THE DETECTION AND DETERMINATION OF PROPIONIC ACID AND SORBIC ACID IN BREAD AND FLOUR CONFECTIONERY

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The Preservatives in Food Regulations, 1962, set maximum limits of 3000 parts per million for propionic acid in bread (calculated on the flour content) and 1000 parts per million in flour confectionery. Detection and estimation of this preservative is far from easy and published methods such as controlled distillation and chromatography on silica gel are not suitable for routine control work. A routine method is described for detecting and determining propionic acid if gas chromatography apparatus with an ionisation detector is available. The number of samples which can be examined daily is mainly limited by the initial steam distillation step. The determination of Sorbic Acid can also be carried out on the same distillate.

Preparation of Sample

The sample is finely crumbled and mixed or is passed through a mincer and then rubbed through a coarse sieve. It is stored in a three-quarter filled, stoppered jar. If the determination cannot be carried out within 48 hours, the sample is preserved by adding 5 ml of chloroform absorbed in one gram of cotton wool per pint capacity of the container.

Isolation of Volatile Acids

This is a slight modification of the method of the A.O.A.C.[1]

Transfer 25 g of the sample to a 350 or 500 ml steam distillation flask with 100 ml of water, 20 ml of N H_2SO_4 and shake well to disperse the sample. Add 20 ml of 20 per cent phosphotungstic acid solution, swirl and add 80 g of $MgSO_47H_2O$. Swirl again and if not acid to congo red paper add more sulphuric acid. Steam-distil 350 ml into a receiver containing 1 ml of N NaOH, keeping the volume in the flask from increasing by using a small flame.

Make sure the distillate is still alkaline and boil down to approximately 5 ml in a 500 ml beaker. Transfer to a small dish and evaporate to dryness on a water bath. To the residue add 10 ml of ether and 0.5 ml of 1 in 6 sulphuric acid, stir well to bring the acid into contact with all the solid material and test for

excess acid with congo red paper. Add approximately 1 g of anhydrous sodium sulphate to absorb the water and stir well. Decant the ether into a 25 ml standard flask and extract the sodium sulphate with further small portions of ether. Make the ether extract up to 25 ml, add a little more sodium sulphate and shake well. This ether solution is now ready for injection on to the gas chromatography column.

Gas Chromatography

Propionic acid can be separated from acetic and butyric acids on a 6 ft column of 5% to 10% poly-ethylene glycol succinate on Celite at 75 - 90° C and detected and determined using either an argon, electron-capture or flame ionisation detector. The electron capture detector is preferable as it can be made more sensitive and selective and also has a better base line with no interference from the trailing edge of the solvent peak. It should perhaps be mentioned that an ordinary argon detector can often be used as an electron capture device by working it at 100 volts or less and substituting nitrogen for argo as the carrier gas. In fact, the authors' argon detector used in this way is appreciably more sensitive for propionic acid than their electron capture detector. Under optimum conditions the argon detector at 85 volts gives a full scale deflection, (100 divisions) of the recorder, at 1/30 of full sensitivity, for 5 micro-litres of 0.1 per cent propionic acid solution in ether. If 5 micro-litres of the prepared ether solution of the sample is injected, then one scale division is equivalent to 10 parts per million propionic acid in the sample (the 25 ml of ether being the extract from 25 g of sample). Because the surfaces of the detectors become contaminated by bleeding from the column, the sensitivity is normally somewhat lower than this.

Using a 6 ft column of 7% polyethylene glycol succinate at 88° C and a nitrogen flow rate of approximately 66 ml per minute the acetic acid peak occurred at 7.2 minutes and the propionic peak at 10.5 minutes. The peak height was reproducible and was proportional to the concentration of propionic acid in the ether (in the range 0 - 0.2%). Three recovery experiments of propioni acid added to bread in the distillation flask (600 to 2000 parts pe million on the bread) each gave a recovery of over 95%.

Notes on the Preparation of the Column

For the gas chromatography of highly polar molecules such as free acids, thorough preparation of the Celite is necessa to prevent adsorption effects and resulting low recoveries and badly tailing peaks. Heat the Celite (100-120 mesh) at 500° C for

at least 2 hours, then digest on a water bath twice with 20% hydrochloric acid for several hours and wash with water until the water is neutral. Treat for one hour with 2% methanolic KOH, wash with methanol until neutral, dry and sieve to remove powder.

Glass apparatus is preferable but the present work was done using a glass flash-heater joined to a copper tube with silicone rubber tubing. The inside of the copper tube is coated with silica by twice treating with a four per cent solution of dimethyldichlorosilane in CCl_4 and heating to approximately 250° C in a bunsen. The Celite can be further improved by exposing it to the vapours of the pure silane in a closed jar until it will float on water. The column is prepared in the usual way by vibration and is pretreated by passing nitrogen through it at 120° C for at least 24 hours before joining to the detector.

Sorbic Acid

Examination for this preservative, which is also permitted in flour confectionery, may be conveniently combined with the above determination. On a routine basis, make 5 ml of the above 350 ml steam distillate just acid, dilute to 50 ml and check the U.V. absorption for the sorbic acid peak at 260-262 mμ. If present, use a $E^{1\%}_{1cm}$ value of 2200 for sorbic acid to give an estimate of the maximum amount present in the sample. This will be somewhat higher than the true value due to the presence of other volatile compounds. If the value obtained exceeds the permitted maximum (1000 parts per million for flour confectionery), the sorbic acid can then be determined on the ether solution from the propionic acid determination by using a 2 - 3 ft column of polyethylene glycol adipate on Celite at 175° C.

References

1. Official Methods of Analysis, Association of Official Agricultural Chemists, Washington, D.C., 1960, (9th Ed.), 171.

NEW FOOD AND DRUGS LEGISLATION DURING 1963

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During the year 1963 some five Statutory Regulations were made which are of interest to Public Analysts and of these, probably the most important relates to bread and flour.

The Bread and Flour Regulations, 1963, were made on the 19th August, 1963, but do not come into operation until the 1st September, 1964. They re-enact the Flour (Composition) Regulations, 1956, with regard to the requirement that flour shall contain specified amounts of chalk, iron, vitamin B_1 and nicotinic acid but, in addition, they cover almost every other possible aspect of the composition and labelling of both bread and flour. In regard to the four nutritive additives mentioned above a prosecution may only be instituted if a sample has been taken in a specified manner at a flour mill or dock. This proviso is introduced because of the admitted difficulty of introducing trace additives evenly throughout small (retail) quantities of flour

Wheatmeal or brown flour is required to contain on the dry weight, not less than 0.6 per cent of fibre. The only colouring matter permitted in any type of flour is caramel and the bleaching agents or improving agents permitted (except in wholemeal flour) are restricted to ascorbic acid, potassium bromate, ammonium persulphate, potassium persulphate, mono-calcium phosphate, chlorine dioxide and benzoyl peroxide (this last in an amount of not more than 50 parts per million). Cake flour may contain chlorine and flour for biscuits may contain not more than 200 parts per million of sulphur dioxide.

The regulations include a comprehensive list of the ingredients which may be present in white or in brown bread. For certain of the ingredients maximum limits are prescribed; for example: soya bean flour in white bread is restricted to not more than two per cent of the flour, the corresponding figure for brown or wholemeal bread being five per cent. Wheat germ bread is required to contain 10 per cent of added processed wheat germ (calculated on the dry matter of the bread) and soda bread, in addition to the ingredients which may be present in white bread, must also contain sodium bicarbonate.

Milk bread is required to contain six per cent of whole milk solids calculated on the dry matter of the bread; but bread containing six per cent of skimmed milk solids may be described as "milk bread: contains skimmed milk solids". Special labelling requirements for milk bread preclude labels from claiming that the bread is rich in milk or that it has improved nutritional value because of the presence of milk solids.

Bread sold as protein bread must contain not less than 22 per cent of protein and gluten bread must contain at least 16 per cent of protein, both calculated on the dry bread. There is a specific regulation relating to slimming and similar claims in relation to bread, biscuits, rusks and cereal breakfast foods. To substantiate a claim that a food is starch reduced it must

contain, on the dry matter, less than 50 per cent of carbohydrate. Specific weight reducing claims are prohibited and a claim that the food is an aid to slimming must be accompanied by a clear statement that it cannot aid slimming unless the total calorie intake in the diet is controlled.

The Soft Drinks Regulations, 1963, were intended to come into operation on the 20th July, 1964, and they follow, with amendments, the form of the previous Food Standards (Soft Drinks) Order, 1953. The new regulations increase the sugar content and decrease the saccharin content of all soft drinks whether they are intended for consumption without dilution or after dilution and the labels of all soft drinks which contain saccharin must bear a declaration of its presence. The description "drinks made from whole fresh Oranges" is no longer used but has been replaced by the less likely to be misleading description "comminuted citrus drinks"; these are now required to contain specific amounts of "potable fruit" instead of, as previously, being required to be made from a minimum quantity of comminuted fresh oranges. Labelling requirements are laid down for the description of all soft drinks containing citrus juice or fruit and these specify when the words "squash", "cordial", "crush" or "drink" must be used in conjunction with the name of the appropriate fruit. Pictorial devices suggestive of fruit are prohibited in relation to flavoured carbonated drinks and words suggestive of fruit may only be used under certain specified conditions on the labels of these products. Although the regulations have not yet come into operation Proposals for Amending Regulations were circulated to interested parties during the latter half of 1963. It is proposed to make exemptions as to composition (if labelled appropriately) for soft drinks for diabetics and for low-calorie drinks. Standards of composition are also proposed for semi-sweet soft drinks and, in soft drinks generally, calcium and sodium cyclamates and saccharin calcium will be permitted as artificial sweeteners in place of, or in combination with, saccharin. Finally the regulations will be amended to require automatic vending machines and counter dispensers for soft drinks to bear the descriptive name of the soft drink and where appropriate, a declaration of the presence of artificial sweeteners.

The Milk (Special Designation) Regulations, 1963, replace the corresponding regulations made in 1960 and the several regulations will come into operation on dates between the 29th September, 1963, and the 1st October, 1964. There are two important amendments in the new regulations, the first is that from 1st October, 1964, "Untreated" will be the special

designation for raw milk and will replace "Tuberculin Tested"; although either designation will be permitted until the 31st December, 1964, in order that stocks of old containers may be used up. The second important amendment is that the Clot-on-Boiling test for milk sold under a producer's licence will no longer be used, and all untreated milk and pasteurised milk will be required to satisfy the half-hour Methylene Blue test for keeping quality. The technical details of the Phosphatase test for pasteurised milk, the Methylene Blue test for untreated milk and for pasteurised milk and of the Turbidity test for sterilised milk are unchanged.

The Liquid Egg (Pasteurisation) Regulations, 1963, came into operation on the 1st January, 1964, and they require that all liquid egg shall be pasteurised and shall satisfy the alpha-amylase test described in the Schedule to the Regulations. There are exemptions for reconstituted dried egg and for liquid egg prepared from shell eggs on the food manufacturer's premises and used within 24 hours. The pasteurising conditions laid down are somewhat different from those prescribed for milk and for ice-cream and require the liquid egg to be retained at not less than 148° F for at least $2\frac{1}{2}$ minutes and then immediately cooled to below 38° F. Enforcement of these regulations is the duty of Local Authorities as distinct from Food and Drugs Authorities.

The last new regulations to be mentioned are the Ice-Cream (Heat Treatment, etc.) (Amendment) Regulations, 1963, which came into operation on the 18th June, 1963, and the purpose of these is simply to permit sugar (i.e. any soluble carbohydrate sweetening matter) to be added to a complete cold mix after the latter has been heat-treated and before it is sent out by the manufacturer of mix.

In addition to Statutory Regulations, three Codes of Practice have been agreed during the year 1963 between the Local Authorities' Joint Advisory Committee on Food Standards and representatives of appropriate trade interests. Code of Practice No. 1 concerns the use of the word "chocolate" in flour confectionery and requires 3 per cent of dry non-fat cocoa solids to be present in the moist crumb of chocolate cakes. The code does not apply to cakes described as "Chocolate Flavoured" or to chocolate covered or chocolate decorated cakes, the crumb of which does not contain chocolate or chocolate colouring. Code of Practice No. 2 relates to the Labelling of Brandy and is a guide to the conditions under which the following descriptions may be used on labels, viz., brandy, Cognac Brandy, Armagnac

Brandy, Cognac, Armagnac, fruit brandy, Cherry Brandy, Apricot Brandy, imitation brandy and Marc Brandy. Code of Practice No. 3 relates to the determination of the crab meat content in Norwegian canned crab products. It assumes the presence of 15 per cent of protein in 100 per cent crab meat (including any natural fat content). Provided, therefore, all the protein determined by the normal Kjeldahl process is derived from crab meat this is then calculated in terms of edible crab meat. If a large amount of cereal filler is present a correction of 2 per cent of the carbohydrate content is made as an allowance for the nitrogen content of the cereal filler.

Reference should also be made to the publication during the year 1963 of the British Pharmacopoeia, 1963, the British Pharmaceutical Codex 1963 and the British National Formulary 1963. The British Pharmacopoeia 1963 became official from the 1st January, 1964, and it is recommended that the B.P.C. should be in force from the same date. The British National Formulary 1963 does not apparently state a specific date from which it should be used but it contains references to the B.P. 1963 and B.P.C. 1963; presumably, therefore, it comes into use on the same date as the B.P. This is apparently the first occasion that new editions of the B.P. and B.P.C. have been published together and become official on the same date. There are some 210 new monographs on drugs and some 105 deletions in the B.P. 1963. Several well known preparations, including Malt Extract with Cod Liver Oil, have been deleted and the additions include maize oil, sodium fluoride and a new radioactive preparation, viz., Sodium Chromate (^{51}Cr) Injection. With regard to changes in methods of assay, examples which may be mentioned include:- Penicillin tablets are now assayed chemically instead of by a microbiological method; Vitamin C tablets are assayed by means of a standard solution of ceric ammonium sulphate instead of by the 2:6 - dichlorophenolindophenol blue dye and an increased use is being made of non-aqueous titrations (as for Cyclizine tablets) and of complexometric methods (as for magnesium sulphate).

THE OCCURRENCE OF LEAD IN TEA

by H.A. Williams
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Responsibility for the examination of imported tea in this country was, by and large, transferred from the Department of Customs and Excise to the Port Health Authorities in January, 1959, and although sampling for analysis has not been continued

on the scale adopted by Customs it is felt that the experience gained at the Port of London of the occurrence of lead in tea since that date may perhaps be of interest.

It will be recalled that lead has been found in tea on several occasions in the past. Very large proportions of metallic lead were reported in 1922 after prolonged storage in lead foil which had become brittle[1], and smaller proportions of 10-20 parts per million have been stated to have been found at the Customs laboratory under ordinary conditions of packing and transport, due to abrasion of the lead linings used in the chests by the hard, sharp fragments of dried tea. As a result lead was replaced by aluminium foil some thirty years ago and, except for a few sporadic instances of contamination discovered since, the risk of any appreciable intake of lead from tea was believed to have been overcome.

Since 1959 routine testing of tea samples has been carried out for the City and Port of London Health Authorities, and although tea from the principal exporting countries, India, Ceylon and Africa, has always been found to be free from significant contamination, the presence of lead has occasionally been found in consignments from certain other countries, notably China, Formosa and Indonesia. Quantities ranging from 10 to 30 parts per million were found in several consignments, and in single ones, respectively, 35, 45, 55, 60, 55-70, 80, 130-140 and 250-280 parts per million. Regarding the China and Formosa teas it should be mentioned that excessive lead was not found in the principal popular grades, such as Keemun, Lapsang Souchong or Oolong, but mainly in the inferior, flaky brown, plain teas and fannings used as "price-reducers" in blending, and occasionally in green teas. In one special hand rolled Formosa green, 350 parts per million of lead was found, probably introduced through the facing used on this tea.

The source of the lead contamination found in the other 98 samples remains something of a mystery. It could only be solved by investigations in the tea factories, and explanations have not been forthcoming. Experiments in this laboratory have shown that the greater part of the lead can usually be removed by sedimentation over carbon tetrachloride; and that it is fairly uniformly distributed, replicate determinations giving reasonably concordant results.

These observations seem to suggest a fine dust from a lead bearing soil, rather than accidental introduction of particles of lead or lead paint, or absorption from the earth by the plant, or the use of fertilisers or pesticides containing lead.

In view of the large quantities of tea consumed by some sections of the population it was obviously desirable to stop the importation of any tea containing lead, and the publication of the Lead in Food Regulations, 1961, came as a useful addition to the somewhat limited powers of the Port Health Authorities under the Public Health (Imported Food) Regulations.

When the new Regulations appeared and before they came into force in April, 1962, the attention of the principal London importers and merchants was drawn to them by circular letter, and an article by the writer on the significance of lead in tea, mentioning possible sources of contamination, was published in the Tea and Rubber Mail (4th January, 1962).

A comparison of the incidence of lead contamination in samples of tea taken on importation before and after January, 1962, is therefore of particular interest. (See figure).

The absence of a single instance of excessive lead contamination in the consignments of tea sampled by the City and Port of London Health Authorities in 1962 is noteworthy and reflects much credit on the producers.

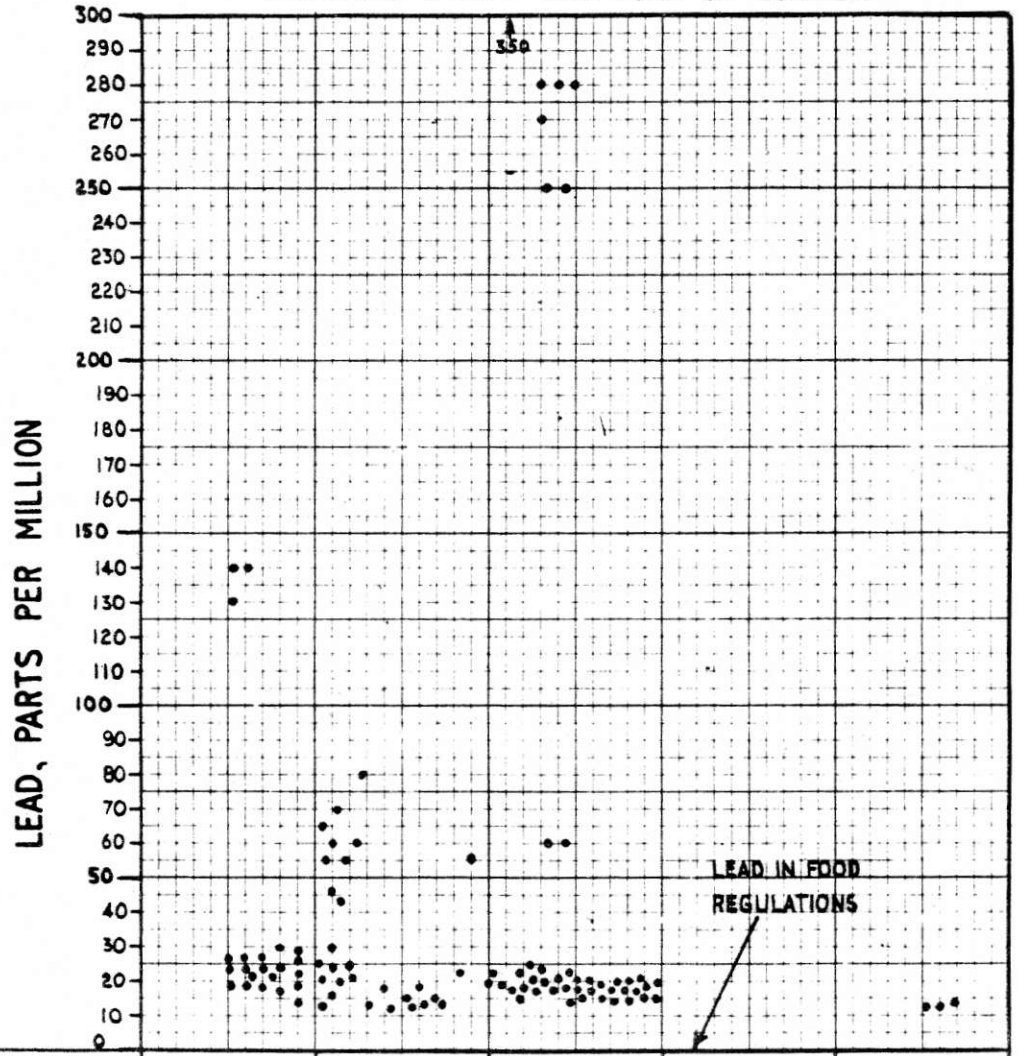
It is also of interest to record the fall in the average lead content of all samples taken since the Regulations came into force. The following Table shows the averages for the principal tea exporting countries, the 1962 averages being calculated separately for comparison with those of the preceding years, 1959-1961, all samples containing lead in excess of 10 parts per million being excluded.

Average Lead Content of Tea Samples

Exporting Country	Lead, parts per million	
	1959-1961	1962
Africa	3.2	2.3
Ceylon	3.2	2.0
China	3.9	2.4
Formosa	4.4	3.0
India	2.6	2.5
Indonesia	3.8	2.1
Japan	5.2	4.2
Malaya	2.7	1.9
Pakistan	-	2.6
South America	3.7	2.5

LEAD IN TEA

SAMPLES FROM THE PORT OF LONDON



YEAR	1959	1960	1961	1962	1963
TOTAL SAMPLES	682	3191	2635	1796	1795
EXCESS LEAD	22	29	45	0	3
PER CENT	3·2	0·9	1·7	0	0·2

In quoting the above averages it should perhaps be emphasized that they are based entirely upon the samples submitted to the analyst. The true average lead content of all tea imported from the countries mentioned will in all probability be slightly lower, because of the preponderance of dusts, fannings, damaged and lower grades of tea in the samples selected for analysis; but while the Port Inspectors' practice continues a comparison between successive year's averages should be valid.

Although the exact origin of every parcel of tea is not always known, information available has been sufficient to enable the analyst to compile records showing the lead content of tea from over 400 different estates. From these records it would appear that appreciable variations occasionally occur between different grades of tea at the same estate, some of the dusts tending to give higher lead figures.

As regards methods of analysis, it has been found that a simple procedure, involving low temperature ignition, extraction with boiling 10% hydrochloric acid, addition of citric acid, and then ammonia until alkaline, followed by potassium cyanide and extractive titration with 0.01% dithizone in chloroform, gives good results for lead in tea and is more suitable for routine purposes than the S.A.C. acid-digestion method.[2] Tea ashes usually give little trouble in ordinary porcelain dishes, but when a high lead content is encountered the dish should not be used again unless tested by repeated ignition and acid extraction until nil "Blanks" are obtained. High leads are invariably confirmed by the S.A.C. method and/or Monier-Williams' method;[3] but strangely enough these more refined methods have not in our experience yielded more concordant duplicates on tea samples, or better recoveries of added lead, than the ignition method mentioned above.

References

1. Wirthle, F. and Amberger, K., Abstract, Analyst 1922, 47, 478.
2. Anal. Methods Committee of the S.A.C., Report on Determination of Lead, Analyst 1959, 84, 127.
3. Monier-Williams, G.W., Lead in Food, Min. of Health, H.M.S.O., 1938.

RADIOACTIVITY OF TEA SAMPLES

The following is an extract from the report of the Medical Officer of Health for the Port of London for 1962.

During May, 1962, a number of samples of tea, selected at

random, from Japan, India, Congo, Tanganyika, Kenya, Ceylon, Argentina, China and Indonesia, were submitted to examination for assessment of radioactive contamination and possible radiation hazard.

At the same time, samples of a particular shipment which has passed through an area of possible fall-out were also submitted.

The examinations were carried out by Dr. F.H. Kendall, F.R.I.C., at the Sir John Cass College, Jewry Street, E.C. 3., who reported as follows :-

> "Comparison of the activities of the supposedly contaminated specimens with those from a range of normal un-contaminated samples shows that there is no detectable difference. Since the measurement technique is sensitive to all forms of radiation except very low energy beta radiation (e.g. tritium) it may be safely concluded that contamination by fall-out is negligible. The low level of activity present in all samples probably consists of naturally occurring radionuclides taken up from the soil, air and water of the tea plants' environment".

A STUDY OF THE DETERMINATION OF p-HYDROXYBENZOIC ESTERS IN FOOD

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The problem of the determination of p-hydroxybenzoic acid resolves itself into two distinct sections (a) the determination of the preservative and (b) the extraction of the preservative from food. Considerable progress has been made with the first part of the problem. The extraction procedure, however, is not yet satisfactory. The purpose of this paper is to stimulate further investigation.

(a) The determination of p-hydroxybenzoic acid and its esters.

A preliminary investigation was made into (1) the U.V. absorption and (2) the pink colour obtained by heating with Millon's reagent in boiling water.

The U.V. absorption of both acid and esters was found to consist of single peaks having the following characteristics in diethyl ether :-

Free acid	$^{2515}E^{1\%}_{1\,cm} = 1200$
Methyl ester	$^{2535}E^{1\%}_{1\,cm} = 1135$
Propyl ester	$^{2530}E^{1\%}_{1\,cm} = 995$

However this approach was rejected since in this region interference is encountered from co-extracted substances.

The Millon's reagent methods suggested by Weiss[1, 2], by Edwards, Nanji and Hassan[3], and later by Johnson[4] were investigated as quantitative methods. In the course of this preliminary investigation the authors found that (1) the reagent was not stable and the colour produced decreased with the age of the reagent, (2) the intensity of the colour increased rapidly after 10 min. in a boiling water bath, reaching a maximum at 15 min. and remaining stable up to 27 min., (3) the colour was produced by the esters without preliminary hydrolysis; the extinction coefficients of the red complexes produced from the acid and from the methyl- and propyl esters were inversely proportional to their molecular weights, (4) a variable amount of yellow mercury compounds was precipitated during the heating, apparently restricting the analyst to visual colour matching, (5) the precipitate of mercury compounds could not be washed after filtration as a turbid filtrate resulted, and (6) Millon's reagent was difficult to prepare and unpleasant to handle.

Quantities of p-hydroxybenzoic ester were dissolved in dilute sodium hydroxide and the colour with Millon's reagent developed by heating in a boiling water bath for 20 min., cooling and filtering rapidly through a cotton wool plug (without washing). Absorption measurements with an E E L absorptiometer gave an approximately straight line over the range 0 - 600 µg.

Examination of the absorption characteristics on the UNICAM S.P. 500 gave a single peak with $^{518}E^{1\%}_{1\,cm}$ approx. 1300 but varying from assay to assay dependent on age and batch of reagent. Extraction of the colour with butanol was quantitative and gave improved reproducibility. However, it became clear that any method based on Millon's reagent involved preparing standards for each assay.

We investigated a method by K. Lemieszek-Chodorowska[5] who proposed heating in a boiling water bath with Denigé's

solution followed by an addition of sodium nitrite solution. At this stage a pink colour is produced with sufficient sensitivity for the range of p-hydroxybenzoic esters anticipated. Lemieszek-Chodorowska, however, adds 15% HCl - when a much paler yellow colour is formed. Yellow colours are unfavourable, since many food extracts are slightly yellow, and in any case the intensity of the colour is too weak to be of value. The authors, therefore, investigated the measurement of the much stronger intermediate red colour, which has very similar absorption characteristics to the red colour produced by Millon's reagent. The reagents appeared stable, more pleasant to handle, and a reproducible curve could be obtained with both acid and esters. It was found that after 5 min. heating in boiling water, a suitable colour development time was 45 min. (See Table I). No precipitation of mercury compounds took place, and the extinction coefficients were of the same order as those obtained with the Millon's reagent.

TABLE I

Determination of p-hydroxybenzoic esters

Variation of extinction with time using Denigé's reagent

Time in mins.	Extinction x 100	Time in mins.	Extinction x 100
2	10.0	20	36.0
4	20.0	40	38.0
6	25.0	60	38.5
8	28.0	80	39.2
10	30.5	100	40.0
16	34.0	120	40.8

This form of the mercury - red colour was not extractable with n-butanol or with other solvents. Addition of comparatively large quantities of mercuric nitrate rendered the colour partially extractable with n-butanol but quantitative extraction was not achieved. Additions of nitric acid were also unsuccessful. Thus, a reasonably colourless extract from the food is essential, which would not be necessary if the red colour could be extracted by a suitable solvent.

Experimental

Quantities of methyl and propyl esters were dissolved

in 0.1 N NaOH and diluted to give from 100 to 600 µg of esters per 5 ml. 5 ml of Denigé's reagent were added and the tube placed in boiling water for 5 min. After cooling, 5 drops of sodium nitrite solution (2%) were added and 45 min. allowed for the colour to develop. The extinctions were then read on the E E L absorptiometer (filter 604). The results are recorded in Table II.

TABLE II

Determination of p-hydroxybenzoic esters

Extinctions obtained using Denigé's reagent

µg of ester	Extinction x 100 (E E L filter 604)	
	propyl ester	methyl ester
100	14.1	19.0
200	28.1	37.2
300	42.9	56.0
400	58.1	77.5
500	72.0	97.7

(b) The extraction of p-hydroxybenzoic esters from foodstuffs.

It was easily demonstrated that the esters of p-hydroxybenzoic acid are not steam volatile. Direct diethyl ether extraction was found difficult with most materials (coffee essence, sauces and pickles, etc.) in which it will be necessary to determine the esters. The authors examined the possibility of direct extraction after grinding with sodium sulphate, vermiculite, or mixtures of the two, and also the use of solvents other than diethyl ether. Finally, the extraction technique suggested by Lemieszek-Chodorowska was investigated. The latter proved more satisfactory than any, although in the authors' hands 3 x 50 ml ether extractions were required and not two as stated. Diethyl ether extraction after grinding with sodium sulphate gave recoveries up to 85% in tomato sauces and pickles. The latter technique failed with coffee essence, giving a recovery of only 50%. However, being less tedious than the Lemieszek-Chodorowska technique it may merit further consideration, if only as a 'sorting-test'.

(c) Tentative quantitative method

Weigh, to the nearest 0.02 g, approx. 2 g of sample. Add 60 ml water at 50° C and adjust to pH 7.5 with sodium hydroxide solution (5%). Heat at 50° C for 30 min. stirring occasionally. Add 2 ml of potassium ferrocyanide solution (15%). Mix carefully, add 2 ml of zinc sulphate solution (30 g $ZnSO_4 .7H_2O$ in 100 ml water). Mix, dilute to 100 ml and set aside for 30 min. Filter, and to 50 ml of the filtrate add 1 ml of sulphuric acid (100 ml of sulphuric acid, S.G. 1.84, made up to 300 ml with water). Extract with 3 x 50 ml of diethyl ether. Wash the combined extracts with water (3 x 5 ml/30 sec.) add 1 drop of phenolphthalein solution (1%) and shake with 3 ml of 0.25N sodium hydroxide solution. Wash with 3 x 1 ml water, combine alkaline extracts and washings, heat in a boiling water bath to remove ether, cool and dilute with water to 10 ml. To 5 ml of this solution add 5 ml of Denigé's reagent (5 g of HgO dissolved in 20 ml conc. sulphuric acid and made up to 100 ml with water). Heat in a boiling water bath for 5 min. Cool, add 5 drops of aqueous sodium nitrite solution (2%), and allow to stand for 45 min. Read on a suitable absorptiometer or spectrophotometer (518 mμ). Dissolve 50, 100, 200, 400 and 600 μg of ester in 3 ml quantities of 0.25N sodium hydroxide, make up to 5 ml and carry out the above method starting from "add 5 ml of Denigé's reagent. . . . ." Prepare a calibration graph or calculate a mean extinction coefficient.

It may be necessary, of course, to know which ester is present (see note (4)), but otherwise the result can be reported as:-

x p.p.m. p-hydroxybenzoic esters as methyl p-hydroxybenzoic ester.

(d) Proposed qualitative method

Mix 2 g of the sample with 1 ml of sulphuric acid (10%). Add anhydrous sodium sulphate until the substance is apparently free from liquid phase. Add a further similar quantity of sodium sulphate (plus sand if necessary), mix and grind in a glass mortar. Add 25-50 ml of diethyl ether, stir well for 30 sec. and decant, filtering through a cotton wool plug. Repeat this extraction with 2 x 25 ml of diethyl ether. Combine the ether extracts and evaporate to dryness. Add 1 ml of 0.1N sodium hydroxide solution, warm to dissolve make up to 5 ml and continue as under (c) commencing at the words "add 5 ml of Denigé's reagent. . . ."

N.B.

(1) Certain foods gave intractable residues by the qualitative method and coffee essence yielded only 50% recovery. Most foods, however, gave 85% recovery and the method provides a useful "sorting-test" for many foods. If 2 portions of 2 g are weighed out and 200 µg of methyl ester added to one portion then the increase in extinction due to 200 µg can be used as an "internal recovery" to make this rapid technique semi-quantitative.

(2) It should be noted that salicylic acid and other hydroxybenzoic acids give this reaction and would interfere if present.

(3) Use of clearing reagents without adjusting the pH involves loss of the esters, by adsorption on the precipitate.

(4) Chromatographic techniques have been reported for the identification of the various esters of p-hydroxybenzoic acid by Bastianutti and Romani [6] and by Höyem[7]. The authors are not able to comment on these methods.

References

1. Weiss, F., Detection and Determination of p-hydroxybenzoic acid methyl ester in Foods. Analyst, 1928, 53, 291.

2. Weiss, F., Detection and Determination of p-hydroxybenzoic acid and its esters in foodstuffs. Analyst, 1930, 55, 584.

3. Edwards, F.W., Nanji, H.R., and Hassan, M.K., The Detection and Determination of p-hydroxybenzoic acid and its Derivatives, with special reference to their distinction from Salicylic and Benzoic acids. Analyst, 1937, 62, 178.

4. Johnson, H.W., The colorimetric determination of p-hydroxybenzoic acid and its esters. Analyst, 1946, 71, 77.

5. Lemieszek-Chodorowska, N., Determination of p-hydroxybenzoic acid esters in foodstuffs. Roczn. Zakl. Hig., Warsaw, 1962, 13, (1), 133-140, (Anal. Abstr., 1962, 9, 5441).

6. Bastianutti, L., and Romani, B., Chromatographic identification of some preservatives in wine. Boll. Lab. Chim. Provinciali, 1960, 11, (3), 362-367, (Anal. Abstr. 1961, 8, 2161).

7. Höyem, T., Separation, Identification and Estimation of Aromatic Food Preservatives and Sorbic Acid by Paper

Chromatography and Ultraviolet Spectrophotometry.
J. Ass. Off. Agric. Chem. 1962, 45, (4), 902.

CHANGES IN FATS DURING LONG STORAGE

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Samples of some fats were 'inherited' by the author upon taking up the post he held from 1922 until the end of 1963. They were in screwed-cap glass jars and bore the date 15th January, 1921. During the years they were stored mainly in the dark in the confines of cupboards with the intention of examining them at the end of the period. The values set forth were ascertained in October, 1963.

THE INFLUENCE OF LONG STORAGE ON THE ANALYTICAL VALUES OF FATS

(Figures in Parentheses indicate corresponding values for fresh samples).

	Coconut Stearin	Nucocos	Nucoline O.S.B.	Cocoa Butter	Illipe Butter
Incipient Melting °C	22.3	21.7	-	34.0	36.2
'Slip' Melting °C	33.6	33.6	15.3	40.0	38.2
Complete Melting °C	47.4	40.3	31.0	52.0+	52.0
R.I.	1.4475 (1.4486)	1.4476 (1.4495)	1.4485 (1.4485)	1.4566 (1.4566)	1.4562 (1.4560)
Sap. Val.	252.6 (252)	256.4 (248)	266.3 (258)	231.9 (192.9)	211.3 (190)
Iod. Val.	0.9 (4.1)	1.60 (9.2)	3.29 (8.15)	11.62 (36.8)	19.66 (34)
Acid. Val.	42.19 (0.1)	71.3 (0.1)	78.5 (0.1)	59.6 (1.7)	39.0 (2.0)
Reichert Val.	5.7 (5.6)	7.5 (7.0)	9.4 (9.2)	-	-
Polenske Val.	10.5 (10.7)	12.4 (13.2)	15.9 (18.0)	-	-

The procedures employed were:-

Melting points: Two capillary tubes of 1 mm bore were inserted into the solid fats so that a column of about 0.75 cm entered. The open end of one tube was sealed by jabbing it into plasticine, thus preventing slip and enabling the complete melting point to be observed. The tubes were mounted in juxtaposition so that the columns of fat were adjacent to the bulb of a thermometer reading to 0.1°C which was placed in water in the left side of an Olberg melting point apparatus. The water was heated by a convection current induced by an immersion heater in the other limb and regulated to raise the temperature by about 1°/min. Fat columns were viewed through a low-power compound microscope suitably mounted for adjustment. Incipient melting could be seen in both tubes, commencement of slip point in one, while clarity in the other denoted complete melting.

Refractive Index: An Abbé refractometer was used at a temperature of 40°C.

Saponification, Iodine, Reichert and Polenske values were determined by the usual standard methods as described by the British Standards Institution and the Society for Analytical Chemistry.

Acid Value: 5 g of sample was weighed at room temperature. A mixture of 20 ml each of I.M.S. and 40°/60°C petroleum spirit, previously neutralised to phenolphthalein were added and the flask warmed to effect melting of the fat. Inherent acidity was titrated with 0.1 N NaOH solution. The petroleum spirit assists the recognition of the end point by keeping the glycerides in solution and forming a barrier against fading due to atmospheric CO_2.

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ERRATUM

Volume 1. No. 4. Page 99.

Line 6. For "1962" read "1963".
Line 8. For "1961" read "1962".
Line 11. For "1961" read "1962".

Page 101.

Line 16. For "1961" read "1962".

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