THE COLORIMETRIC ESTIMATION OF SALICYLIC ACID IN FOODSTUFFS.

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THE work was carried on with a view to obviating the difficulties experienced in obtaining a good colour with Fe_2Cl_6 , when using the present methods employed for estimating small quantities of salicylic acid in foodstuffs containing tannin bodies.

The test is based upon the fact that lead tannate is insoluble in caustic alkalies, while lead salicylate is readily soluble, and resolves itself into the separation of the lead salts of both acids by means of potash or soda.

The process which gave the best results with preserved fruits, fruit pulps, or jams is the following: Fifty grammes of the crushed sample to be tested are placed in an ordinary flask which has previously been marked at 300 c.c.; a small quantity of water is added, then 15 to 20 c.c. of a saturated solution of basic lead acetate, and the whole made alkaline by the addition of about 25 c.c. of roughly normal soda.

The alkali first throws down the excess of lead, afterwards dissolving the hydroxide and a quantity of albuminoids, etc., which are reprecipitated on adding acid, giving a clear colourless filtrate; to effect this, 15 to 20 c.c. roughly normal HCl are added.

The above procedure was found necessary in order to obtain complete solution of the lead salicylate, which is not reprecipitated by the acid.

The contents of the flask are then made up to the 300-c.c. mark, well shaken, and filtered cold through Rhenish paper, 200 c.c. of the filtrate being collected. The filtrate is acidified with HCl, refiltered if necessary, and extracted three times with ether; HCl has been used in preference to H_2SO_4 , as lead chloride is easily filtered off and washed, and if present in small quantities only, does not interfere with the extraction.

The ether is distilled off, the salicylic acid dissolved in a small quantity of dilute alcohol, made up to 100 c.c., and estimated colorimetrically with Fe_2Cl_6 in the usual way.

The result multiplied by three will bring the amount to percentage.

The presence of alcohol in the liquid to be extracted prevents complete solution of the salicylic acid in ether; in cases where large quantities of alcohol are present this must be driven off before proceeding with the test. To effect this, the sample must be made alkaline and boiled gently; afterwards the process is continued as before. It is advisable to neutralize the alkali before adding lead acetate, or too great an excess of the former may result.

Different samples require different treatment (with regard to the quantity of lead acetate and alkali used), in order to insure the maximum rapidity of filtration combined with the smallest quantity of lead hydroxide dissolved; there is one point at which this takes place, a point which can readily be judged by practice.

If the contents of the flask are made too alkaline, filtration is rapid, but a large excess of lead and albuminous bodies come through into the filtrate, making a second filtration necessary; on the other hand, if too acid, filtration is also rapid, but the

complete solution of the lead salicylate is then doubtful. After the addition of the lead acetate and alkali the contents of the flask may be raised to boiling with safety; this will materially assist filtration, but has the disadvantage of allowing a large amount of lead to go through into the filtrate. We do not recommend this unless absolutely necessary; in any case, the filtration should be conducted in the cold.

For beers the process used was the following: 100 c.c. of beer are placed in a flask marked at 200 c.c., made alkaline with 5 c.c. roughly normal NaOH, and all the alcohol driven off; this should be done at a temperature just below the boiling-point, as vigorous boiling appears to result in loss even when the solution is alkaline.

After cooling, 5 c.c. of roughly normal HCl are added, and then at least 20 c.c. basic lead acetate; the whole is then made alkaline with about 20 c.c. normal NaOH, and made up to the mark. At this stage the whole may be raised to boiling and allowed to cool before filtering, but this may be omitted if thought advisable.

One hundred c.c. of the filtrate are collected and acidified as before; in this case a precipitate of lead chloride is thrown down, which is filtered off, and the filtrate extracted with ether as before.

The process which gave the best results with wine was the same as with beer—taking 50 c.c. of the wine, making up to 100 c.c., and filtering off 50 c.c. As with beer, it was necessary to add a large excess of lead acetate, and to have the whole fairly alkaline, in order to exclude all tannin bodies from the filtrate.

If any tannin colours present themselves in the process of matching, they are probably due to using an insufficient quantity of lead acetate.

The lead acetate used was a saturated solution made in the usual manner by boiling with litharge.

The tendency which beers and wines have to emulsify when shaken with ether is entirely obviated by this process, it being impossible to produce an emulsion with the filtrate even with the most vigorous shaking.

With regard to the actual colorimetric readings, these were observed in ordinary 50-c.c. Nessler tubes, using a very weak solution of ferric chloride, which should be made up fresh when required. The standard salicylic acid solution used was 0.01 per cent., which could be kept without change for a very long time.

In a paper read before this Society on November 5, 1902, Mr. Harvey recommended the use of ferric alum instead of ferric chloride, claiming that it gives with salicylic acid a deeper and more permanent colour. We have tried several samples of ferric alum, and are unable to confirm Mr. Harvey's results.

On allowing two tubes to stand over night, one containing ferric alum and the other ferric chloride, we found that the former had become quite turbid and the iron had separated out, while the latter, although not the same tint as at first, was just as clear.

The use of sulphuric acid in the ferric alum solution as recommended is also a drawback, since using one drop in excess will prevent the colour with salicylic acid appearing at all.

We find our process gives excellent colours with black beers and wines containing large quantities of tannin, and works perfectly satisfactorily, even after the addition of about 0.5 gramme tannic acid in solution.

The subjoined table will show the degree of accuracy which may be expected :

Sample.	Salicylic Acid added in Grains per Pound or per Pint.	Salicylic Acid found in Grains per Pound or per Pint.	Error.
Apple pulp	0.23	0.22	0.01
„	0.45	0.36	0.09
„	0.45	0.43	0.02
„	0.45	0.32	0.13
„	1.06	0.93	0.13
„	1.50	1.36	0.14
Blackberry jam	0.40	0.30	0.10
Apple pulp	0.70	0.54	0.16
Blackberry jam	0.56	0.42	0.14
Black currant jam	3.80	3.77	0.03
Preserved apricot	3.80	3.77	0.03
Bitter beer	1.14	0.91	0.23
„	1.14	0.92	0.22
„	1.14	0.91	0.23
Preserved apricots	1.50	1.40	0.10

The working error of the method applied to beers, etc., appears to be slightly larger than in the case of fruit pulps, jams, etc., but this is no doubt due to the boiling required at the commencement of the operation and the subsequent filtration. Again, any method that depends on an extraction with ether and a colorimetric matching must of necessity possess an inherent minus error. In the case of jams, etc., the average error is about 0.09 to 0.1 grain per pound, while in the case of beers, etc., it is about 0.22 grain per pint.

We may, perhaps, point out that we have in all cases worked with quantities representing about 1 grain per pint, or 1 grain per pound in the case of solids, partly because in the estimation of larger quantities the percentage error due to the interference of tannin bodies is very much smaller, and such cases consequently present no difficulty. We have also been influenced by the consideration that the above-mentioned quantity is the limit recommended by the Preservatives Committee.

In conclusion, we should like to take this opportunity of thanking Mr. Chapman for his kindness in not only allowing us the use of his laboratory, but also for the personal assistance he has given us in carrying out this work.

DISCUSSION.

The CHAIRMAN (Mr. Blount) having invited discussion,

Mr. BEVAN said that the difficulty of getting the salicylic acid in a state of purity was well known to those who were in the habit of estimating it, either directly or by titration or colorimetrically, and they were therefore much indebted to the authors for showing how that difficulty might be overcome. The authors had mentioned, in the case of beer, an error of 0.23 in 1.14, or something like 25 per cent. He should like to hear whether, supposing the quantity of salicylic acid present to be 11.4, the error would be 2.3. If so, it would be a rather serious error.

Dr. SCHIDROWITZ said that in his experience there was an exaggerated view as to the frequency of the occurrence of salicylic acid in beer. He should like to know whether the authors had ever met with a sample of beer which did contain salicylic acid; he himself never had.

Mr. BEVAN: In the case of lager beer, certainly.

Dr. SCHIDROWITZ said that some little time ago attention had been strongly directed to the use of salicylic acid in imported beer, and he had taken the trouble to obtain samples of nearly every kind of lager beer in London, but had not been able to detect salicylic acid in any of them. If, however, beer or wine containing salicylic acid was kept for some time, the salicylic acid undoubtedly disappeared gradually.

Mr. CHAPMAN said that the paper to which they had listened that evening had had its origin in the fact that in his laboratory it had been found absolutely impossible to detect salicylic acid in one or two samples of fruit pulp in which it was known to be present in small amounts. Some fruits contain relatively large quantities of tannin and other bodies capable of producing a dark coloration with ferric chloride, and this in some cases entirely masks the fainter colour due to the salicylic acid. It was with the object of surmounting this difficulty that the authors had tried the method now described, and had found it to be very satisfactory. The method worked well with beer, but, as in the case of most analytical processes, some experience of it was essential for its successful application. A very interesting point in connection with the method was that the solutions to be extracted showed no tendency whatever to emulsify. This was, of course, highly important, inasmuch as the formation of emulsions is one of the most fruitful sources of error in processes involving extraction by the employment of immiscible solvents.

Mr. A. E. PARKES said that he had not quite followed the way in which the authors treated the ether extract. If the solvent were distilled off, the amount of salicylic acid obtained would be lower than when the ether was extracted with alkaline water or allowed to evaporate spontaneously.

Mr. LING inquired whether a clean crystalline residue was obtained after the first extraction, or whether it was necessary to extract again. In the ordinary process one had to make two extractions, one with ordinary ether and one with petroleum spirit, in order to obtain anything like a clean residue.

Mr. HARRY, in reply, said that they had only tried the method with small quantities of salicylic acid, but were certainly of opinion that with larger quantities the error would not be larger in proportion. There was no great difficulty in estimating large quantities of salicylic acid in beer, as the tannin was but small in quantity and did not interfere; in fact, they doubted whether it would be necessary to use this method at all. With regard to the treatment of the ether extract, they distilled off the ether in an ordinary Soxhlet apparatus, and then made up with dilute alcohol, the estimation being proceeded with as described. In some cases the residue might not be quite dry, but where a fairly dry extract was obtained it was quite crystalline and generally colourless. In some beers it might be slightly yellow, but this was not noticeable when it was in solution.