ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Essential Oils from Mushrooms. F. W. Freise. (*Perfumery and Ess. Oil Record*, 1935, **26**, 91–92.)—Fresh, well-developed individual specimens of the genera *Clitocybe*, *Cortinarius*, *Hydnum*, *Hygrophorus*, *Hypholoma*, *Lactarius*, *Pleurotus*, *Polyporus*, and *Psalliota* (urupês, orelha de páo, or "woodear") found in Brazil, gave the following analytical data (as percentages):—Nitrogenous substances, 1·68 to 2·82; fat, 0·31 to 0·55; nitrogen-free extractive, 3·33 to 4·72; crude fibre, 0·88 to 1·65; mineral residue, 0·92 to 1·63; moisture, 92·88 to 88·63; sulphur in mineral residues (as SO_3), 3·65 to 9·25. Well-dried mushrooms (10 per cent. of moisture) contained 4·0 to 4·5 per cent., 11 to 13, and 26·0 to 29·8 per cent. of substances soluble in ether, 70 per cent. alcohol, and hot water, respectively. Soon after the maximum dimensions of the fungus are reached autolysis occurs, and 80 to 82 per cent. of the material is converted into water, ammonia and carbon dioxide, the solid residue being mainly chitin and cellulosic matter. Most of the essential oils exist in glucosidal combination in the stem and caps, but a small proportion occurs combined in the wax-like outer surface of the cap. These oils differ in composition in the case of the *Cortinarius*, *Hydnum* and *Pleurotus* species, and oils in the former class, without exception, contain sulphur compounds. Steam-distillation yields 0·00125 to 0·035 per cent. of oil (*cf.* Aye, *Arch. Pharm.*, 1931, **269**, 246; *Apoth.-Ztg.*, 1932, **47**, 1027), but this is considered too drastic a method of separation. It is preferable to obtain the glucosides by careful evaporation under reduced pressure of an extract in water, but specific enzymes are required for hydrolysis, and these may be obtained from the plant by irrigation with water for several days, followed by extraction with glycerin, and precipitation of the extract with benzene. The material is dried over phosphorus pentoxide, and the enzymes of *Clitocybe*, *Hypholoma* and *Polyporus* were then obtained as a white powder (sp.gr. 0·950 to 1·110), which produced an opalescent, foaming, non-dialysable solution with water. They were not homogeneous, and acted rapidly on glucosides, cellulose, hemicellulose, starch and sugars. The maximum hydrolysis occurs at 35° to 42° C. and *p*H 6·2 to 6·6, and it proceeds without variation in energy throughout the period of decomposition. If the concentration of glucoside is less than 10 per cent. and foreign enzymes are absent, the essential oils are easily separated, since they are heavier than water. It is considered that the glucosides and enzymes occur in separate groups of cells, and that widely different types of reaction may be initiated according to the age and vitality of the fungus. No relation appears to exist between the constitution of the products of enzymic decomposition and the organic matter on which the fungi grow. The principal constituents of the oils are *Clitocybe* (*clavipes*) and *Cortinarius* (*var. spec.*), 82·5 to 86·2 per cent. of benzyl *iso*thiocyanate, with phenyl-hydroxy-acetonitrile; *Hydnum* (*cyathiforme*) and *Hygrophorus* (*sp.*), 75·8 to 81·3 per cent. of phenylethyl *iso*thiocyanate; *Polyporus* (*melanopus*), 88·85 per cent. of benzyl *iso*thiocyanate.

J. G.

Determination of the Diastatic Power of Malt by Potassium Ferricyanide Titration. F. W. Norris and W. A. Carter. (*J. Inst. Brewing*, 1935, **41**, 167–171.)—For the final titration of the starch conversion liquid obtained in the determination of the diastatic power of malt, Cole's method (Abst., ANALYST, 1933, **58**, 616) is recommended. This procedure gives a much sharper end-point than titration with Fehling solution in presence of methylene blue alone. The solutions required are: (i) 1·333 per cent. potassium ferricyanide solution, which keeps for some weeks when stored in a dark bottle away from the light; (ii) 2·5 *N* sodium hydroxide solution, standardised against hydrochloric acid solution in presence of methyl red; (iii) aqueous 1 per cent. methylene blue solution.

A preliminary titration is first carried out: 20 ml. of solution (i) and 5 ml. of solution (ii) are placed in a small conical flask, with a pinch of powdered pumice or broken porcelain. The liquid is heated to boiling on a wire gauze and the conversion solution is run in from a burette at the rate of 1 ml. every 15 seconds until the yellow colour diminishes in intensity. One drop of solution (iii) is then added from a tube drawn out to deliver 25 drops per ml. The titration is continued until the purple solution becomes colourless. For the final titration, a volume of the conversion liquid less by about 0·2 ml. than that used in the preliminary titration is run into the mixture of 20 ml. of (i) and 5 ml. of (ii) containing pumice. The liquid is heated to boiling (in not more than 2 minutes), and kept boiling steadily for 1 minute. The titration is completed, by adding the conversion liquid dropwise, in not more than a further 2 minutes. As the preliminary titre is usually smaller than the final one, about 0·5 ml. will be required for the completion. The times indicated should be closely adhered to, and the total time from the commencement of heating should not exceed 5 minutes. Shortly before the end-point is reached the purple colour of the liquid becomes very marked, and, within one or two drops of the end-point, changes to a delicate pink; after this, one or two additional drops render the solution colourless. The titration values thus obtained agree closely with those given by the standard method, so that the ordinary formula for Lintner value is applicable directly. T. H. P.

Comparative Composition and Colour of Commercial Tomato Juice. J. S. Mitchell. (*J. Assoc. Off. Agr. Chem.*, 1935, **18**, 128–135.)—Samples of tomato juice, packed in 1932, and produced in various parts of the United States, have been examined for composition and colour. Total solids were determined by drying 5-g. samples of the well-mixed juice, in covered aluminium dishes *in vacuo* at 70° C. overnight (usually for about 16 hours). The total solids were calculated as percentage by weight of the fresh juice. For the determination of soluble solids, 37·5 g. of the well-mixed juice were weighed into 250-ml. flasks, diluted to the mark with water, and thoroughly mixed. This mixture was then filtered. Twenty-ml. aliquot portions of the filtrate were dried *in vacuo* at 70° C. overnight. The weight of the residue of soluble solids was calculated as percentage by weight of the original juice. The salt-content of the same filtrate was determined by titrating 20-ml. portions with 0·1 *N* silver nitrate solution, with potassium chromate as an indicator. The results were again calculated to percentage by weight of the original juice. Again on the same filtrate, the total acid was determined by the

titration of 20-ml. portions with 0·1 *N* sodium hydroxide solution, with phenolphthalein as an indicator. The total acid was calculated as citric acid and expressed as a percentage by weight of the original juice. The refractive index was determined on filtrates of the undiluted juice at 20° C., allowances of ±0·0001 being made for each degree above or below 20° C. Colour measurements were made by means of standard colour discs prepared and calibrated after the Munsell system (see also *A Practical Description of the Munsell Colour System*, Munsell Color Co., Baltimore, **1921**; Nickerson, *U.S.Dept. Agr. Tech. Bull.*, 1929, **154**; and MacGillivray, *Purdue Univ. Agr. Expt. Sta. Bull.*, 1931, **350**). The method of MacGillivray consists in subtracting the chroma number from 13 and adding the result to the hue number. This method was used in expressing results. It was also found possible to calculate the total soluble solids from the refractive index by means of the formula:

$$\text{Total soluble solids} = (662{\cdot}25 \times n_D) - 883{\cdot}18.$$

Determinations of the *p*H values were made by means of the glass electrode, the quinhydrone electrode, the hydrogen electrode, and colorimetrically by the standards of Michaelis, and also of La Motte. All five methods gave results in fairly good agreement, values varying from *p*H 3·8 to 4·38 for different samples. The following table gives the maximum, minimum and average values found for juice from the Western and the Eastern parts of the United States.

TOMATO JUICE SAMPLES

	Total solids Per Cent.	Total soluble solids Per Cent.	Refractive index n_D^{20}	Salt Per Cent.	Total acidity, as citric acid Per Cent.	Hue	Colour: Chroma	Colour: Value	Rating
From Western United States									
Average	6·74	6·19	1·3430	0·555	0·388	6·62	10·38	2·96	9·28
Maximum	8·31	7·71	1·3445	1·071	0·563	5·32	11·85	2·52	6·47
Minimum	5·28	4·85	1·3411	0·022	0·233	7·76	8·49	3·29	11·94
From Eastern United States									
Average	6·16	5·68	1·3423	0·687	0·383	6·98	9·92	3·02	10·09
Maximum	8·71	8·00	1·3464	1·059	0·496	6·05	11·29	2·44	8·33
Minimum	4·72	4·12	1·3396	0·000	0·269	8·15	7·21	3·32	12·38

S. G. S.

Determination of the Acid Value of Flour-fat for judging the Age of Flour. N. P. Kosmin and K. A. Alakrinskaja. (*Das Muhlenlaboratorium*, 1935, **5**, 33–36.)—The most sensitive indication of the degree of freshness of flour is the magnitude of the acid value of the flour-fat, this being independent of the fat-content of the flour. The acid value of the fat is relatively constant for freshly-ground flours, varying only from 15 to 20 mg. of potassium hydroxide per 1 g. of fat. The theoretical maximum for this acid value is, on the average, 180 mg. of potassium hydroxide per 1 g. of fat. Thus, the acid value of the flour (by which the authors mean that of the fat) gives a measure of its age or degree of maturation, on which the consistence of the dough depends. Complete removal of the fat from flour is achieved only after extracting with ether for

8 hours in a Soxhlet extractor, but a sufficient quantity of the fat having the same acid value as the total fat is obtainable in a much shorter time. The procedure recommended is as follows: 20 g. of the flour are introduced, in a filter-paper thimble, into a weighed 350- to 400-ml. Erlenmeyer flask, to which 200 ml. of petroleum spirit (b.p. 80° C.) are then added. The flask is heated for an hour under a reflux condenser on a boiling water-bath, the thimble being then removed by means of tweezers and rinsed with 20 ml. of the petroleum spirit. The solvent is evaporated from the flask, which is dried in an oven at 75° to 80° C. for 2 hours, and weighed. The known quantity of fat is dissolved in 40 ml. of a 1 : 1-mixture of neutral alcohol and ether, and the solution titrated against 0·01 *N* potassium hydroxide, phenolphthalein (or, with a dark fat, thymolphthalein) being used as indicator. The values thus obtained agree well with those found by the usual method, in which the fat is first completely extracted.

The following results, expressed as mg. of potassium hydroxide per 1 g. of fat, were obtained with different flours: 100 per cent. extraction (2 samples) immediately after grinding, 14·5, 17·9; 75 per cent., 17·9 (just ground), 26·2 (after 8 days at room temperature); 85 per cent., 41·0 (25 days at room temperature), 92·4 (6 months at room temperature), 114·3 (45 days at 30° C.); 70 per cent. (all kept for 90 days at about 12° C.), 45·1, 51·2, 55·4, 55·6. T. H. P.

Component Glycerides of Piqui-a Fats. T. P. Hilditch and J. G. Rigg. (*J. Soc. Chem. Ind.*, 1935, **54**, 109–110T.)—Piqui-a (*Caryocar villosum*), a tree indigenous to Brazil, and cultivated to some extent in Malaya, has a fruit which contains fatty matter both in the mesocarp and in the kernel, and these fats bear a resemblance to palm oil in consistence and composition, and form one of the somewhat rare instances in which endosperm (or embryo) and fruit-coat fats from the same fruit are almost similar in composition. The fruit consists of about 74 per cent. of hard outer pericarp with one to 4 seeds, each surrounded by mesocarp (13 per cent. of fruit) containing about 47 per cent. of fat, inside of which is the shell (11 per cent.), enclosing the kernel (usually less than 2 per cent. of the fruit), containing 45 per cent. or more of fat. The general characteristics of the fats were (*a*) kernel, (*b*) fruit coat fat; saponification equivalent, (*a*) 284·0, (*b*) 281·6; iodine value, (*a*) 48·6, (*b*) 47·8; acid value, (*a*) 0·9, (*b*) 10·6; unsaponifiable matter, (*a*) 1·7; (*b*) 0·8 per cent., with iodine value, (*a*) 183, (*b*) 282. The mean mol. wt. of the mixed fatty acids was (*a*) 271·4, (*b*) 268·7; iodine value, (*a*) 50·0, (*b*) 50·0; setting point, (*a*) 48·5°, (*b*) 48·7° C. The composition of the mixed fatty acids was myristic, (*a*) 1·6, (*b*) 1·8; palmitic, (*a*) 50·7, (*b*) 47·3; stearic, (*a*) 0·8, (*b*) 1·7; oleic, (*a*) 43·7, (*b*) 47·3; linolic, (*a*) 3·2, (*b*) 1·9 per cent. (mol.). The average unsaturation of the fruit-coat fatty acids is slightly greater than that of the kernel fatty acids. The kernel-fat conforms to the usual seed-fat rule of even distribution of fatty acids throughout the glycerol molecules. The fruit-coat fat resembles the corresponding Sterculia fat (ANALYST, 1934, **59**, 632) in corresponding with the kernel-fat type of distribution, thus differing from palm oils. A portion of the fruit-coat fat was hydrogenated almost completely and systematically crystallised, and the fractions were examined. A partly hydrogenated fat was also analysed. The component glycerides (wt. percentages) of the original fat are given roughly

as: tripalmitin 2; dipalmito-oleins 42; palmitodioleins 56 per cent. Both α- and β-palmitodioleins and α- and β-oleodipalmitins are probably present in appreciable quantity. D. G. H.

Koryan (Kaoliang) Oil. S. Ueno and R. Yamasaki. (*J. Soc. Chem. Ind. Japan*, 1935, **38**, 113–116B.)—Koryan corn (*Andropogon Sorghum* var. *vulgaris* Hack.), a very important product of Manchukow, from which an increasing amount of germ and shells are likely to be available as bye-products, yields 3·3 per cent. of a deep yellow oil with the following characteristics: Sp.gr. at 20°/4° C., 0·9260; n_D^{20}, 1·4655; saponification value, 187; iodine value (Wijs), 121; unsaponifiable matter, 8·6 per cent.; acid value, 18·5; saponification value of neutral oil free from unsaponifiable matter, 204; neutralisation value of mixed fatty acids, free from unsaponifiable matter, 210; iodine value of the unsaponifiable matter, 62·0. The composition of the unsaponifiable matter varied according to the solvent used, and with (*a*) ether and (*b*) benzene extraction was found to be, "takakibyl" alcohol, (*a*) 2, (*b*) 6; "koryanyl" alcohol, (*a*) 12, (*b*) 28; sitosterol, (*a*) 21·6, (*b*) 50; cetyl alcohol and $C_{30}H_{48}O_3$ (?), etc., (*a*) 63, (*b*) 15·6 per cent. This oil appears to be very suitable for the preparation of sitosterol, owing to the high proportion present, the ease of purification and the absence of other phytosterols. Three new alcohols were isolated from kaoliang oil: koryanyl alcohol ($C_{28}H_{58}O$), apparently the next higher alcohol to ibotaceryl alcohol, mol. wt. 410; takakibyl alcohol ($C_{44}H_{90}O$), m.p., 71 to 71·5° C., mol. wt. 634, iodine value 11·9; and an alcohol $C_{30}H_{48}O_3$ (?). D. G. H.

Fatty Substances of Japanese Shell-fish. M. Tsujimoto and H. Koyanagi. (*J. Soc. Chem. Ind. Japan*, 1935, **38**, 118–120B.)—*Kumanokogai.*—This somewhat conical shell-fish, weighing 1·7–7·9 g., yielded, on extraction with ether, an acetone-insoluble, dark yellow-green viscous material (phosphatides) and a liquid acetone-soluble portion with solid in suspension. The fatty acids had m.p. 32·5 to 33·5° C., iodine value (Wijs) 153·4, ether-insoluble bromide 40·3 per cent. The unsaponifiable matter, after recrystallisation from methanol, consisted of long, white prismatic crystals, m.p. 145 to 146° C., giving no depression of m.p. when mixed with cholesterol, and an acetyl compound of m.p. 118 to 118·5°C. It is regarded as consisting chiefly of cholesterol, probably mixed with a small amount of conchasterol. *Shako.*—These very large shell-fish, weighing 345 to 783 g. each (3 specimens), gave an ethereal extract nearly all of which was soluble in acetone. The fatty acids had m.p. 42 to 43° C., iodine value 91·3, and ether-insoluble bromide 16·3 per cent. The proportion of highly unsaturated acids was small. The low iodine value, compared with that of the fatty acids of other shell-fish, was to be expected from the tropical origin of shako. The unsaponifiable matter was found to contain a new sterol, called "shakosterol," an isomer of cholesterol, giving an acetyl compound with high m.p., 156 to 157° C. D. G. H.

Characteristics of Halibut-liver Oils. N. Evers and W. Smith. (*Pharm. J.*, 1935, **134**, 417.)—Analytical data for halibut-liver oil, which were scanty when the monograph in the B.P.C. 1934 was written, have now

accumulated to an extent which makes it possible to prescribe more definite limits than previously. Analytical figures for this oil from known sources have already been given by Haines and Drummond (*J. Soc. Chem. Ind.*, 1934, **53**, 81T; Abst., ANALYST, 1934, **59**, 358; and *Brit. Med. J.*, 1933, i, 558), and also by the present authors (*Quart. J. Pharm. and Pharmacol.*, 1934, **7**, 482), while Lovern, Edisbury and Morton (*Biochem. J.*, 1933, **27**, 1461) have published figures for the vitamin-*A* content. The present paper gives the results of the analyses of 33 oils of known origin. All of these results are summarised in the table, which also indicates the extreme values obtained.

	Blue value	Vitamin *A* $E^{1\%}_{1cm.}$ 328$m\mu$	Sp.gr. 15·5/15·5°	Ref. ind. 40° C.	Iodine value	Saponification value	Unsaponifiable matter Per cent.
Evers and Smith	205–7100	6·8–144	0·922–0·9286	1·470–1·488	111–171 (R–K)	150–175	8·3–21·5
Haines and Drummond	650–8700	—	—	1·474–1·478	116–161 (R–K)	172–180	9·2–12·7
B.P.C.	400–3000	—	0·922–0·925	1·470–1·478	120–136 (Wijs)	170–180	8 –13
Lovern, Edisbury and Morton	60–7630	3·5–164	—	—	—	—	—

The Rosenmund-Kuhnhenn pyridine sulphate bromide method for determining iodine values (*Z. Unters. Nahr. Genussm.*, 1923, **46**, 154–159; Abst., ANALYST, 1934, **49**, 105) was found to give the most consistent results, and is recommended as a standard procedure for halibut-liver oil. S. G. S.

Colorimetric Micro-Determination of Morphine in Opium and its Preparations. J. A. Sanchez. (*J. Pharm. Chim.*, 1935, **21**, 366–376.)—The solutions required are: (i) Wavelet's reagent: 70 g. of recently calcined molybdic acid are dissolved in a solution of 140 g. of sodium carbonate and 20 g. of disodium phosphate in 500 ml. of water; 200 g. of tartaric acid are then dissolved in the solution, the whole being made up to 1 l. with water, and filtered after 24 hours. (ii) Morphine hydrochloride solution of 1 : 10000 strength. (iii) Ammonia solution of sp.gr. 0·980. To prepare a scale for comparison, ten similar test-tubes of colourless glass are charged with 1, 2 . . . . , 10 ml. of solution (ii), 1 ml. of (i), and 1 drop of nitric acid. After 10 minutes the liquids are mixed, diluted with water to give the same depth in all the tubes, and again mixed; 20 drops of (iii) are then mixed into each. The blue colours thus obtained reach their maximum intensity within a minute and remain unchanged for about 15 minutes. Of the solution to be tested, 1 ml. is made up to 100 ml. with water, 1 and 6 ml. respectively of this solution being transferred to two test-tubes and treated with the various reagents, as described above. The two tubes are then compared with the scale of standards. The method is not applicable to solutions containing adrenaline, which also reduces Wavelet's reagent, even in absence of ammonia. Details are given for applying the method to morphine hydrochloride syrup (Codex Argentin), opium powder, Laudanum Sydenhami, and tincture of opium. T. H. P.

Assay of Ext. Cocae Liq. B.P.C. and Ext. Cocae. W. A. N. Markwell. (*Pharm. J.*, 1935, **134**, 416–417.)—The assay of coca and its preparations may yield different results by different methods, and even if a standard method (such

as that in the B.P.C. 1934) is used, different analysts may not obtain the same result. These discrepancies have been found to be due to insufficient extraction with immiscible solvents and also to the presence, or absence, of volatile bases in the final residue. The author suggests that the B.P.C. assay process for Ext. Cocae Liq. should be modified to include repeated extractions of the ammoniacal solution by successive portions of ether until the extraction is complete, and that the extraction of this ethereal solution with dilute acid should be repeated until the extraction is complete, the solution being tested in each case with Mayer's reagent. It is also suggested that the final ethereal solution be washed with a little water to remove traces of ammonium salts, as recommended by Self (*Pharm. J.*, 1915, **40**, 585; Abst., ANALYST, 1915, **40**, 329), and that the final residue be dehydrated with absolute alcohol. In addition, the volatile bases should be excluded by heating the alkaloidal residue at 80° C. for 2 hours before titration. Since the physiological action of coca is essentially that of cocaine, the author considers that the assay of this drug should be based on the content of non-volatile alkaloids calculated as cocaine, rather than upon the total alkaloids, including the volatile bases. S. G. S.

New Method for the Determination of Pilocarpine and its Salts. J. A. Sanchez. (*Ann. Chim. anal.*, 1935, **17**, 122–123.)—The method is based upon the fact that, when pilocarpine is heated with sodium or potassium hydroxide, the internal ester, of which it consists, is decomposed and a salt of pilocarpic acid is formed. This acid is monobasic and can be titrated with phenolphthalein as an indicator. For the determination of the free base, a quantity of not less than 0·05 g. is dissolved in 10 ml. of a mixture of two volumes of 95–96 per cent. alcohol and one volume of water in a flask with a long neck. To this is added 10 ml. of $N/10$ sodium hydroxide solution and 111 drops of 2 per cent. phenolphthalein solution, the mixture being heated in a boiling water-bath for 5 minutes. After the flask and its contents are cooled, 5 ml. of 95 per cent. alcohol are added and the whole titrated with $N/10$ sulphuric acid. The number of ml. of acid used, subtracted from 10 and multiplied by 0·0208, gives the amount, in grams, of the pilocarpine present. For the determination of the salts of pilocarpine, a solution containing 0·5 g. per 100 ml. of the hydrochloride or nitrate is prepared with the alcohol-water mixture described above. Into a small flask having a long neck, 10 ml. of this solution is placed, followed by 111 drops of the phenolphthalein solution. $N/10$ sodium hydroxide solution is then added until a pale rose colour is obtained. At this stage the hydrochloric or nitric acid is present as the sodium salt, and the pilocarpine, which does not affect the indicator, is liberated. To this mixture, 10 ml. of $N/10$ sodium hydroxide solution are added, the flask is heated in a boiling water-bath for 5 minutes, then cooled, 5 ml. of alcohol added as before and the solution titrated with $N/10$ sulphuric acid. The factor for the hydrochloride is 0·02445, and for the nitrate 0·0271. S. G. S.

Assay of Papain. A. K. Balls, T. L. Swenson and L. S. Stuart. (*J. Assoc. Off. Agr. Chem.*, 1935, **18**, 140–146.)—The method advocated depends upon the titration, in alcohol, of casein which has been digested at its *iso*-electric point by the papain. Thirty g. of a suitable casein are rubbed with a little water

in a large mortar, 30 ml. of *N* sodium hydroxide solution are then added, and finally water to a total volume of 1 l. The casein solution must not be heated, and is kept in an ice-box under toluene. In making the determination 10 ml. of the casein solution are placed in a 125-ml. glass-stoppered bottle together with a number of small glass beads, and the bottle is placed in a thermostat at 40° C. After the lapse of sufficient time for the temperature of the bottle and its contents to reach 40° C., 4 ml. of a buffer solution consisting of 280 ml. of 0·2 *M* disodium citrate solution and 850 ml. of 0·1 *N* hydrochloric acid, are added at 40° C. These are followed at once by the enzyme solution, whose volume should not exceed 3 ml. The bottle is shaken vigorously for 10 to 20 seconds, replaced in the thermostat, and incubated for 20 minutes. At the end of this time the contents of the flask are titrated with 0·1 *N* alcoholic potash solution. To the solution in the bottle 1 ml. of a 1 per cent. solution of thymolphthalein in alcohol is added, and then just enough alkali to turn the mixture a distinct blue. The alkali must be added slowly, with shaking, until the blue colour is permanent, and the shaking is continued until all the solid matter has dissolved. The contents of the bottle are then transferred to a 500 ml.-flask, and the bottle is washed out two or three times with a total volume of 25 ml. of 95 per cent. alcohol. To this mixture are added 175 ml. of boiling 95 per cent. alcohol, which causes the colour to disappear, and the titration is continued carefully until the blue colour is restored; this is best seen in daylight. A blank determination is also made in exactly the same manner, except that the solution in the bottle is titrated immediately after the addition of the enzyme solution and not incubated for the 20 minutes. If a series of determinations is made with different amounts of papain, it is possible to plot a graph, with the amount of papain used on the one axis and the amount of 0·1 *N* alkali required in the titration on the other. It is suggested that the amount of papain which is found to be equivalent to 1·0 ml. of 0·1 *N* alkali should be taken as the unit of papain (proteinase activity). S. G. S.