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A Review of Three Modern Techniques Available for the Determination of Soya Protein in Meat Products

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Three techniques for the determination of soya protein in meat products are reviewed, respectively based on microscopy, HPLC and Enzyme Linked Immunosorbant Assay (ELISA). The cost, speed and accuracy are compared by applying these techniques to standard mixes of hydrated soya material, meat and hydrated rusk which resembled sausage meat in composition.

Processed foods based on traditional meat products may contain vegetable proteins added as binders, to improve texture and retention of water or fat, or as meat protein extenders or substitutes. The control of such products can only be undertaken if adequate analytical methods are available.

Native "soya protein" consists of three major storage proteins designated as 7S, 11S and 15S, many specific minor proteins such as lipoxygenases and other enzymes, haemaglutinins and over 15 trypsin inhibitors, in the presence of greater or lesser amounts of carbohydrate, lipid and other biochemicals. The proportions are subject to biological variation before the food technologist processes it, thereby changing its composition yet again, mixes it with meat and other ingredients, and manufactures a product.

During processing the protein macromolecules interact, to different extents, physically and chemically with each other and with other components to form an intractable and partly insoluble agglomerate[1]. The food analyst is then set a highly complex problem. How much of the modified protein originates from the soya bean?

In an effort to solve this problem, three procedures have been developed in the area of soya analysis:

(a) the stereological approach[2]
(b) the peptide approach[3,4,5,6], and
(c) the enzyme-linked immunosorbant assay (ELISA) approach[7,8].

These three approaches were investigated for the purposes of this review, to determine the percentage of soya material incorporated in differing amounts within model mixtures. These mixtures also contained beef, fat and hydrated rusk and resembled sausage meat in composition.

Stereological Technique

Microscopical methods for the determination of soya material were qualitative in nature[9,10,11,12] until the paper published by Flint and Meech[2]. The object

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of their investigation was to produce a quantitative technique that could be used as a routine method of analysis, and this appears to have been reasonably successful for certain types of soya material.

Stereology is the term given to a collection of methods that enable information about three-dimensional quantities, including volume ratios, to be obtained from the study of two-dimensional sections. The particular stereological technique chosen by Flint and Meech was the two-dimensional systematic point count[13], first used by Glagolev[14] in 1933. This particular stereological technique provided the experimental details used for this first method.

The Peptide Technique

Currently the two main approaches to protein characterisation are immunochemical techniques[15,16,17] and gel electrophoresis[18,19,20,21]. Other techniques have been suggested, for example, *iso*electric focusing[22] and density gradient centrifuging[23].

These methods are relatively sound and reliable on uncooked products but they do not work well with materials which have been cooked or sterilised during production.

One alternative to working with whole proteins is to hydrolyse them and examine the fragments. Proteins can be distinguished from one another by differences in their primary structures. Therefore any method which detects differences in the primary structure of proteins ought to be applicable to examining unique soya proteins in the presence of meat. One of the most commonly applied primary structure methods in this field is total amino acid analysis where the proteins are completely hydrolysed. The main drawback to total hydrolysis of the protein is that much of the structural information is lost. However partial hydrolysis (for example, using an enzyme) fragments the protein at specific points in its structure and produces a wide variety of peptides each representing a portion of the original molecule. Provided the enzymic reactions go to completion, the peptide pattern obtained in this way is unique to the protein hydrolysed and is frequently called a "finger print".

Provided characteristic peptides can be detected and adequately fractionated, it should be possible to use them as a soya protein marker in mixtures, in much the same way as hydroxyproline is used as a marker for collagen in meat.

This procedure, using the enzyme trypsin to hydrolyse the proteins, was used as the second method. Several papers by Bailey and Hitchcock[3,4,5] on the method have shown that the peptide profiles of meat and soya digests were similar, there being a large number of positively charged and neutral peptides, but few negatively charged peptides. Detailed examination of the profiles showed that the soya digests contained groups of positively charged peptides which could be readily distinguished from those of meat. A specific method was developed to analyse just a small portion of the entire profile and among the main differences detected between meat and soya peptides, a component designated SP_1 was selected as the most suitable marker. The SP_1 fraction was found to be heterogeneous, consisting of one main component (60 per cent.) and at least seven minor ones. The major peptide was found to be seryl-glutaminyl-

glutaminyl-alanyl-arginine. This information enabled Bailey[6] to synthesise SP_1 which was supplied for use as a marker.

In a series of experiments it was shown that only the 11S portion of soya proteins possessed an SP_1 fraction on analysis by the routine analytical method and it was established that SP_1 from a variety of soya protein isolates had basically the same structure.

These papers[3,4,5] provided the experimental details for this second method. However, Bailey and Hitchcock employed a standard Technicon NCI automatic amino acid analyser for this technique, but as this was not available in our laboratory, it was decided to carry out this step of the analysis using a High Pressure Liquid Chromatograph with post-column derivation (see details in Figure 1).

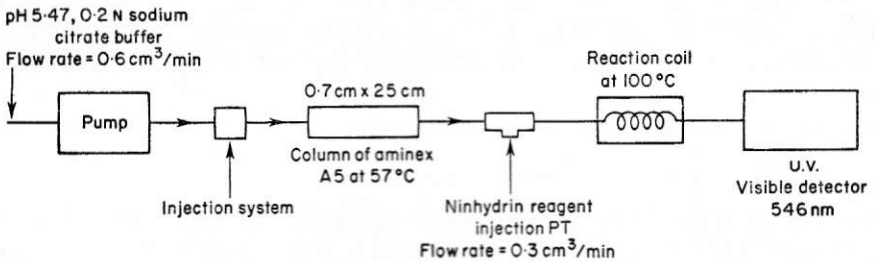

Fig. 1. High pressure liquid chromatograph with post-column deviations.

An example of the chromatogram obtained by this method for a model sample is given in Figure 2.

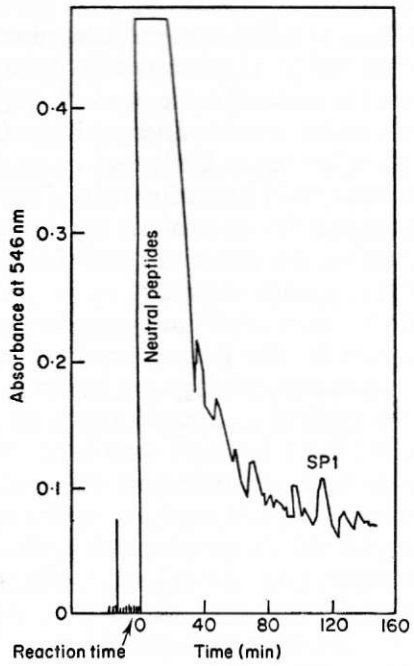

Fig. 2. Chromatograms showing the fractionation of meat and soya peptides in sample F.

The Elisa Technique

Procedures are now well established in clinical analysis[22,23,24,25,26,27] using radio-immunoassay, Enzyme Linked Immunosorbant Assay and Fluorescence-immunoassay techniques; these employ markers to detect antigen–antibody interactions. These techniques do not depend on precipitation and the assay can be carried out using small amounts of sample; such small volumes of antisera are required (1 cm^3 can be sufficient for 1000 assays or more) that standardisation becomes less of a problem.

Since the soya antigens to be assayed will have been treated under different processing conditions, they can be solubilised and then allowed to re-nature using procedures established by Koh[20].

A recent paper by Crimes, Bailey and Hitchcock[28] used an extraction procedure similar to that of Koh and raised antibodies against a "re-natured" soya isolate antigen. With these antibodies, and using an inhibition ELISA procedure, an assay for soya protein has been developed[3] and this is the method employed here.

Discussion

At the present time, as the three methods represent complementary approaches, it is not possible to recommend any single method for the characterisation of soya material in processed foods. However, the following general conclusions can be drawn.

It appears obvious from the work undertaken that estimation of soya material by the stereological technique presents problems as it can give a low accuracy in the determination of certain types of soya material and is very time-consuming, which leads to a high cost per determination. However, a quick microscopical examination will yield much needed information in determining what type of soya material is present in the product. This, used in conjunction with the other methods under consideration, will assist in yielding a more accurate final result. It therefore seems likely that, as methodology increases for the other methods, microscopy will be used more and more in this complementary role, and unless automatic image analysis can be perfected to relieve the rather tedious point counting, the future of quantification of soya material by the stereological technique appears uncertain.

The peptide technique in terms of accuracy appears to yield the best results and seems to hold promise for the future, provided analytical times can be reduced and less cumbersome methodology can be devised. At present it would be impossible to use this method as a rapid screening procedure for a large number of samples without incurring high costs in terms of equipment.

However, this review does demonstrate how this method can be transferred from the use of expensive amino acid analyser equipment to the more readily available High Pressure Liquid Chromatograph systems. It also seems likely that, with adequate research into new column packing materials to increase speed, and with a more specific means of detection[28], the way could be cleared to a rapid estimation of not only soya protein but also other proteins by adaptation of this technique (Table I).

TABLE I

COMPARISON OF STEREOLOGICAL, PEPTIDE AND ELISA DETERMINED HYDRATED SOYA MATERIAL IN MODEL MIXTURES

Sample description	Theoretical added soya material *per cent.* w/w	Stereological results* *per cent.* v/v		Peptide results *per cent.* w/w		ELISA Results *per cent.* w/w	
		WMCC Lab.	Mean of MAFF collab. trial	1 Using soya isolate standard	2 Using soya flour and TVP standards	1 Using soya isolate standard	2 Using soya flour and TVP standards
A	2·5	1·3	2·9	2·6	1·9	2·8	3·3
B	7·5	0·1	4·3	8·0	5·8	5·8	6·2
C	7·5	0·1	3·8	6·9	5·9	5·1	5·5
D	15·0	0·2	5·7	17·8	12·8	10·9	10·9
E	18·0	16·4	15·6	21·0	17·9	17·1	16·1
F	18·0	20·4	16·2	18·8	15·5	11·9	12·8
G	16·5	13·1	11·3	17·8	14·1	13·4	14·2

* Note on stereological results. Results for samples ABC+D obtained in the West Midlands County Laboratory are atypically low. The most likely reason for this appears to be mechanical damage to soya structures at the microtome sectioning stage. As the results are obtained on a v/v basis they have been reported as such. The figures on a w/w basis should be very similar.

Enzyme Linked Immunosorbant Assay procedures provide a very exciting and promising new field of food analysis. From the results obtained, it appears that a rapid semi-quantitative screening procedure for the detection of soya material can be easily achieved by this technique at a very low cost per sample. The method uses comparatively inexpensive equipment and it should be possible to achieve reasonable results using relatively inexperienced personnel. It seems likely that errors associated with this technique could be reduced by the raising of more appropriate antibodies with a resultant increase in accuracy, hopefully leading to a fully quantitative method for soya determination. Although at first sight antiserum appear expensive, the high dilutions involved make the cost per determination insignificant. It is important to note however that variations occur from one batch to another of antisera, and for each new batch extensive standardisation and optimisation of conditions need to be employed. This problem can be overcome by buying antiserum in bulk, so that enough from one batch is available for thousands of determinations (Table II).

Both the peptide and ELISA techniques suffer from the problem of expressing experimentally determined soya protein as soya material. In many cases the food analyst is faced with the problem of not knowing what type of soya material is used.

The soya protein content as soya material can vary from as low as 40 per cent. in a soya flour to 95 per cent. in a soya isolate. Assuming an average protein content of an unknown material is obviously not ideal. Fortunately, however, soya material can be classified by a quick microscopical examination into roughly three types; soya flour or grit, textured vegetable protein and soya isolate. If average soya protein figures are assigned to these types such as 50 per cent., 60 per cent. and 90 per cent. soya protein respectively a better approximation of

TABLE II

COSTING OF THE THREE TECHNIQUES INVOLVED IN THIS REVIEW AT APRIL 1981 PRICES

Stereological technique specialised equipment cost Description	Cost £	*Peptide technique specialized equipment cost* Description	Cost £	*ELISA technique specialised equipment cost* Description	Cost £
Freezing microtome	1035	2 × High pressure pumps for HPLC	4880	Manual plate reader	1300
Binocular microscope capable of 400× magnification	800	Injection system for HPLC	897	Set of 50–200 µl pipettes	200
		Detector	2130	96 Well microtitration plates with lids per 100	82
CO_2 cylinder	65	Empty stainless steel chromatographic column	110		
Total cost	1900		8017		1582
Reagents		*Specialised reagents*		*Specialised reagents*	
The reagents required for this method do not contribute significantly to the analytical costs.		Aminex A5 column packing material (25 g) = £280. All other reagents required for the above method do not contribute significantly to the analytical cost.		1 cm^3 Goat -anti rabbit antibody/enzyme conjugate (sufficient for 2000 individual determinations) = £92. All other reagents required for the above method do not contribute significantly to analytical cost.	
Man-hours per determination = 18		Man-hours per determination = 6		Man-hours per determination = 3	

NOTE: It is assumed that all normal food laboratory equipment would be available.

soya material present calculated from a determined soya protein content can be arrived at. Alternatively, once the type of soya material present is ascertained, a standard soya flour, textured vegetable protein or soya isolate can be used for calibration in the peptide or ELISA technique.

In conclusion, it would seem wise to rely on more than one approach for the determination of soya material in a meat product. A quick microscopical examination to ascertain what type of soya material is present, could be followed by an ELISA screening procedure. From this screening procedure samples of further interest could then be taken for more extensive analysis, either involving the peptide technique or a more extensive ELISA procedure. It must also be pointed out that all these techniques lend themselves to the determination of other proteins in processed foods, and further research is at present under way, particularly in the area of meat protein determination.

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Rapid Differentiation Between Vinegars and Non-Brewed Condiments: Part II

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The methods described in Part I of this study to differentiate vinegars from non-brewed condiments were evaluated. Using improved methodology, it was envisaged that differentiation between different vinegars might also be possible. Whilst all vinegars could be differentiated from non-brewed condiments using the improved osmotic pressure/freezing point depression method, and all except spirit vinegars using the ultra-violet absorption method, it was not found possible to differentiate between different vinegars using any of the techniques.

Part I of this paper describes two methods for the rapid differentiation of vinegars and non-brewed condiments, one based on osmotic pressure/freezing point depression measurements, and the other on ultra-violet (u.v.) absorption[1]. The former method was able to differentiate between brewed and non-brewed samples, but the u.v. method produced ambiguous results. It was found that when a graph of osmotic pressure against freezing point depression was plotted for this original data, linearity was very poor, but with different groups of samples apparently lying above or below the regression line (Figure 1).

It was considered possible that the position of the sample in relation to the regression line, in conjunction with the osmotic pressure data could be used to differentiate between different types of vinegar as well as between brewed and non-brewed samples. Using improved methodology, determinations of osmotic pressure and freezing point depression on a wide range of samples (brewed and non-brewed) were carried out to test this hypothesis. Simultaneous measurements of u.v. absorption were also made

Materials

Forty-two commercial samples labelled as vinegar and nine samples of non-brewed condiment were used in all determinations. All commercial samples of vinegar were purchased from retailers in the Weybridge area, five of the non-brewed samples were prepared in the laboratory and four commercial non-brewed samples were supplied by British Vinegars Ltd, London.

Vinegars were sub-divided as follows: malt, which included distilled samples; wine, which included red and white samples; cider; spirit.

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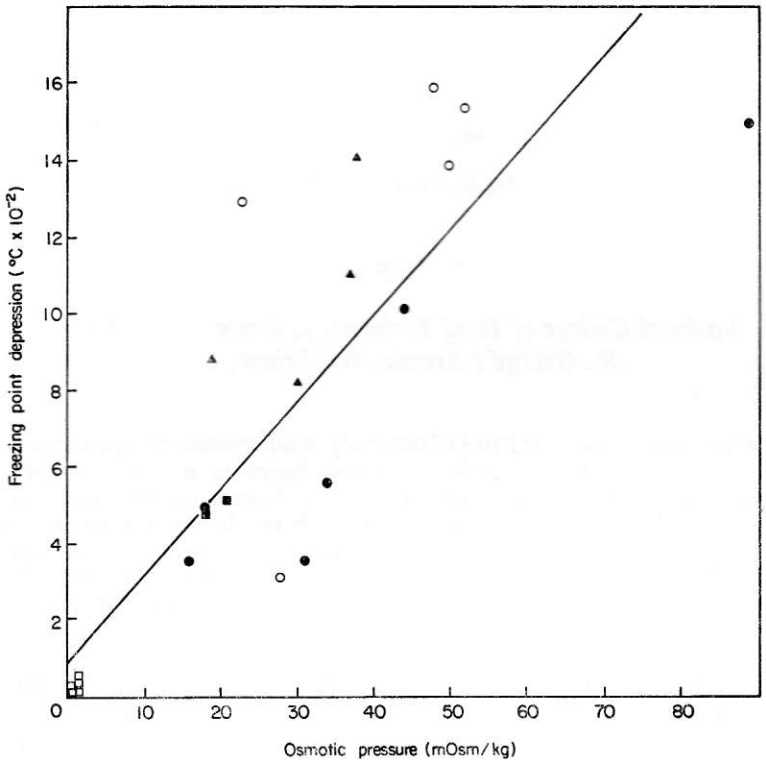

Fig. 1. Osmotic pressure v. freezing point depression for vinegars and non-brewed condiments. Values reported by Kearsley and Gibson (1981)[1].
(○) Cider vinegar; (●) wine vinegar; (■) spirit vinegar; (□) non-brewed sample; (▲) malt vinegar.

Experimental

Sample preparation was identical for all determinations, and a neutralised, distilled sample was used.

A 35-ml volume of sample was measured into a 150-ml round-bottomed flask and adjusted to pH 8·2 using molar sodium hydroxide with the aid of a pH meter. The flask was connected to a simple distillation apparatus, the flask was heated using a bunsen burner, and 35 ml of distillate collected. Osmotic pressure, freezing point depression and absorbance (at 200 nm) of the samples were determined using the equipment described in Part I.

Results and Discussion

U.V. ABSORPTION

The absorption ranges for the samples at 200 nm were:

Malt vinegars	1·900	$\longrightarrow \infty$
Cider vinegars	1·950	$\longrightarrow \infty$
Wine vinegars	1·420	$\longrightarrow \infty$
Spirit vinegars	0·296	$\longrightarrow \infty$
Non-brewed condiments	0	$\longrightarrow \infty$

In the original method the un-neutralised sample was distilled and absorption of the distillate determined at 275 nm. It was not possible to measure absorption at 200 nm owing to the presence of acetic acid in the distillate. When neutralised distilled samples were used, absorption at 200 nm readily distinguished malt, cider and wine vinegars but not spirit vinegars from non-brewed condiments. Spirit vinegars presumably contain a lower concentration of minor components (which absorb strongly at 200 nm) than the other brewed samples, and the range overlaps with that for non-brewed condiments.

OSMOTIC PRESSURE AND FREEZING POINT DEPRESSION

The results are given in Table I with a summary in Table II.

Several observations may be made from these results. The data for the non-brewed condiments appear to be completely different from the data for the brewed samples since the mean is so much smaller and the extreme spread so

TABLE I

RESULTS OF OSMOTIC PRESSURE AND FREEZING POINT DEPRESSION MEASUREMENTS ON VINEGARS AND NON-BREWED CONDIMENTS

Malt vinegar		Wine vinegar		Cider vinegar		Spirit vinegar		Non-brewed condiment	
OP	FPD	OP	FPD	OP	FPD	OP	FPD	OP	FPD
27	0·075	61	0·140	50	0·100	51	0·112	0	0·004
19	0·060	49	0·120	33	0·080	25	0·059	0	0
40	0·120	68	0·155	27	0·070	15	0·065	0	0·003
32	0·088	42	0·128	41	0·102	16	0·065	0	0·001
18	0·049	36	0·090	26	0·054	20	0·063	0	0
21	0·060	28	0·065	23	0·052			1	0·005
23	0·050	12	0·032	23	0·060			1	0
24	0·061	73	0·175	42	0·105			2	0·003
50	0·100	25	0·071	56	0·112			3	0·007
25	0·065	17	0·030	15	0·022				
28	0·060	13	0·020	25	0·070				
22	0·048	29	0·072						
15	0·053								
41	0·095								

OP = Osmotic pressure (mOsmol/kg).
FPD = Freezing point depression (°C).

TABLE II

SUMMARY OF RESULTS FOR OSMOTIC PRESSURE AND FREEZING POINT DEPRESSION DATA

Sample	Range for OP	Range for FPD
Malt vinegar	15–50	0·048–0·120
Wine vinegar	12–73	0·020–0·175
Cider vinegar	15–56	0·022–0·112
Spirit vinegar	15–51	0·059–0·112
Non-brewed condiment	0–3	0–0·007

OP = Osmotic pressure (mOsmol/kg).
FPD = Freezing point depression (°C).

much less. Analyses of variance were carried out both including and excluding the results for non-brewed condiments. Including these data, a highly significant difference was found ($P<0{\cdot}001$), but excluding them, there are no significant differences between any of the true vinegars as regards osmotic pressure or freezing point depression.

The best fit regression line (x upon y) was calculated for the data as a whole and this is shown in Figure 2. Regression lines were also calculated for each type of vinegar separately, and these are shown in Figure 3. There is no apparent relationship between the sample's position in relation to the regression line and the type of sample, as was first proposed. Spirit vinegar samples were shown to be different from the malt and wine vinegars but it is debatable whether this is an

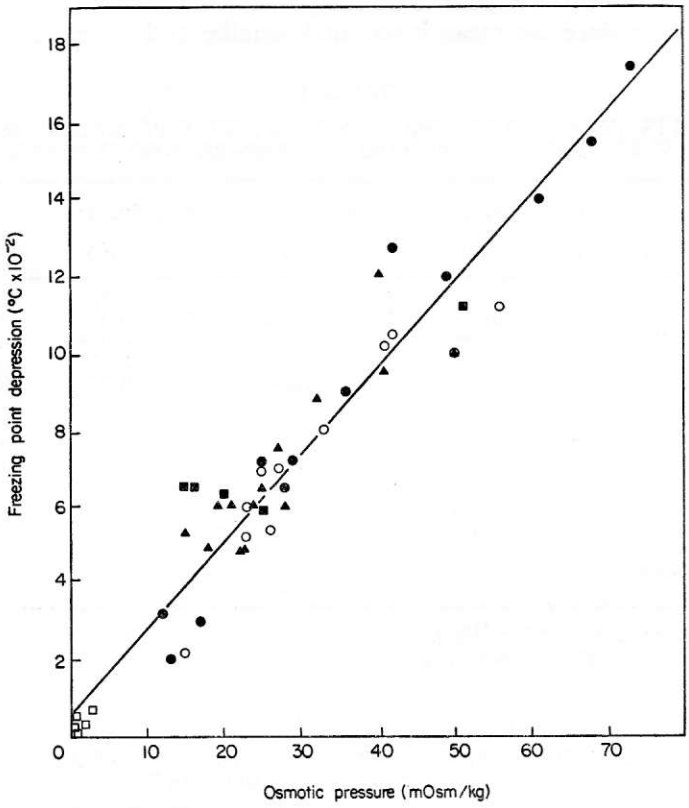

Fig. 2. Osmotic pressure v. freezing point depression for vinegars and non-brewed condiments. Present study.
(○) Cider vinegar; (●) wine vinegar; (■) spirit vinegar; (□) non-brewed sample; (▲) malt vinegar.

authentic result since so few spirit samples were analysed. Comparing Figures 1 and 2 it can be seen that there is a much better agreement between osmotic pressure and freezing point depression in the latter case. This can be attributed to improved methodology and the facts that all determinations in this present paper were carried out on the same sample and a larger pre-distillation sample

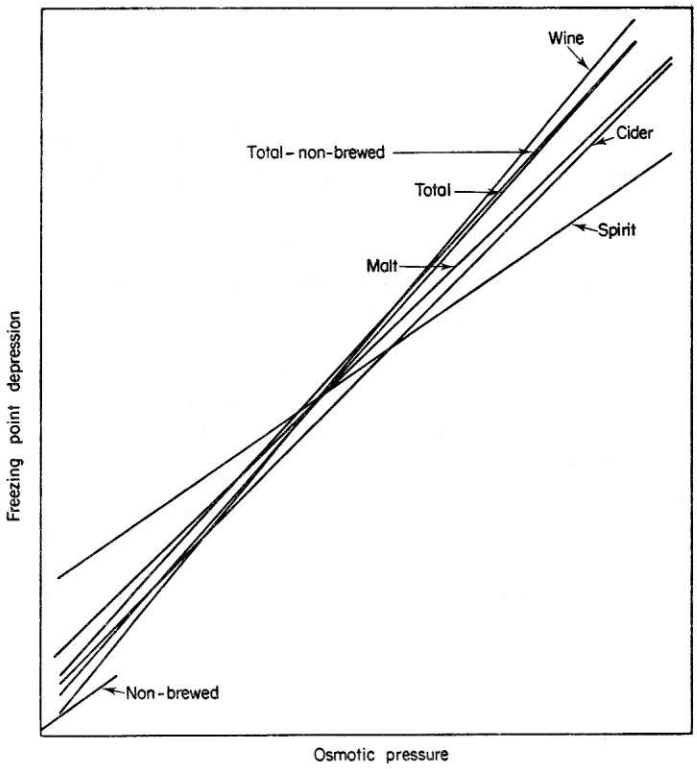

Fig. 3. Regression lines for different types of vinegar and non-brewed condiments.

used for all determinations, reducing any likelihood of breakdown during heating which may have occurred in the previous series of results.

Conclusions

Both osmotic pressure and freezing point depression measurements on neutralised, distilled samples of vinegars and non-brewed condiments will readily distinguish any of the former from the latter. Ultra-violet absorption measurements on the same samples can be used to differentiate malt, cider and wine vinegars from non-brewed condiments. Neither method will distinguish one type of vinegar from another. Spirit vinegars produced anomalous results in some instances and more analyses of this type of product would be useful.

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Determination of Rapeseed Oil in Mustard Oil from the Critical Solution Temperature

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Both mustard oil and rapeseed oil mixed with an equal volume of ethylene glycol monomethyl ether, exhibit a characteristic critical solution temperature. Mustard oil adulterated with rapeseed oil shows a decrease in the critical solution temperature. This decrease in critical solution temperature is linearly related to the amount of the adulterant. This provides a very simple and rapid method for the determination of rapeseed oil in mustard oil and requires only 3 ml of the oil.

When two partially miscible liquids are mixed together, two layers are formed. If the temperature of the system is increased, the two layers approach each other in composition and finally at a particular temperature, the system becomes homogeneous. This temperature is called the "critical solution temperature" (C.S.T.). Since the C.S.T. is a characteristic of each specific system, it has been successfully used for the identification of vegetable oils[1]. In this present investigation an attempt has been made to determine rapeseed oil in mustard oil with the help of this phenomenon. Since mustard oil (Brassica nigra and B. juncia or Sinapsis alba) and rapeseed oil (B. campestris, subspecies napas, etc.) are rather similar in nature[2,3], it is difficult to detect the presence of rapeseed oil in mustard oil by routine tests, though thin layer chromatography can be applied successfully[4]. Once the adulterant has been identified as rapeseed oil, the suggested method of determination by C.S.T. is very simple, rapid and reproducible.

Experimental

APPARATUS

1. *Hot plate* with thermostatic control and magnetic stirrer.
2. *Conical flask*, 10 ml.
3. *Pipette*, 3 ml, calibrated.
4. *Thermometer* (range—10 to 110°C) with graduations of 0·1°C.

REAGENT

1. *Ethylene glycol monomethyl ether*, A.R.

Method

Pipette 3 ml, accurately measured, of the oil into the dry conical flask. Add 3 ml of ethylene glycol monomethyl ether. Place a magnetic stirrer bar into the

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TABLE I

CRITICAL SOLUTION TEMPERATURE OF PURE AND KNOWN MIXTURES OF OILS

Samples	C.S.T. °C	Average C.S.T. °C
Pure mustard oil, 10 samples	104–105·7	—
Particular pure mustard oil	104·4	104·2
	104·1	
	104·1	
10 per cent. Rapeseed oil in mustard oil (v/v)	102·1	102·1
	102·3	
	102·0	
20 per cent. Rapeseed oil in mustard oil (v/v)	100·4	100·2
	100·1	
	100·2	
30 per cent. Rapeseed oil in mustard oil (v/v)	98·0	97·8
	97·9	
	97·7	
40 per cent. Rapeseed oil in mustard oil (v/v)	95·9	95·9
	95·9	
	96·0	
50 per cent. Rapeseed oil in mustard oil (v/v)	93·9	93·8
	94·0	
	93·7	
60 per cent. Rapeseed oil in mustard oil (v/v)	92·0	91·9
	91·9	
	91·9	
80 per cent. Rapeseed oil in mustard oil (v/v)	87·8	87·7
	87·7	
	87·7	
Particular pure Rapeseed oil	83·5	83·5
	83·5	
	83·6	
Pure Rapeseed oil, 10 samples	83·2–84·7	—

conical flask. Introduce the thermometer through a cork so that the bulb of the thermometer is completely immersed in the liquid and the stirrer can rotate freely. Cork the flask tightly. Now place the conical flask on the hot plate and fix by a clamp. Increase the temperature slowly, keeping the mixture continuously stirred. Note the temperature at which the two layers become homogeneous. This temperature (C.S.T.) measured with mustard oil is 104·0 to 105·7°C and with rapeseed oil it is 83·2 to 84·7°C. The experiment is repeated each time with a fresh mixture of oil and the reagent and also for various control samples containing different known percentages of rapeseed oil in mustard oil. A calibration graph is prepared by plotting the C.S.T. against the known percentage of rapeseed oil in mustard oil.

Results and Discussion

C.S.T.s were determined for mixtures of 10, 20, 30, 40, 50, 60 and 80 per cent. rapeseed oil in mustard oil and also for the pure oils. Each experiment was repeated three times using fresh mixtures of oil and reagent. The results are shown in Table I.

If the C.S.T. is plotted against the percentage of rapeseed oil in mustard oil a linear graph is obtained as shown in Figure 1. Using this graph, an estimate of the percentage of rapeseed oil in a sample of mustard oil can be made with good sensitivity.

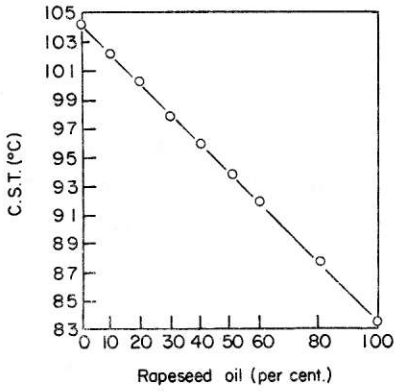

Fig. 1. Variation of critical solution temperature with different mixtures of mustard oil and rapeseed oil.

The C.S.T. is dependent on the exact composition of the system. Therefore, the measurement of oil and reagent should always be accurate. The C.S.T. for a particular system remains constant as long as the pressure remains constant, provided other factors remain the same. Changes of pressure, however, will normally be negligibly small under ordinary conditions.

The author thanks Dr K. Goswami, M.Sc., Ph.D., Director, Forensic Science Laboratory, Assam for his interest and valuable suggestions in this work.

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Thin Layer Chromatographic Detection of Some Common Preservatives Encountered in Foods Sold in India

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A method has been devised for the qualitative detection of benzoic, *p*-hydroxy benzoic, salicylic and sorbic acid used as preservatives in food. The qualitative procedure is based on a TLC separation, using Silica Gel G as the adsorbent and cyclohexane : acetic acid (9 : 1) as the mobile solvent. The separated preservatives are identified by spraying first with 3 per cent. hydrogen peroxide and then with 2 per cent. ferric chloride solution after heating the plate at 95°C for 5 min.

Benzoic, *p*-hydroxybenzoic, salicylic and sorbic acids and their salts have been used as preservatives for a very long time. When added to food, they are capable of retarding, inhibiting or arresting the process of fermentation, acidification or other decomposition of food[1]. Growth of yeasts, moulds and sometimes bacteria are inhibited in food containing suitable levels of such preservatives.

In India, because of its hot, humid climate, fruit products, juices, squashes, pickles, jams, etc., must contain preservative, to prevent food spoilage due to microbial action which would occur very soon after manufacture. The Prevention of Food Adulteration Act, Government of India[2], has classified "preservatives" into two classes. Benzoates, sulphites and nitrites, etc., fall into Class II and glucose, fructose, sugar (sucrose), salt and spices fall into Class I.

There is no restriction in regard to use of class I preservatives which may be used for enhancing the aroma, colour, and palatability of foodstuffs in addition to their natural preservative action.

As sulphites and nitrites are very unstable, particularly in food articles with high acidity, so the addition of benzoates, salicylates and sorbic acid salts in fruit products are preferred for prolonged preservative action. However, Class II preservatives used solely for increasing the storage life of food have some limitations. Potential toxic effects of the benzoates and salicylates cannot be completely ruled out. In India, salicylates are not permitted to be used as a preservative. The others too, although permitted should be present in food below a maximum specified level[2]. In view of the widespread use of Class II benzene derivative preservatives either permitted or non-permitted, a suitable routine method for their detection was devised.

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Experimental

APPARATUS

1. Standard TLC set.
2. Spraying equipment.
3. Glass plate: 20 cm × 10 cm × 0·3 cm.
4. Capillary tubes.
5. Separating funnels, 250 and 500 ml capacity.
6. Volumetric flasks, 10 ml capacity.
7. Oven.
8. Macerater.

REAGENTS

1. *Silica gel G.* Analar.
2. *Cyclohexane.* Analar.
3. *Glacial acetic acid.* Analar.
4. *Hydrogen peroxide.* 3 *per cent. w/w, aqueous.*
5. *Ferric Chloride solution.* 2 *per cent. w/w, aqueous.*
6. *Ammonia solution.* 10 *per cent. w/w.*
7. *Sulphuric acid.* 10 *per cent. w/w.*
8. *Ether, solvent.*
9. *Standard preservatives: benzoic, p-hydroxy benzoic, salicylic and sorbic acids.*

SAMPLES EXAMINED

a. Mango pickle in sealed bottle (retail sample).
b. Jaffa orange powder in sealed packet (retail sample).
c. Jaffa orange liquid concentrated in sealed bottle (retail sample).
d. Mixed pickle in loose packet (retail sample).
e. Control sample of orange juice (prepared in Laboratory).

Method

PREPARATION OF SAMPLE: EXTRACTION AND CLEAN-UP

Take 25 g of sample, macerate finely with 200 ml of distilled water and proceed as follows:

Add 10 per cent. sulphuric acid such that the whole suspension is acid to litmus. Transfer to a 500-ml separating funnel, extract with 25 ml of ether twice and combine the ether extracts in a second separating funnel. Add 100 ml of distilled water and 10 per cent. ammonia with thorough shaking such that the aqueous layer is alkaline to litmus. Transfer the alkaline aqueous layer to another clean separator, acidify the contents with 10 per cent. sulphuric acid and extract thrice using 25-ml portions of ether each time.

Combine the ether extracts. Then allow the extracts to concentrate to approximately 2 ml on a warm water bath, which is then ready for chromatography.

THIN LAYER CHROMATOGRAPHY

Spot 20 μl of the concentrated extract on to a Silica gel G plate (layer thickness 0·3 mm) previously activated at 120°C for 5 min and cooled to room temperature in a desiccator. Develop with solvent, cyclohexane : acetic acid (9 : 1), by allowing the solvent front to run approximately 15 cm from the base line. Dry the plate and spray intensively with 3 per cent. hydrogen peroxide. Heat at 95°C for 5 min in an oven. Over-spray with 2 per cent. ferric chloride[3], solution and observe the spots developed on the plate and measure their Rf value (Table I).

PREPARATION OF STANDARDS

Prepare 50 mg/litre standards each of benzoic, *p*-hydroxybenzoic, salicylic and sorbic acid in ether. Prepare the control sample in the following way:

Press and squeeze fresh oranges and take the juice after filtration. Consider this as the negative control sample. Prepare a positive control sample by adding 1 ml of ether solution of each preservative. Proceed with positive and negative control samples as described above in "Thin Layer Chromatography" beginning with "macerate" and ending with "Rf values". The coloured spots observed and Rf values calculated in the case of the positive control sample as well as simple preservative solutions are recorded in Table I.

TABLE I

Rf VALUES OF PRESERVATIVES WHEN PRESENT IN PURE STATE, CONTROL POSITIVE SAMPLES AND IN RETAIL SAMPLES

Types of sample	Colour of spot	Rf value
Benzoic acid	PP	0·72
p-Hydroxy benzoic acid	Y	0·04
Salicylic acid	DP	0·49
Sorbic acid	Y	0·67
Pure mixture of four	Y, DP, Y, PP	0·04, 0·50, 0·66, 0·73
Sample a	PP	0·71
" b	PP	0·72
" c	—	—
" d	Y	0·68
" e negative control	—	—
" e positive control	Y, DP, Y, PP	0·05, 0·51, 0·66, 0·72

PP = Pale purple; Y = Yellow; DP = Deep purple.

Results and Discussions

It was observed that the Rf value of the spots were very similar in case of pure mixture and control positive sample. Furthermore the resolution was clear. However, to obtain this, the experimental conditions specified under "Method" should be strictly adhered to.

The author thanks P. K. Das, M.Sc(London), D.I.C., Public Analyst, Government of Assam, India, for his interest and advice in this work.

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Book Review

CHEMICAL METHODS OF ROCK ANALYSIS. 3rd Edition. By P. G. Jeffrey and D. Hutchinson. Pergamon, Oxford, 1981. 379 pp. Price £25. ISBN 0-80-023806-8.

It is quite obvious that this book has been written by practising analysts with similar readers in mind who wish to make reference to an up-to-date handbook on the analysis of elements of silicate rock and like materials.

The methods, arranged alphabetically in relation to the elements, are well set out in standard form and are frequently described, with alternative procedures. The techniques used include spectrophotometry, flame emission spectrometry, atomic absorption spectroscopy, gravimetry, volumetric analysis and specific ion electrodes. The procedures themselves are sufficiently detailed to be of immediate use, often without need to delve into the numerous references.

It is a pity that such a professional work should have to be criticised on the quality of the script, which appeared to have been reproduced by a photocopying process from typewriting; this is not altogether attractive in a textbook. The diagrams were neat and clear but there was a small number of trivial mistakes which should have been picked up at the proof-reading stage.

On balance, it is a book, well worth having in the laboratory and the text is good value at £25.

D. J. TAYLOR

J. Assoc. Publ. Analysts, 1981, **19**, 137–138

Letters to the Editor

The Chlorination of Swimming Pools and the Presence of Apparent Nitrogen Trichloride

Sir,

I have studied the contents of this paper which appeared in *J. Assoc. Publ. Analysts*, 1980, **18**, 125–128.

I must advise you that the DPD method, especially where NCl_3 is concerned, is not being applied in accordance with current recommended procedures. I can appreciate that in the circumstances the author might well ask in connection with KI "What is a small crystal?" and later add "The results show that without a prescribed weight of potassium iodide the test is of limited value".

On a general point I think it should first be made clear that the validity of the DPD method for the various chlorine species, including NCl_3, has been established by independent workers throughout the world and it is adopted in many countries as the standard procedure for these determinations.

Coming to the particular point about KI the amount to be used is in fact specified in such standard methods and I would, for example, refer the author to "Chemical Disinfecting Agents in Water and Effluents, and Chlorine Demand", HMSO, London, 1980. If difficulty arises in judging what is a "very small crystal" of the weight specified, i.e. about 0·5 mg then the alternative procedure given therein as note b against Ca.7·2 should be followed.

If we now look at Table IV of the author's report it can be demonstrated from the figures there presented that if extrapolated using a log scale the "NCl_3

TABLE I

EFFECT OF NCl_3 ERRORS ON DPD CALCULATIONS

(NH_2Cl assumed absent to simplify the comparative calculations)

	Assumed correct	Readings With partial breakthrough into the NCl_3 reading*		
		20 per cent.	*40 per cent.*	*80 per cent.*
A =	1·0	1·0	1·0	1·0
N =	1·1	3·1	5·1	9·1
C =	10·0	10·0	10·0	10·0
		Calculations		
Cl_2	1·0	1·0	1·0	1·0
NCl_3	0·2	4·2	8·2	16·2
$NCHl_2$	8·9	6·9	4·9	0·9

*Due to increased quantities of KI being added beyond the specified amount.

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indication as Cl_2" would be 0·10 to 0·15 mg/l for the correct amount of added KI (i.e. 0·5 mg/100 ml sample).

It seems quite evident that the errors reported arise from the use of too much KI at this stage of the method. The calculations I have given in Table I indicate the effect of such errors in determining NCl_3.

Perhaps the definition of a "small crystal of KI" should have been given more precisely by the responsible committee in "The Purification of Water for Swimming Pools", 1975, HMSO London, the booklet to which the author refers.

So far as chlorine–amino acids and chlorine–urea reactions are concerned the response of the chloro-derivatives in the DPD method has been well established by many workers including myself in 1949 (University of London, Thesis). In the case of creatinine, to which the author makes special reference, this is known to produce a fairly stable chlorcreatinine compound of a "dichloramine" type which so responds in the DPD method.

When the analytical procedures are investigated I am sure the results will lead to very different conclusions with regard to both nitrogen trichloride and chlorcreatinine.

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J. Assoc. Publ. Analysts, 1981, **19**, 139–140

The Composition of Foods

Sir,

In a book review, (*J. Assoc. Publ. Analysts*, 1981, **19,** 31), the reviewer comments on the usefulness of the tables presented in "The Composition of Foods – First Supplement" by McCance and Widdowson with particular reference to the amino acid composition of fish. Two points emerge from this review article.

(1) The values presented in the book supplement under review especially for raw species of fish indicate that considerable variation exists between the amino acid compositions of the different species. Whilst this fact is indeed true the values given in the supplement should be seen in the context of the manner in which they have been derived. The data for each species have originated from a single set of data points namely:
"No 2435 White and fatty fish – all kinds";
"The Composition of Foods" McCance and Widdowson 4th revised edition.
The data which appear in the supplement to this volume have been calculated for each species by multiplying this "utility" amino acid set of data for all the species by a factor representing the total nitrogen value for each individual species.

Although it is an acceptable fact that the *true* protein contents of all species are similar[1], due account should be taken of factors which contribute to variations in the total amino acid composition, e.g., seasonal variation, species variation and non-protein nitrogen[2] and contribute to the nitrogen content, e.g., biological variation, non-protein nitrogen and iced storage time[3].

As with all biological material great care should be exercised in espousing theories based on what are after all minor differences in dynamic biochemical systems and absolute values expressed in the supplement should only be viewed as a general guide.

(2) The second point arising from this review is the statement by the author that "other fish, indeed many other comestibles, have similar levels of histidine to mackerel which have been associated with most of the outbreaks of poisoning in the U.K., yet it is mainly with scombroid fish that outbreaks are associated and not dried eggs, grilled pork or lamb cutlets or parmesan cheese".

It is obvious from this statement that the author is not aware that the data to which he is referring gives the total histidine content of the fish in question (i.e., mainly bound as part of the protein molecule) whereas the decarboxylation

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process in the formation of histamine relates to the free or unbound histidine only[4]. The values for the free histidine content in a few species of fish are shown Table I[5].

TABLE I

HISTIDINE CONTENTS OF FISH

	Cod *Gadus morhua*	Haddock *Melanogrammus aeglefinus*	Whiting *Merlangius merlangus*	Herring *Clupea harengus*	Mackerel *Scomber scombrus*	Tuna *Thunnus thynnus*
mg of Histidine per 100 g of fish	3–7	6–10	5–9	60–160	300–600	500–1000

The easy availability of this free histidine makes its conversion to histamine by spoilage bacteria relatively simple, especially if elevated temperatures are used for the storage of these fish products. From these histidine values it can be observed that the pelagic species are able to generate over 100 mg of histamine per 100 g of flesh from the free histidine pool without any contribution from the proteinaceous histidine derived by proteolysis of the bound amino acid[6].

Furthermore wine and cheeses, indeed most foods involving a curing process or period of maturation, voluntary or otherwise, are all products which have been implicated in the production of biologically active amines but the levels of histamine in these foodstuffs do not generally rise to such high concentrations as those for scombroids[7-9].

A. H. RITCHIE

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Editor's Note

The above letter is much appreciated, as is the clarification, but what a pity the free histidine content of the various substances had not been quoted in the book supplement, thus increasing the value of such an esteemed and useful set of tables.