Mr. Heich (ANALYST, vol. xi., p. 188), from 5 to 9 per cent., Mr. Winter Blyth, from 1.8 to 5.5 per cent., Dr. Muter in his "Organic Materia Medica" (third edition), from 2 to 3 per cent., and Ure's Dictionary (sixth edition) a fraction of a grain, and lastly, Dr. Stevenson, in the ANALYST, vol. xii., p. 144, from 6.62 to 7.14 for black pepper, and 6.47 per cent. for white pepper.

Owing, therefore, to such conflicting evidence, with considerable variation in the amount of piperine said to be contained in similar peppers, I determined to investigate the subject myself.

I have done so, and the results obtained in that investigation furnish me with the contents of this paper, and although I have not been able to reduce the limits of variation (but on the other hand to increase them), I have been rewarded by the discovery of the fact that pepper contains a volatile alkaloid, and I have every reason to believe that it is piperidine, from the results I have obtained upon the analysis of its platinum salt:—

					Found.	Calculated.
C	..	..	..	120.0	20.02	20.60
H	..	..	..	24.0	4.32	4.15
N	..	..	..	28.0	5.01	4.80
Pt	..	..	..	197.4	33.93	33.89
Cl	..	..	..	213.0	36.62	36.56
$2(C_5H_{11}N.HCl)PtCl_4$				582.4	99.90	100.00

In entering upon this investigation I had in the first place to obtain genuine specimens of pepper, and through the kindness of my friend Mr. Worcester, I obtained from Messrs. Lewis and Peat, well-known brokers in the City, thirteen specimens, and I now take this opportunity of thanking them for their liberality and the trouble they took to obtain them for me.

The samples as received by me were taken from the bulk, that is, dock or cargo samples, and are therefore undoubtedly genuine, and are representative samples of the pepper which is sent to this country, having undergone no particular process of cleaning other than having simply been screened by means of a sieve, containing seven meshes to the inch, before being ground.

Having obtained such magnificent samples, my next consideration was the adoption of a process for the estimation of the piperine, which would give constant and accurate

results, as I had grave doubts as to the accuracy of the alcohol method usually followed, that is, extracting with alcohol and then purifying by potash, and subsequently again crystallising from alcohol.

At this point I therefore directed my attention to the old but well-known fact, that piperine, when treated with caustic potash and alcohol, splits up into piperidine and pipric acid, as represented by the following equation :—

Piperidine, as you are all well aware, is a volatile alkaloid possessing strong alkaline properties (B.P. 106°C .), so the idea immediately occurred to me that if the above equation could be made to work in practice so as to yield the theoretical quantity of piperidine, the difficulty would be solved, there being no difficulty experienced in distilling off the piperidine and titrating the distillate with standard acid.

The next thing, therefore, was to prove the above reaction, so having some very fine crystals of piperine in my possession, which I had obtained from Messrs. Macfarlan and Co., of Edinburgh, some years ago, and which I had every reason to believe were pure, but erring on the safe side I re-crystallised them, and subjected various quantities to the action of caustic potash and alcohol.

A series of misfortunes and unsatisfactory results followed, so unsatisfactory that I got quite disheartened, concluded that it was a miserable failure, and allowed the subject to remain at rest for over eighteen months, when fortunately one idle day I again returned to the subject with increased vigour.

The trouble I had to encounter before was the difficulty of keeping the stoppers of the bottles tight when subjected to heat and pressure, as the digestion was carried on in the water-bath at a temperature of 100°C .

If one was fortunate enough to get them to remain tight under pressure it was 100 to 1 that when allowed to cool, and you attempted to remove the stopper, it would be found hard and fast, no alternative being left but to knock off the neck; otherwise, if it was got out without breaking the bottle, I always found a great loss.

After a dozen bottles had been used up in this manner, not counting the ones that burst, I found that in three instances I had obtained practically theoretical results (which will be seen by referring to the three first estimations in the accompanying table), so encouraged by my results, and at the same time determined to overcome the difficulty, if possible, and make the process a working success, I designed the following little arrangement, with which I have obtained actually theoretical results, which I have tabulated in the diagram before you, the first three estimations, as already stated, having been made in an ordinary stoppered bottle.

PIPERINE.		
Taken.	Found.	Per Cent.
·8400	·83075	98·89
·2520	·24937	98·95
·5900	·58450	99·06
·4320	·43170	99·93
·7640	·76380	99·97
·5288	·52212	99·85
·3530	·35197	99·70
1·0108	1·01052	99·97

This instrument, as you will observe, consists merely of an ordinary 4-oz. bottle with its mouth ground flat, and is simply closed by placing a circular piece of sheet india-rubber over it, and then placing it in its holder, putting the clamp in position, and finally making it perfectly tight by screwing down the two screws made for the purpose, put into the water-oven, digested at a temperature of 100° C. for from four to six hours, when the reaction is complete, then removed from the oven and allowed to cool.

When cool it will be found that the bottle has remained perfectly tight, none of the contents having escaped, so all that now remains to be done is to unscrew the clamp, and transfer the contents of digestion bottle to a distilling flask fitted with a condenser, and distil the piperidine and titurate the distillate with standard acid.

Having, therefore, satisfied myself of the accuracy and adaptability of the process, the next thought occurred to me to try the estimation of the piperine contained in the pepper direct, that is, digesting the ground pepper with caustic potash and alcohol for six hours, and then distilling as before.

Ten grms. of ground pepper was carefully transferred to the digestion bottle, 30 c.c. of alcohol added, and then about 3 grms. of caustic potash dissolved in 25 c.c. of water also added, the indiarubber placed in position and the clamp screwed down, and then digested for six hours, allowed to cool, opened at convenience, and contents of the bottle washed into large flask with plenty of water, distillation commenced and carried on until the distillate coming over is neutral, aliquot portions of which are then titrated with $10 \frac{\text{N}}{\text{H}_2\text{SO}_4}$ using methyl orange as an indicator.

Several experiments were performed in this manner on one sample of pepper, concordant results being obtained in every instance, which established the reliability of the process; consequently all the samples of pepper were treated in this manner, and the results so obtained are tabulated in the table before you.

The piperine estimations having proved successful, I decided to make a complete analysis of the various samples, the results of which I have the pleasure of laying before you this evening.

I will now, therefore, describe in detail the processes employed by me so as to obtain the figures I have placed upon the diagram.

Moisture.—The moisture is estimated by the ordinary method of drying a weighed portion of the ground pepper in a platinum capsule at 100° C. in the water-oven until the weight remains constant, and then noting the loss in weight.

Oil.—The volatile oil is estimated by distilling 20 grms. of the ground pepper with water in a large flask, shaking the distillate several times with ether, and evaporating the separated ether in a weighed flask at a very low temperature, and finally drying over sulphuric acid.

Piperidine.—Twenty grms. of the ground pepper are distilled with water in a similar manner as that for the oil estimation until the distillate is neutral (450 c.c. being about the quantity necessary to distil). An aliquot portion is then titrated with $10 \frac{\text{N}}{\text{H}_2\text{SO}_4}$ using methyl orange as an indicator. In the piperidine estimation it occurred to me that the process of distilling with water might be done away with, and the pepper

put into a boat and then into a hot-air tube, heated to 230 to 240° F., a constant current of air being aspirated through it all the while, the air ultimately passing through a bulb of standard acid.

Working in this manner, and after an hour's treatment, the acid was washed out of the bulb and its equivalent of soda added, the excess of alkali being titrated back with $10 \frac{N}{H_2SO_4}$ using methyl orange, as usual, as indicator. The results obtained by working in this manner are interesting on account of only a third of the piperidine being obtained in this way, or rather only a third of that obtained when the pepper is distilled with water.

The following are the results obtained in duplicate when working in the manner just described, using 5 grms. of the pepper:—

No. Sample	I.	II.	III.	IV.	V.	VI.	VII.
	0.13	0.106	0.16	0.25	0.11	0.19	0.19
	0.13	0.105	0.12	0.25	0.10	0.20	0.17
No. Sample	VIII.	IX.	X.	XI.	XII.	XIII.	XIV.
	0.29	0.17	0.127	0.093	0.076	0.085	0.272
	0.25	0.17	0.127	0.093	0.076	0.085	0.271

These results tend to suggest that the piperine was to some extent hydrolysed by the action of prolonged boiling with water, but such is not the case for if pure piperine is boiled with water no piperidine is obtained, and it is also disproved by the fact that there is no difficulty in getting the distillate neutral; the amount of piperidine distilled represents a mere fraction of the piperine remaining in the pepper.

I am rather disposed to the theory that there may be a particular ferment contained in the pepper capable of producing the hydrolysis of piperine when boiled with water, or it may be due to the existence of another alkaloid more easily hydrolysed than piperine, anyhow these are the results obtained, and any one is welcome to continue the investigation further if they should feel so disposed.

Ash.—The dried sample used for the moisture estimation is incinerated at a very low temperature, the most convenient arrangement for this work being a jar-muffle, which is infinitely superior to any kind of burner. The residue obtained is the mineral or inorganic matter, and this in turn is then treated with water and then with dilute acid, so as to obtain the portions soluble and insoluble in those menstrua, the insoluble in acid portion, representing the proportion of sand present, and is of special importance.

Piperine.—Ten grms. of ground pepper are digested in a closed bottle similar to that already described, from four to six hours, with 3 grms. of caustic potash dissolved in 25 c.c. of water and 25 c.c. of alcohol. When the digestion is finished, the apparatus is allowed to cool and its contents then carefully washed into a large flask, a few pieces of tobacco-pipe stalks being also put into the flask so as to prevent the violent bumping which would otherwise occur, distillation commenced and continued until the distillate coming over no longer shows signs of alkaline reaction, which is generally the case when about 750 c.c. have passed over. The distillate is then made up to one litre, and titrated as before with $10 \frac{N}{H_2SO_4}$ using methyl orange as indicator.

Fibre crude.—Between 3 to 5 grms. of the ground pepper are placed in a flask and covered with 50 c.c. of dilute sulphuric acid (5 per cent. or 50 grms. of sulphuric acid

per litre), to this 150 c.c. of water are added and the liquid boiled for half an hour, the flask having previously been attached to an upright condenser.

After subsidence, the supernant liquid is run off by means of a syphon into a beaker, and the residue is then twice boiled for half an hour with 200 c.c. of water. After removing as much of the clear liquid as possible, by decantation, the residue, to which is added any sediment that may have become deposited in the beaker containing the acid extract, is next boiled in the same manner with 50 c.c. of dilute caustic potash (50 grms. of KHO in 1,000 c.c.) and 150 c.c. of water for half an hour, and after subsidence the supernant clear liquid is syphoned off into another beaker, and the residue is again extracted by boiling twice with 200 c.c. of water.

The residue obtained after the above process of extraction has been carried out, together with any sediment deposited in the beaker containing the alkaline extract, is now thrown upon a weighed filter and washed well with boiling water, alcohol and ether. The filter and residue are then dried at a temperature of 105 to 110° C. and weighed, and then, in order to ascertain the small proportion of ash which the woody fibre contains, also the accompanying sand, the filter is ignited and the ash found deducted from the previous weight.

This is merely an approximate determination, as the term *crude fibre* designates nothing beyond the fact that it does not consist of pure cellulose, but that a certain amount of ligenous substances are insoluble in acid and alkaline solutions of definite strength, and alcohol and ether, after definite treatment, such as already described, furnishing results which are of some comparative value, but still leaves much to be desired.

Nitrogen and Albuminoids.—The nitrogen was determined in the usual manner with soda-lime.

Alcoholic Extract.—Two grms. of the ground pepper are extracted for 24 hours with 95 per cent. alcohol.

The pepper under examination is placed in a tube, which in turn is inserted in a Soxhlet's extraction apparatus.

The tubes used for this purpose are ordinary test tubes cut in two, the upper half having a piece of fine linen (previously washed and extracted) tied over the mouth end of the tube, then a wad of extracted cotton-wool of sufficient thickness is placed inside the tube, upon the linen so as to prevent any of the solid particles of the sample finding their way into the receiving flask, and then another wad of cotton-wool is packed on the top of the sample and the whole inserted in the Soxhlet apparatus, and the extraction continued, as already stated, for 24 hours. The alcohol is then distilled off, and the flask dried in the water-oven until weight remains constant.

I must here remark that it is necessary to distil the recovered alcohol with dilute sulphuric acid if it is to be again used for any other purpose, so as to get rid of the piperidine which it now contains.

Starch.—Before proceeding to convert the starch into dextrine, the pepper must be carefully extracted with 95 per cent. alcohol, so as to remove substances other than starch which are acted upon by the acid, and also which have the power of reducing Fehling's solution, such as piperine and piperidine, both substances being possessed of the power of reducing Fehling's solution.

The residue contained in the Soxhlet tube is, therefore, readily available for this purpose, having been thoroughly exhausted with alcohol.

Without drying, therefore, it is carefully washed off the cotton-wool into a suitable flask, with about 200 c.c. water and 20 c.c. HCl (sp. gr. 1.121) added, the flask connected with an upright condenser and heated in the water-bath for 3 hours.

After cooling, the liquid is filtered, and the filtrate very carefully neutralised with caustic soda and diluted, so as to measure 500 c.c.; finally titrated with Fehling's solution, and the results calculated to starch or any other desired basis.

A few drops of chloride of zinc may be added, so as to assist clarification of the liquid before filtering.

Extraction with alcohol is indispensable, without which the results are most uncertain and unreliable. Proper and careful extraction with uninterrupted boiling are the two most important conditions to be attended to.

These results, therefore, which I have this evening placed before you, were obtained by the methods I have just described, and although individually I am disappointed with them, I must admit, nevertheless, they are of considerable value, as they are representative analyses of several varieties of pepper, obtained from various quarters of the globe, and as met with in commerce and as shipped to this country.

The moisture and oil determinations are of no practical value, and the same may be said of the piperidine and piperine; the piperidine being in too small quantity to be of any use, the piperine, on the other hand, exhibiting large variations, showing a maximum of 13.03 per cent., with a minimum of 5.21 per cent., and giving a mean of 8.25 per cent.

The albuminoids and matter soluble in alcohol are also of little practical value (except that an excessive amount would reveal the presence of some foreign seed rich in nitrogen), so that we are left with the starch, fibre, and ash as the only data which approaches to anything like reliable, constant, or uniform amount. Fortunately in two of these we have very reliable data, viz., in the ash and fibre. Too great a value, in my opinion, has been placed upon the amount of starch, as will be seen in referring once more to the diagram, a maximum of 42.45 per cent. being obtained, with a minimum of 29.60 per cent., giving a mean of 36.02 per cent. for the black peppers.

It may be of more value in judging white peppers, but as I had only three samples of white, my remarks do not, therefore, apply to white pepper.

An increase of fibre indicates the addition of a more fibrous material than pepper. The fibre will be observed to reach a maximum of 15 per cent., with a minimum of 10 per cent., and a mean of 12 per cent., so that we can easily accept 20 per cent. as a fair basis for calculating the amount of fibrous material.

When we come to the consideration of the ash, however, we have very reliable data, the amount of ash immediately indicating the addition of any material containing a larger proportion of inorganic matter than pepper.

The ash is undoubtedly a most reliable and valuable constituent, and it will be observed that not in a single instance has it reached 5 per cent. If we leave out the Long pepper, we obtain a maximum of 4.65 per cent., with a minimum of 3.51 per cent., and which gives a mean of 3.96 per cent. Anything, therefore, above 5 per cent. must be looked upon with suspicion.

Another valuable indication is the solubility of the ash in water and in hydrochloric acid.

In water we have a maximum solubility of 2.72 per cent., a minimum of 1.41 per cent., with a mean of 1.93; and the insolubility in hydrochloric acid gives a maximum of .62 per cent., with a minimum of 0.06 per cent., and a mean of 0.82 per cent. (Long pepper gives 1.47), so that anything over 1 per cent. of insoluble matter in hydrochloric acid ought to be reported against as containing sand or dirt.

	Moisture.	Oil.	$C_6H_{11}N$	Ash.	$C_{17}H_{19}NO_3$	Fibre.	Albumi- noids.	Starch.	Soluble in C_2H_6O .	Undeter- mined.	Total.		Solubility of Ash in		
											N.		H ₂ O.	HCl.	SiO ₂
Acheen ..	15.15	1.51	0.50	3.79	12.21	10.00	2.34	42.45	0.84	11.21	100.	1.06	1.41	1.74	0.62
Alleppey ..	15.36	1.87	0.44	4.66	13.03	13.10	2.62	29.60	2.12	17.20	100.	1.34	2.72	1.87	0.06
Kampoot ..	13.82	1.63	0.55	3.51	8.13	11.65	5.37	37.50	9.57	8.27	100.	1.42	1.50	1.70	0.31
Lampong ..	15.22	1.42	0.77	4.46	11.05	15.05	7.25	30.80	4.55	9.43	100.	1.82	2.15	2.10	0.21
Long Pepper ..	12.26	1.56	0.34	7.57	7.15	13.75	6.93	32.10	3.50	14.84	100.	1.51	2.18	3.92	1.47
Penang, W.C...	15.04	0.98	0.46	4.14	6.41	11.70	8.37	41.65	5.44	5.81	100.	1.72	1.71	1.63	0.56
Siam ..	14.06	1.29	0.76	3.65	6.89	12.25	6.93	40.15	6.26	7.76	100.	1.57	1.64	1.67	0.34
Singapore ..	14.72	0.99	0.72	3.64	5.72	10.75	6.31	32.18	5.55	19.42	100.	1.40	2.00	1.48	0.16
Tallicherry ..	14.24	1.01	0.39	3.87	8.25	12.15	5.87	33.33	2.60	18.29	100.	1.40	2.26	1.55	0.06
Trang ..	14.02	1.40	0.47	3.96	5.21	10.70	6.68	37.50	4.54	15.52	100.	1.49	1.74	1.92	0.30
Penang, W. ..	14.94	0.53	0.34	2.47	8.87	4.35	2.62	51.00	0.46	14.42	100.	0.90	0.31	1.97	0.19
Siam, W. ..	13.13	1.41	0.21	2.22	7.79	4.45	6.00	53.50	0.11	11.18	100.	1.37	1.18	0.95	0.09
Singapore, W..	15.62	1.14	0.42	1.07	8.66	4.20	7.00	52.00	1.76	8.13	100.	1.61	0.30	0.62	0.15
Husks, etc. ..	12.54	1.74	0.74	16.34	6.32	22.88	6.50	11.80	4.23	16.91	100.	1.47	1.10	6.71	8.53