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Ministry of Agriculture, Fisheries and Food
Food Labelling and Standards Division

An Evaluation of Analytical Methodology for Characterising Olive Oils and Olive-pomace Oils

Prepared for publication by

Peter N. Gillatt[a], Dr.Roger Wood[b] and Dr.Gregory C. Hodson[c][1]

The primary aim of this study was to evaluate the methodology described in a proposed EC Regulation later adopted as 2568/91 on the characteristics of olive oils and olive-pomace oils.

An informal survey of thirty-five olive oils on retail sale was conducted as a means of establishing the applicability of the proposed methods in the analysis of olive oils. The samples were labelled either "extra virgin olive oil" (twenty-four samples) or "pure olive oil" (eleven samples). Analysis was carried out according to methods published in "MAFF Information Bulletin for Public Analysts on EC Methods of Analysis and Sampling for Foodstuffs. No. 110. Methods of Analysis for Olive Oil", (which anticipated those in EC Regulation 2568/91 [1]) with the objective of assessing and commenting on their suitability for judgement of olive oil authenticity. The samples were analysed for peroxide value and free fatty acids to demonstrate the quality of the samples at the time of purchase. The oils were also analysed for overall fatty acid composition, fatty acids at the triglyceride 2-position, sterols, aliphatic alcohols and specific extinction at 232 run and 270 nm. For those oils described as "extra virgin" one sample (4%) was found not to comply with analytical criteria subsequently specified in the EC Regulation; the remainder would have been judged to be satisfactory. Five (45%) of those sold as "pure olive oils" were found not to satisfy the proposed EC Regulation's requirements for authentic oils. However, caution should be exercised in concluding that these products may have been adulterated, for the following reasons:-

***(a)** The analytical methods used in this study were not always identical to those in the Regulation 2568/91, nor is it clear that these have been successfully collaboratively tested.*

1 Author to whom communications relating to this report should be addressed.

(a) P. N. Gillatt, Ministry of Agriculture, Fisheries and Food

(b) Dr. R.Wood, Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, Food Science Laboratory, Norwich Research Park, Colney, Norwich, UK, NR4 7UQ

(c) Dr. G.C.Hodson, Ministry of Agriculture, Fisheries and Food, Food Labelling and Standards Division, Room 316, Ergon House, 17, Smith Square, London, SW1P 3JR

(b) *No guidance has been provided within the Regulation on how the compositional limits specified should be applied. For example, should they take account of the inter and intra laboratory variation that might be expected? Have tolerances been incorporated within the minimum and maximum limits laid down for certain criteria?*

(c) *The work reported here indicates that if an oil fails to meet the prescribed limits, it might be a result of one or more of the following:-*

the oil being impure (or not as labelled),

a fault in the analytical methodology,

inappropriate purity criteria in the Regulation.

(d) *Finally, it should be noted that the oil samples were purchased prior to implementation of the Regulation* [1].

S.1 INTRODUCTION

1. Olive oil is categorised according to its quality, which is dependent to a large extent on organoleptic properties. The initial olive pressing yields the much prized virgin oils (e.g. extra virgin). These must be obtained only by mechanical or other physical means, such that the oil is not harmed. In the preparation of virgin oils, the use of solvents (e.g. hexane), re-esterification or mixing with other oils is forbidden. Other grades of olive oil such as refined, blends of refined and virgin, and olive-pomace (residue) oils are also traded. Greater detail is provided in Annex 2. The following figures indicate the difference in the cost of olive oils and other vegetable oils [2].

Imports of Selected Refined Vegetable Oils in 1990 [2]

Vegetable oil	Volume ('000 tons)	Value (£m)	Cost (£/ton)
Palm oil	82	17.7	220
Soyabean oil	5.2	1.6	310
Rapeseed oil	6.1	2.2	360
Maize (Corn) oil	6.7	3.5	525
Sunflowerseed oil	33.6	20.1	600
Groundnut oil	3.0	2.0	670
Virgin Olive oil	3.0	6.4	2,200

2. Data published in 1985 [3] have indicated that a high proportion of olive oils sold in the USA was not of the grade stated on the label. Discussions took place in the EC on the methods for analysis of olive oils and olive-pomace oils, a number of which required collaboratively testing. To aid this process, the methodology was published by MAFF in 1990 [4] and used in this study.

3. The EC proposed a Regulation on the analytical characteristics of olive oils and olive-pomace (residue) oils. This led to considerable discussion between Member States. As part of the UK's contribution to this discussion, MAFF commissioned studies at the Leatherhead Food RA (BFMIRA); the results of these are reported in the following text. The objectives were primarily to evaluate these methods [4] and to establish whether the purity criteria quoted in the draft Regulation were appropriate for these commodities. Of secondary importance, the values obtained from the analyses were used in an attempt to assess whether the oils were correctly described on the label at the point of sale by comparison of the results with the criteria laid down in EC Regulation 2568/91 (then in draft form). During the latter stages of this work, the European Commission published Regulation 2568/91[1] in the Official Journal of 5 September 1991. A number of amendments to this Regulation have since been published, some of which are relevant to this study [5,6,7,8,9].

4. The study had the following detailed objectives:-

(i) To ascertain whether the methods of analysis being proposed for establishing olive oil purity and grading were satisfactory.

(ii) To analyse olive oils by the seven methods in proposed legislation.

(iii) To determine the overall fatty acid composition (FAC) of the oils.

(iv) To compare the results with the purity criteria specified in the Regulation.

5. The approach taken was to purchase thirty-five samples of olive oil from retail outlets in south-east England during the last two weeks of November 1990. Of these, eleven were labelled as "olive oil" and twenty-four as "extra virgin olive oil".

Samples were analysed in coded form.

A number of analytical difficulties were encountered during the analyses, which made it necessary to enlist the assistance of the MAFF Food Science Laboratory at Norwich. As a consequence, the analytical studies took considerably longer than originally envisaged which has resulted in the delay in publication of this reporl.

6. The following analytical methods, described in MAFF Information Bulletin No. 110 [4], were employed in this study:-

Method 1 - Determination of the fatty acids in the 2-position in the triglycerides.

Method 2 - Determination of sterols by capillary GC.

Method 3 - Determination of aliphatic alcohols by capillary GC.

Method 4 - Determination of refined oils in virgin oils by spectrophotometric analysis.

Method 5 - Determination of the acid value and the acidity.

Method 6 - Determination of the peroxide value.

In addition, fatty acid compositions were determined by ISO 5508/9 [10,11] using capillary GC.

A determination of wax-content was not undertaken during this study because, although it was present in the MAFF Information Bulletin, it was omitted from the adopted text of EC Regulations. A method for wax, which replaces the method for aliphatic alcohols, has since been introduced and new limits for the wax content published [9].

7. The findings of this research are considered in two sections. Firstly, the analytical techniques employed are briefly described, much greater detail being given in Annex 1. The second section contains the analytical results and conclusions. This section contains a comparison of the analytical data with the criteria subsequently adopted in the Regulation.

For completeness, the raw data, on which the conclusions are based, are presented in Annex 5.

8. The results of analysis indicate that some oils might not comply with all the analytical criteria specified in Regulation 2568/91. It might, therefore, be suggested that these oils are impure. However, care should be taken before making this assumption for the following reasons:-

(a) The analytical methods used in this study were not always identical to those in the Regulation 2568/91, nor is it clear that they have been successfully collaboratively tested.

(b) No guidance is provided within the Regulation on how the compositional limits it specifies should be applied. For example, should they take account of the *inter and intra* laboratory variation that might be expected? Have tolerances been incorporated within the minimum and maximum limits laid down for certain criteria?

(c) The work reported here indicates that if an oil fails to meet the prescribed limits, it might be a result of one or more of the following:-

- the oil being impure (or not as labelled),
- a fault in the analytical methodology,
- inappropriate purity criteria within the Regulation.

(d) Finally, it should be noted that the oil samples were purchased prior to implementation of the Regulation [1].

S.2 DESCRIPTION OF ANALYTICAL METHODOLOGY

The methodology is essentially as published in the EC Regulation [1]; where significant differences exist these are shown in Annex 3.

Analysis of the Fatty Acids in the 2-position of the Glycerol (2-position analysis)

9. This involves the partial enzymic hydrolysis of glycerides to produce 2-monoglycerides. These are separated by thin layer chromatography (TLC), derivatised and their fatty acid composition (FAC) determined by GLC. The results of this measurement may indicate the presence of re-esterified oils.

Determination of Sterols by Capillary GLC

10. The oil is saponifed and the unsaponifable matter (USM) extracted. The sterols are separated from the other unsaponifiable components by TLC, derivatised and the individual components quantified by GLC against an internal standard (IS). The results of this measurement can indicate whether an olive oil has been adulterated by vegetable oil (e.g. high-oleic sunflowerseed oil).

Determination of Aliphatic Alcohols by Capillary GLC

11. The oil is saponified and the USM extracted. The aliphatic alcohols are separated by TLC, derivatised and the individual components determined by GLC against an IS. The presence of elevated concentrations of these compounds in olive oils is indicative of their adulteration by olive-pomace oils.

Spectrophotometric Analysis of Oils to Determine Specific Extinctions (SE)

12. A solution of the oil is prepared and the specific extinction (SE) determined in the usual way. Measurements are made at 270nm and 232nm. Unless shown to contain significant quantities of oxidation products, an oil with a high SE at 270 nm is likely to contain refined oil and would not be considered as a pure virgin oil. Oils that have SEs at 232nm greater than 2.5 would similarly not be considered as pure virgin oils.

Determination of the Free Fatty Acid (FFA) and Acidity Value

13. The free fatty acid (FFA) content of an oil is a guide to its quality. In general, the greater the FFA the lower the quality of the oil. However, virgin olive oils cannot be classified in the same manner as fully processed oils because their characteristic taste is attributable, in part, to their FFA content. An oil's FFA is measured by titration.

Determination of the Peroxide Value (PV)

14. The peroxide value (PV) is a measure of the amount of lipid hydroperoxide present in the sample as a result of oil oxidation. The PV is therefore a good indicator of oil quality. However, it should be remembered that pure olive oils (which by definition are mixtures of virgin and refined oils) would be expected to have lower PVs than virgin oils. The PV is determined by titration. Since virgin olive oils do not experience the extensive processing that most vegetable oils undergo, their PV will tend to be significantly greater than would be acceptable for a vegetable oil.

Determination of the Overall Fatty Acid Composition (FAC)

15. The oil is saponified and derivatised. The concentrations of the resulting methyl esters are determined by capillary column GLC. This is generally a very useful method of identifying impure oils.

S.3 INTERPRETATION OF RESULTS

(i) Interpretation of the Results of the Analysis of the Fatty Acids at 2-Position of the Triglyceride

16. Data illustrating the repeatable nature of this technique are presented in Table 1. These data indicate that the methodology is precise and will provide accurate results.

17. The data in Table 2 indicate that there are very large differences in the 2-position

profiles of genuine and re-esterified olive oils for palmitic and stearic acids. These differences have been exploited in the development of standards to prevent fraud. For example, the current EC Regulation [1] states that for Extra Virgin and Pure Olive Oils, the maximum permissible sum of these concentrations are 1.3 and 1.5% of the total fatty acids at the 2-position, respectively.

18. There is only one sample in this present study that fails to meet this criterion (Annex 4). The sum of the concentrations of palmitic and stearic acids at the 2-position of D-1 is 2.7%. This sample is described as an extra virgin olive oil and the maximum permitted concentration of stearic and palmitic acids at the 2-position is therefore 1.3%. The value obtained clearly exceeds this limit. The suspicion that this sample is not as labelled is increased by the specific extinction data **(see part 34)**.

TABLE 1

Statistical Data Regarding the Analysis of Fatty Acids at the 2-Position of the Triglyceride

Fatty Acid	Mean (%)	Range (%)	Std. Dev. (%)	CV (%)
C16:0	0.69	0.5-0.8	0.11	15.9
C16:1	0.59	0.5-0.6	0.04	6.40
C18:0	0.11	0.1-0.2	0.04	36.4
C18:1	83.1	82.9-83.4	0.19	0.23
C18:2	14.4	14.0-14.4	0.21	1.45
C18:3	1.01	1.0-1.1	0.04	3.74

TABLE 2
Typical Profiles of Fatty Acids at the Triglyceride 2-Position for Various Grades of Olive Oils

Oil Type	Virgin Olive oil	Virgin Olive oil	Virgin Olive oil	Esterified oil	Pomace oil
Fatty Acid					
C16:0	0.8	1.0-1.2	0.7-1.6	9.0	2.0
C16:1	0.5	0.5	-	1.1	-
C18:0	0.2	0.2-0.5	trace-0.2	2.6	0.6
C18:1	89.1	85.5-86.3	-	75.6	-
C18:2	10.9	1.3-11.7	-	11.6	-
C18:3	-	0.6-0.9	-	-	-

(ii) Determination of Sterols by Capillary GLC

Introduction

19. Analytical detail is presented in Annex 1. However, before evaluating the results of this determination, the following background information should be considered.

20. Early analyses for sterols by gas chromatography involved the use of packed columns and stationary phases such as SE32 and SE52. Using these stationary phases, six sterols, namely cholesterol, brassicasterol, campesterol, stigmasterol, ß-sitosterol and Δ-7-stigmastenol, were resolved. Improved stationary phases such as OV-17 were introduced in the 1980s, and it became possible to resolve Δ-5-avenasterol from ß-sitosterol and Δ-7-avenasterol from Δ-7-stigmastenol. Further improvements based on capillary column GC with bonded OV-17-like stationary phases (e.g. CP SIL 19) eventually enabled the resolution of sixteen components in the desmethyl sterol band namely Cholesterol, Brassicasterol, 24-Methylenecholesterol, Campesterol, Campestenol, Stigmasterol, Δ-7-Campesterol, Δ-5,23-Stigmastadienol, Chlerosterol, ß-Sitosterol, Sitostanol, Δ-5-Avenasterol, Δ-5,24-Stigmastadienol, Δ-7-Stigmastenol, Δ-7-Avenasterol.

The IOOC [37] laid down the following criteria for sterol composition of olive oil:-

ß-sitosterol	> 93% of total sterol band
campesterol	< 4.0% of total sterol band
cholesterol	< 0. 5% of total sterol band

This standard was based on separation achieved on a SE32 stationary phase, and is in need of revision.

21. At an IOOC meeting in 1990 [12] it was agreed that the reference to ß-sitosterol in the above standard should be modified, indicating that ß-sitosterol is understood to mean the sum of the following sterols:

ß-sitosterol	Δ-5-avenasterol	Δ-5, 23-stigmastadienol
Δ-5, 24-stigmastadienol	chlerosterol	sitostanol

The sum of the above individual concentrations is referred to as the *apparent* ß-sitosterol concentration. This has been adopted by the EC. The concentration of individual sterols may be expressed in absolute terms (e.g. mg/kg oil), or as a percentage of the total sterol concentration. The EC Regulation quotes most values for sterols as a percentage of total sterols and consequently, the latter system is used predominantly in this report.

Apparent ß- Sitosterol Concentration

22. The results of sterol analysis undertaken in this study, indicate that there are 66 determinations in which the 'apparent' ß-sitosterol content is below the 93% statutory limit and 50 determinations where it is above the limit. Interpretation of sterol data is one of the most difficult areas of the EC Regulations [1], especially with regard to the apparent ß-sitosterol content. This is exacerbated since the regulation does not provide guidance on tolerance limits for this or other criteria. Considering all the replicate analyses for a particular sample, there are many cases where replicates fall on either side of the 93% limit; for example, for A-6 the apparent ß-sitosterol value ranges from 90.8 to 94.1 %. Where analytical values in excess of 93% apparent ß-sitosterol have been obtained, it is prudent to conclude that the sample is genuine in respect of this analysis, particularly if other analytical parameters laid down in EC Regulation [1] are met. Table 3 shows those samples that failed to meet criteria as laid down in this Regulation. For the purposes of this report, those samples that had apparent ß-sitosterol contents that fell below the 93% limit were considered individually and account was taken of the values obtained for other purity criteria.

23. The determination of sterols in olive oil facilitates the detection of seed oil additions. Some of the likely adulterants have a natural sterol content greater than olive oil, and their presence would increase the total sterol concentration in the resulting blend. It is also likely to reduce the apparent level of ß-sitosterol as a proportion of the total sterols. This will naturally affect the percentage of other sterols present. It has been calculated that the addition of 1% rapeseed oil would reduce the apparent ß-sitosterol value by nearly 3%, increase the campesterol level by a similar amount, and increase the brassicasterol by a small but probably insignificant amount.

24. The sterol results from oils considered to be pure include a proportion whose apparent ß-sitosterol content is below the 93% limit. For some oils a wide spread of results either side of the limit was detected; for example, with B-4, the range is 91.9-95.1% from six satisfactory results. Statistical analysis of results has been carried out for several oils, which are tabulated below.

Sample Code	No. of replicates	Mean (% of total sterols)	Range (%)	SD (%)
A-2	5	94.2	93.1-95.0	0.74
B-3	5	94.1	93.4-94.8	0.73
B-4	6	93.6	91.9-95.1	1.18
B-5	5	92.1	91.5-93.3	0.80

SD = standard deviation

Additional Sterol Criteria

25. The Regulations [1] confirm the IOOC criteria above (**21**), including the revised definition of ß-sitosterol. Additionally, stigmasterol must be present in a lower concentration than the campesterol; maximum concentrations are set for brassicasterol (0.2%) and Δ-7-stigmastenol (0.5%); a minimum absolute concentration of 1,000 mg/kg total sterols is prescribed for olive oils (1,800-2,500 mg/kg for olive-pomace oils). The results of this study indicate that:

i) All samples comply with the requirement that there should be more campesterol than stigmasterol.

ii) All samples comply with the requirement that the total sterol concentration should exceed 1,000 mg/kg.

iii) The requirement for a maximum of 0.2% brassicasterol is achieved by all samples in at least one of the analyses with the exception of C-4. However, in view of the known occasional co-elution of this peak with an artefact, it would be imprudent to conclude that an oil was adulterated or impure on this parameter alone.

iv) Similarly, most samples meet the 0.5% max. requirement for Δ-7-stigmastenol, but again interferences by an artefact are known to occur.

v) The campesterol concentration must be less than 4% (approximately 50-70 mg/kg) of the total sterols. A number of analyses narrowly fail to meet the criterion, but only by 2 or 3 mg/kg. For example, in sample B-4, the absolute value for campesterol is 51 mg/kg. If, however, the true value was 49 mg/kg it would meet the 4% criterion. This is within experimental error. However, a number of samples fail on this criterion, e.g. C-4, C-5, A-5, A-6, A-11, A-15, Blend-l, Blend-2, Blend-3 and Blend-4.

vi) There is also a requirement for a maximum of 0.5% cholesterol. All samples meet this criterion in at least one of the replicate analyses.

26. In the past there has been a lack of suitable desmethylsterols standards which has hampered attempts to obtain satisfactory statistical data regarding this analysis. In an attempt to overcome this difficulty, the Community Bureau of Reference (BCR) produced standard oils. The desmethyl sterol concentration and composition of these standards have been established following analyses by a number of laboratories throughout the EU. Analyses of these BCR standards by BFMIRA in the current study, have enabled further statistical information to be calculated. These are reported in Tables 4 and 5.

27. Close examination of the individual sterol concentrations, indicated that a trend existed in Δ-5-avenasterol content. This is illustrated in Tables 6 and 7. Of the twenty-four samples labelled "extra virgin", only two, B-2 and E-2, contained less than 100 mg/kg. By comparison, of the eleven samples labelled "olive oil", only C-5 and A-6 contained more than 100 mg/kg. This suggests that the absolute

Δ-5-avenasterol content might be related to the quality of the oil; for example it might be preferentially destroyed by processing. Literature information concerning the absolute values of sterols in olive oil is scarce. Research results [13] for nine samples of solvent-extracted oil showed that they contained less than 63 mg/kg of Δ-5-avenasterol. This is consistent with trends shown above.

TABLE 3

Samples Failing to Comply with All Sterol Criteria as Laid Down in EC Regulation 2568/91

Sample	ß-Sitosterol (>93%) %	Campesterol (<4.0%) %	Brassicasterol (<0.2%) %	Cholesterol (<0.5%) %
A-2	-	-	-	0.7
A-3	92.5+	-	-	-
A-4	92.0	-	-	0.8
A-5	91.5	4.3	0.4	-
A-6*	92.5+	4.7	0.3	-
A-11	92.9+	4.3	-	-
A-12*	92.4+	-	-	0.6
A-13*	90.2	-	0.3	0.7
A-15	92.3+	4.4	-	-
C-3	92.8+	-	-	0.6
C-4	91.7	4.3	0.6	0.9
C-5*	90.3	5.3	-	-
D-1	92.7+	4.1	-	-
Blend-1*	91.6	4.5	-	1
Blend-2*	90.9	4.7	0.4	-
Blend-3*	88.4	6.4	-	0.6
Blend-4*	90.4	5.1	0.6	-
Blend-5	92.2	-	-	-
E-1	92.5+	-	-	-
E-2	-	-	-	0.8
E-4*	-	-	0.4	-
B-1	92.8+	-	-	0.6
B-2	92.2	-	-	0.7
B-5*	92.1	-	-	0.7

+ certain beta-sitosterol results >93%

* blended olive oils

28. The total sterol content will also give some indication of the quality of the oil. Generally, a lower total sterol concentration was found in pure oil compared with virgin oil. In this study, the average values for total sterols are 1,565 mg/kg for extra virgin oil and 1,394 mg/kg for pure oil (i.e. a blend of virgin and refined oils). This decrease might be expected after refining. However, the absolute concentration of an individual sterol may decrease less than that of another sterol owing to isomerisation during processing [14, 15]. The new Regulations for olive oil [1] set a minimum sterol concentration of 1,000 mg/kg for both virgin and refined olive oils. It is considered that relatively high total sterol contents are generally encountered in olive oils if they have been contaminated with olive-pomace (residue) oil. Values in the range

1,800-2,000 mg/kg are typical. Wessels noted that [38] olive-pomace oils contained higher total sterols. Therefore, if the individual sterol concentrations and percentage values of an olive oil are satisfactory, but the total sterols encountered are higher than expected, adulteration with olive-pomace oil may be the explanation. Under these circumstances it would be prudent to determine the uvaol and erythrodiol contents. These are much higher in the olive-pomace (residue) oil and the sample should exceed the 4.5% maximum for these parameters, if residue oil has been added in significant quantities (10-20%).

29. Based on the findings of the desmethylsterol analysis, it is possible to state that certain oils are suspected of being mislabelled. This applies to C-5, Blend-2, Blend-3 and Blend-4 because in each case the apparent ß-sitosterol concentrations are consistently and significantly below 93% and also because the levels of campesterol are greater than 4% of total sterols.

TABLE 4

Statistical Data for Sterol Determinations

Analysis of BCR Reference Material RM162 (mg/kg)

Sterol	BCR Mean (mg/kg)	Study Mean (mg/kg)	Range (mg/kg)	Std. Dev. (mg/kg)	CV (
Cholesterol	40	25	17-40	6.6	26
Brassicasterol	30	30	22-39	4.7	15
Campesterol	1480	1410	1190-1524	95.2	6
Stigmasterol	680	618	518-677	43.5	7
ß-sitosterol	4340	3974	3171-4392	370	9
Δ-5-avenasterol	330	222	152-297	51.4	53
Δ-7-stigmastenol	60	47	33-71	12.4	26
Δ-7-avenasterol	60	54	42-64	7.3	13

TABLE 5

Statistical Analysis of Sterol Determinations

Analysis of BCR Reference Material RM162 (mg/kg)

Sterol	BCR Mean (mg/kg)	Study Mean (mg/kg)	Range (mg/kg)	Std. Dev. (mg/kg)	CV
Cholesterol	0.6	0.4	0.3-0.6	0.1	2
Brassicasterol	0.4	0.5	0.4-0.7	0.1	2
Campesterol	21.8	22.2	20.9-23.6	0.8	
Stigmasterol	9.7	9.8	9.0-10.3	0.4	
ß-sitosterol	61.8	62.0	60.4-63.5	1.0	
Δ-5-avenasterol	4.7	3.5	2.6-4.2	0.7	2
Δ-7-stigmastenol	0.9	0.8	0.5-1.2	0.2	2
Δ-7-avenasterol	0.9	0.9	0.6-1.0	0.1	1

TABLE 6

Comparison of the Concentrations of Δ-5-Avenasterol in Extra Virgin Olive Oil

Sample	Conc. (mg/kg)	Sample	Conc. (mg/kg)
A-1	197-203	B-1	111-117
A-2	188-242	B-2	78-98
A-3	131-165	B-3	177-201
A-4	112-118	B-4	89-166
A-5	136-161	C-1	255-314
A-7	114-130	C-2	222-269
A-8	105-111	C-3	225-237
A-9	248-254	C-4	223-301
A-10	127-157	D-1	220-251
A-11	117-143	E-1	113-147
A-14	92-128	E-2	58-84
A-15	101-112	E-3	125-133

Overall Range 58-314 mg/kg, Mean 164 mg/kg n=90 samples

TABLE 7

Comparison of the Concentrations of Δ-5-Avenasterol in Olive Oils

Sample	Conc. (mg/kg)	Sample	Conc. (mg/kg)
Blend-1	28-49	A-6	93-109
Blend-2	45-54	A-12	43-60
Blend-3	45-59	A-13	53-58
Blend-4	32-57	E-4	25-51
Blend-5	35-38	C-5	122-128
		B-5	37-50

Overall Range 25-128 mg/kg, Mean 55 mg/kg n=43 samples

(iii) Determination of Aliphatic Alcohols by Capillary GLC

30. This technique is no longer part of the Regulation having been replaced in 1993 by a method for the measurement of wax content [9]. Nonetheless, the results are of value and are discussed below. Approximately 90% of the linear aliphatic alcohol content is found in the even carbon number alcohols in the ratio 20% C22; 30% C24; 30% C26; 10% C28 approximately. The Regulation [1] sets a maximum total of 300 mg aliphatic alcohol/kg oil for the extra virgin oils and 350 mg/kg for pure olive oils. A-12 (an olive oil blend) is the only sample that appears to fail these regulations, with an average of 444 mg/kg. A possible explanation of this high concentration is contamination with lampante oil or with olive-pomace (residue) oil. The concentration of aliphatic alcohols in A-12 suggests that the oil may be mislabelled. Data obtained in this study are similar to those observed in other such studies [16, 17].

31. Little repeatability or reproducibility data regarding aliphatic alcohol determination are available. However, from the repeatability data derived from this

study and presented in Table 8 it is clear that the determination of aliphatic alcohols may be undertaken precisely.

TABLE 8

Statistical Data for Aliphatic Alcohol Determination

Sample	Total Aliphatic Alcohol Content (mg/kg)**	Range (mg/kg)	Std.Dev. (mg/kg)	CV (%)	Number of replicate anal
B-5	125	104-146	14.1	11.3	9
A-2	248	210-303	42.8	17.2	6
C-2	278	232-317	31.0	11.2	5
E-4	252	227-275	19.9	7.9	5

Note:-** = Sum of C22,C23,C24,C25,C26,C27 and C28.

E-4 = Pure Olive Oil, all others Extra Virgin Olive Oil

(iv) Interpretation of the Specific Extinction Results

32. Specific extinction has been used as a simple, rapid method for establishing whether virgin oils contain refined oils (if measured at 270nm) or olive-pomace oils (if measured at 232nm). However, a complication exists. Lipid oxidation will increase the extinction at 270nm which could cause a pure virgin oil to be wrongly considered as adulterated. Consequently, oils that initially give rise to high values at 270nm should be treated with alumina which will remove oxidation products. If the resultant extinction is below 0.11, the oil is considered to free from refined oil, but adulterated if the value is greater than 0.11

33. The results obtained in this study for extra virgin olive oils indicate that the bulk have specific extinctions at 270nm below 0.25. This meets the unofficial limit at the time the oils were purchased. However, D-1, A-9 and C-2 failed to meet this limit. They were therefore subjected to treatment with alumina, and further analysed at 270nm. In all cases the specific extinction was reduced to below the limit of 0.11. It is considered therefore that these oils were oxidised rather than adulterated with refined oils. Furthermore, the peroxide values for these oils were amongst the highest of the oils tested, an additional indication that the products were oxidised rather than adulterated (Table 9).

TABLE 9

Specific Extinction of Extra Virgin Olive Oils at 270nm.

Sample	Before Alumina Treatment	After Alumina Treatment	Peroxide Value ($MeqO_2$/kg)
D-1	0.29	0.09	20.0
A-9	0.27	0.09	13.5
C-2	0.25	0.09	16.0

34. As described above, D-I met the specific extinction criteria at 270 nm, following treatment with alumina. This was not the case at 232 nm as shown below (Table 10). This suggests that the oil might be adulterated with unrefined olive oil or unrefined olive-pomace oil.

TABLE 10

Specific Extinction Data for D-1

	K_{270}	K_{270}+Alumina	K_{232}
(i) D-1	0.29	0.09	3.23
(ii) Maximum permitted	0.20	0.10	2.40

(v) Interpretation of the Free Fatty Acid Results

35. The FFA content of an oil is a measure of its quality. With regard to processed vegetable oils, the lower the FFA content, the more acceptable the oil becomes to the human palate. However, virgin olive oils can not be classified in the same way as fully refined oils, because their characteristic flavour involves a sharp component, which is attributable, in part, to the FFA in the product. Consequently, higher levels of FFA are acceptable in virgin olive oils.

36. The FFA of the extra virgin olive oils tested in this study varied from 0.21 to 1.07% (as oleic acid) and those of the pure olive oils from 0.18 to 0.81%. Only one sample (C-3), labelled extra virgin, had an FFA content in excess of the 1% limit prescribed by the EC Regulation. With this exception, all other samples should be considered satisfactory with regard to FFA.

(vi) Interpretation of the Peroxide Value Results

37. Peroxide value (PV) is a measure of the amount of lipid hydroperoxide present in an oil due to oxidation. It is therefore a good indicator of the quality of the oil. As might be expected, the pure olive oils, which by definition are a mixture of virgin and refined oils, have lower PVs, mostly below 10 meq O_2/kg, whereas only one virgin oil has a PV of less than 10.

38. The EC Regulation [1] limits extra virgin olives oils to maximum peroxide value of 20 meq O_2/kg, and pure olive oils to a maximum limit of 15 meq O_2/kg. On this basis all oils tested were satisfactory

(vii) Interpretation of the Results of the Overall Fatty Acid Composition

39. The fatty acid compositions of the oils in this study were found to be in accord with those in the literature [16,18,19,20,21]. A number of compositions were slightly outside the limits stated in the EC Regulation, these are shown below in Table 11, but are considered to be of little significance with regard the oil purity.

TABLE 11

Fatty Acid Compositions - Samples that fail to meet the criteria in EC Regulation 2568/91.

Sample Limit Value	Myristic acid 0.1% max.	Behenic acid 0.3% max.	Lignoceric acid 0.5% max
A-3	0.2%	-	-
E-1	-	-	0.6%
E-2	-	-	0.6%
A-6	-	-	0.6%
A-12	0.2%	-	-
A-13	0.2%	-	-

Note: The above samples met all other fatty acid limits.
All other samples fully met the requirements of the fatty acid ranges, etc.

40. The use of fatty acid compositions to describe the purity of olive oils has been criticised because of the very wide variations in the concentrations of particular fatty acids. This tends to make it more difficult to authenticate the purity of an olive oil in comparison with other single seed oils. The oleic acid content of B-3 at 64.9% is almost 5% lower than that of any other in this study. However, this is within accepted limits e.g. the Codex range for oleic acid is 55-83%.

S.4 CONCLUSIONS

41. It would appear that the methodology quoted in the current and past versions of the EC Regulation [1] is generally time-consuming, and can give rise to inconclusive and ambiguous results. It is also unclear the extent to which the methodology has been successfully collaboratively tested.

42. Methodology, based on the determination of dehydrated sterols (steroidal hydrocarbons or sterenes), already exists and permits the identification of refined oils in virgin oils; this methodology has now been incorporated into the Regulation. Additional techniques involving the analysis of oils for their n-alkane profile and using artificial neural networks may also prove valuable in the prevention of fraud involving olive oils.

43. When making judgements on an oil's purity it is important to consider the following aspects. The methodology and limits laid down in the Regulation do not indicate tolerance levels. For example, the apparent ß-sitosterol concentration of a pure olive oil should exceed 93% of the total sterols. It is unclear, however, whether the repeatability of the sterol determination has been taken into account when establishing this limit and whether any tolerance values have been or should be added to this value. Furthermore, there will clearly be natural variation in olive oil composition which will be dependent on climatic and geographical conditions, etc. but it is not clear to what extent this is included in the limit set in the Regulation [1].

44. It is strongly recommended that the analytical limits for certain criteria be developed to permit conclusive authentication of olive oils. In particular, for the determination of sterols (including uvaol and erythrodiol) and saturated fatty acids at the 2-position, ranges rather than only maxima or minima should be presented. This would permit the calculation of means and standard deviations, etc. Consequently, the degree to which an oil was considered pure (or impure) could be calculated with a degree of confidence i.e. ± 2 standard deviations from the mean for 95% confidence; ± 2.3 standard deviations from the mean for 99% confidence.

45. The analytical results of the fatty acid composition, FFA and PV determination are in accord with the manner in which the oils were labelled.

46. The desmethylsterol results indicate that C-5, Blend-2, Blend-3 and Blend-4 were suspect because their contents of ß-sitosterol were consistently and significantly below 93% and the levels of campesterol exceeded that permitted (4%).

47. The aliphatic alcohol contents of the oils were acceptable for all but one sample. A-12, a blended olive oil, contained 444 mg/kg which is considerably greater than the permitted concentration of 350mg/kg for such products. This oil was, therefore, suspect.

48. D-1 failed to meet the criteria for saturated fatty acids at the 2-position and the specific extinction at 232 nm. This oil is suspect and might be adulterated with an

unrefined olive oil or olive-pomace oil.

49. Should an oil fail to meet the appropriate specification, it is not fully clear whether this is a result of the analytical criteria within the specification being inaccurate, faults within the methodology, or the oil not being of the quality stated on the label. It is also possible that it could be a combination of two or more of these factors. As a result of these uncertainties and because the samples were purchased before the Regulation came into force, the commercial identities of the oils are not presented. Furthermore, the main objective of the study was an evaluation of the methodology in determining olive oil purity, rather than a representative surveillance exercise to establish whether the olive oil on the UK market was as labelled. It should also be appreciated that a limited number of samples were studied (thirty five) and the collection area was relatively small.

S.5

ANNEX 1

DISCUSSION OF ANALYTICAL METHODOLOGY EMPLOYED

(i). Analysis of the Fatty Acids at the 2-Position of Glycerol (2-Position Analysis)

50. This technique involves the partial enzymic hydrolysis of the glycerides to produce 2-monoglycerides. These are separated by thin layer chromatography, derivatised and their fatty acid composition determined by GLC. The technique is of particular use in determining whether olive oils have been adulterated with re-esterified olive oils.

51. The analysis of an oil for the fatty acids at the 2-position is important for the following reasons:-

(a) For vegetable oils, the 2-position profile is often different to the overall fatty acid composition (FAC). It is believed that this is due to saturated fatty acids being preferentially incorporated at the 1,3-positions by the biosynthetic process. Therefore, the 2-position provides an alternative composition which is indicative of oil purity.

(b) The enrichment factor or EF (see below) is different for different oils and may assist in their authentication. For example, this factor may be of value in distinguishing groundnut oil from maize germ oil or sunflowerseed oil using the linoleic acid EF, or palm oil from its fractions by using the palmitic acid factor.

EF = % fatty acid X in 2-position / %fatty acid X in overall composition

(c) The EF is also a useful indicator of the presence of re-esterification, which is particularly relevant to olive oils. For example, in olive oils of poor quality, fatty acids may be hydrolysed from the triglyceride. These may be recovered during oil refining, and recombined with glycerol to form an oil with the same overall FAC, but having a different distribution of fatty acids within the triglycerides. In such a case the 2-position distribution is altered and the re-esterified oils have a considerably higher concentration of palmitic and stearic acids at the 2-position in comparison with a virgin olive oil. This is illustrated in Table 2. Re-esterified oil is sometimes illegally blended with more valuable grades of olive oil for commercial gain. For example, a study in the US in 1985 [3] found that of twenty-five oils labelled as being "pure" or "virgin", only seven were believed to be genuine. Of the others, fourteen contained re-esterified olive oil, which was detected by 2-position analysis.

52. In studies on palm oil, [22] it was shown that the large quantities of solvents in the oil during the lipolysis, (an original requirement of the method) gave erroneous results. The reasons for this have not been established.

53. Short-chain fatty acids (butyric, capric and caprylic, etc.) are readily hydrolysed

by water under the conditions of the test. Therefore, the test is unsatisfactory for fats containing measurable quantities of short-chain fatty acids, such as butterfat, palm kernel oil and coconut oil.

54. Accurate results are obtained only if the hydrolysis stages are accurately timed, and sufficient hydrochloric acid added to inactivate the enzyme after the specified time. If these precautions are not taken when analysing olive oils, i.e. lipolysis is prolonged, high and false concentrations for palmitic (C 16:0) and stearic (C 18:0) acids will be recorded.

55. Integration of peak area from the GLC chromatogram is an important consideration. Since the method specifies that only 0.1 g of oil should be analysed and less than one-third of the fatty acids are extracted and analysed under the test conditions (i.e. those in the 2-position), the concentration of fatty acid methyl esters in the final hexane extract may be too small for the integration to be undertaken satisfactorily. Three modifications to improve this situation have been developed:-

a) increase the concentration of the methyl esters injected onto the GLC by reducing the volume of solvent;

b) ensure complete removal of the released fatty acids at the 2-position by increasing the volume of extraction solvent. If combined with concentration step in (a), this gives rise to improved chromatograms;

c) increase the weight of sample from 0.1 to 0.2 g together with corresponding increases to the quantities of reagents used.

56. The very large concentration differences between the adjacent peaks of C18:0 (0.01- 0.2% for all but one oil) and C18:1 (76-91%) can lead to the C18:0 peak being unresolved by the integrator although it can be seen by the naked eye. Column performance should be monitored to ensure satisfactory resolution and integration are achieved. The column conditions used in this study were capable of resolving stearic methyl ester from oleic acid methyl ester when present at 0.5% but not at 0.01%.

57. Evidence is available, although unpublished, that demonstrates that an artefact which elutes with a similar retention time to C15:1 is sometimes observed in the reagent blank; care should be exercised to ensure that this peak is not mistaken for that attributable to C16:0.

58. Difficulties have been experienced resulting in poor TLC separation of the 2-monoglyceride band (Rf = 0.035) from the non-eluting material on the TLC baseline. This has also been shown using 99% pure mono-palmitin. Removal of only the 2-monoglyceride should be made. Separation may be improved by using dried chromatographic quality solvents.

59. The method contained no instruction to the analyst that the TLC plates should be cleaned prior to use in order that artefactual contamination be avoided. This is however stated in the EC Regulation [1] and was undertaken in this study.

Analysis of (ii) Sterols and (iii) Aliphatic Alcohols

60. The determinations of desmethyl sterols and aliphatic alcohols involve similar techniques and consequently will be considered together. The large number of stages involved in each tends to reduce the degree of accuracy when determining absolute concentrations. This is borne out by a collaborative trial undertaken by the Community Bureau of Reference (BCR). The results showed a wide divergence for the absolute sterol concentrations (mg/kg) between laboratories (CV = 20%) compared with relative percentages (CV = 5%). The methods therefore need to be written in a way that assists the analyst to minimise losses and a number of improvements could be made to the sterol protocol.

61. For example, the initial stages of the sterol and aliphatic alcohol analyses should be combined since these tests are essentially identical up to identification of the individual bands on a TLC plate.

Comments on each stage of the combined procedure are given below.

Step 1 (Section 5.1.1) Choice of Internal Standard (IS) for Sterol Analysis

62. Three IS are commonly used.

a) 5-α-cholestane. This has frequently been used. However, sterols contain a ß-hydroxy group in the ring A at carbon 3 which is absent in 5-α-cholestane rendering it less polar. If added at the beginning of the analysis 5-α–cholestane does not appear on the final sterol chromatogram because it has a different RF on the TLC plate than sterols and is therefore lost from the analysis. Consequently, it should only be added when derivatising the sterol band and is then not a true IS for the full procedure.

b) ß-cholestanol (alternative names dihydrocholesterol, 5-α-cholestan-3-ß-ol). This is the IS recommended in the current study. The disadvantage with this compound is its elution in close proximity to cholesterol in the GC chromatogram (Relative Retention Times (RRT) cholesterol = 0.66; ß-cholestanol = 0.67; ß-sitosterol = 1.00). Consequently, complete resolution of the two compounds is not always achieved, casting doubts on the accuracy of the absolute concentrations obtained.

c) Betulin (alternative name Lup-20(29)-ene-3,28 diol). This is the IS recommended by BCR. However, a number of problems may arise in its use. For example

- Silanised betulin has a very long retention time. This makes it more suitable for determining uvaol and erythrodiol than for sterols (RRTs betulin = 1.61; uvaol 1.52; ß-sitosterol 1.00).
- It is not of consistent purity.
- Derivatisation is slow and sometimes incomplete.
- If the sterol band contains oxidised material or if oxidation occurs, e.g. because of analytical delays etc., artefact peaks will elute with retention characteristics similar to

those of betulin. There is a danger of undetected co-elution with the IS giving rise to erroneous results.

- Betulin has limited solubility in common solvents. If betulin was employed as IS considerable modifications to the procedure would be necessary. For example,
- Di-iso propyl ether (DIPE) should be added to the reaction mixture before saponification, to ensure complete dissolution of betulin. If DIPE is omitted, the betulin peak area is decreased, giving rise to false high absolute sterol concentrations.
- Betulin is insoluble in all the recommended TLC developing solvent mixtures (Table 12), and the procedure can only be satisfactorily accomplished by use of a mixture of toluene (95 parts): acetone (5 parts).
- Betulin does not co-elute with the sterol band on the TLC plate (RFs:- betulin 0.14; cholesterol 0.18). This necessitates the removal of a wider band, increasing the risk of incorporating interferences from other oil components.

In these studies, ß-cholestanol was used. The IS for aliphatic alcohols (1-eicosanol) appears to be satisfactory.

63. Repeatability is improved if the IS solution is added by weight rather than by volume.

Step 2 (Section 5.1.1) - Sample Weight

64. The method implies that a sample weight of 5 g be used, which is acceptable in the present work, where considerable amounts of sample are available. However, in many commercial and enforcement situations very limited amounts of sample are available and it may be necessary to carry out several analytical procedures. If this is the case, a combined analysis for sterols, uvaol, erythrodiol and aliphatic alcohols can be carried out adequately on a 1g sample.

Step 3 (Section 5.1.2) - Saponification of Sample

65. Initially the method stated that methanolic rather than ethanolic potassium hydroxide (KOH) should be used. Methanol boils at a lower temperature (64.5°C) than ethanol (78.3°C) and the reaction is consequently undertaken at a lower temperature. The rate of breakdown of sterol containing lipids (i.e. steryl esters) might, therefore, be reduced if methanol rather than ethanol was used as solvent. Since any unreacted steryl ester would not behave as a free sterol the use of the lower boiling solvent might lead to incomplete saponification and therefore inaccurate results.

(The EC Regulation [1] reinstated ethanol as the solvent in the saponification stage). Analyses reported here were carried out using ethanol, since comparative studies using methanolic and ethanolic KOH indicated that use of the former gave rise to variable and inaccurate results.

Step 4 (Section 5.1.3) Extraction of Unsaponifiable Matter (USM)

66. The USM was extracted with three portions of diethyl ether. Emulsions that may arise can be destroyed by adding a small volume (5 ml) of the alcohol, followed by gentle inversion of the separating funnel.

Step 5 (Section 5.1.4) Moisture Removal

67. The procedure for this step is satisfactory, but the sodium sulphate and filter paper should be washed on at least four occasions with diethyl ether to remove all the unsaponifiable matter.

Alternatively, the drying process could equally be carried out by adding 100 ml dry acetone and evaporating to dryness. This removes the traces of water present by azeotropic distillation. This technique is preferred because it eliminates possible losses of unsaponifiable matter due to insufficient washing of the sodium sulphate.

Step 6 (Section 5.1.5) Determination of mass of USM

68. This is carried out to ensure that sufficient derivatising agent is added to the USM to ensure complete derivatisation of the free sterols. During drying at 100°C, care should be taken to avoid sterol oxidation. To overcome this possibility, if an excess of derivatising agent is added there is little need to dry and weigh the USM. Should a determination of total unsaponifiable matter also be required, it is more appropriate to carry out a separate determination [13].

Step 7 (Section 5.2.1) TLC separation of components of the USM

69. FFA present in the USM are removed by dipping plates in 0.2 N ethanolic potassium hydroxide thereby allowing retention of the resulting soaps on the baseline.

70. A benzene/acetone mixture is stated in the method as developing solvent. However, owing to benzene's highly toxic nature, its use must be avoided. A hexane-ethyl ether (65:35) solution may be used as an alternative. Equally effective developing solvents are available as shown in Table 12.

TABLE 12

Alternative Developing Solvents for Sterol and Aliphatic Alcohol Analysis

Developing Solvent Mixture	Ratio
40-60 Petroleum ether: diethyl ether: acetic acid	80:20:1 or 90:30:2
Hexane: ethyl acetate	4:1 or 85:15
Methylene chloride: carbon tetrachloride	1:5
Chloroform: diethyl ether	9:1
Chloroform: diethyl ether: acetic acid	94:5:1
40-60 petroleum ether: diethyl ether	1:1

71. With regard to the extraction of the sterols and aliphatic alcohols from the TLC plates, the standard is acceptable but improvements as described below should be made:-

For glass plates.

After removal of the band from the TLC plate, transfer to a 50 ml round-bottom flask containing 10 ml chloroform. Attach a condenser and reflux on a steam bath for 1 min. Cool and decant the chloroform through a filter paper into a 100 ml conical flask. Repeat this procedure three times. Evaporate the solvent and proceed with the derivatisation.

For plastic plates.

The losses due to dust formation may be reduced considerably by the following. The required band should be cut into small pieces and transferred to a 100 ml glass beaker and soaked in hot chloroform (4h10 ml) and filtered. The solvent should be evaporated and the sample derivatised. The inhalation of silica dust is a health hazard. The above procedure should reduce the likelihood of inhalation.

72. Some workers recommend that the triterpene dialcohol components uvaol and erythrodiol, be examined along with the sterols. High levels of these two compounds indicate the presence of olive-pomace (residue) oils.

It has been shown [23a, 23b, 24] that pressed oils from Spain, Tunisia and Greece all contain less than 5% uvaol and erythrodiol, expressed as a percentage of sterols plus uvaol plus erythrodiol. This limit is prescribed by Italian law and by various trade bodies. These two compounds were not, however, studied in the present work. The triterpene dialcohols were shown to elute from the TLC plate fractionally before the sterol band. Therefore, the two bands can be removed from the TLC plate, combined, and the GLC separation of derivatised (silanised) components undertaken.

If levels above 5% in the combined sterol/triterpene dialcohol band are found, this is an indication that the virgin olive oil is contaminated with olive-residue oil. There are obvious limitations to the detection of residue oil by this process. Most of the oils studied by Paganuzzi [23a, 23b, 24] were well below the Italian 5% forensic limit, enabling some residue oil to be added without detection. The Regulation [1] has a more rigid limit of 4.5%. Passaloglou-Emmanouilidou[25] claims that the method is only suitable for detecting adulteration with 20% or more of residue oil.

Step 8 (Section 5.3) Derivatisation of Sterols and Aliphatic Alcohols Prior to GLC

73. In this study, both sterols and aliphatic alcohols were converted to their silyl derivatives. The procedure is that used traditionally[26]. The reagent used is bis(trimethyl-silyl)trifluoroacetamide (BSTFA) containing 1% trimethyl chlorosilane; derivatisation of sterols and aliphatic alcohols occurs within 15 min at room temperature. The Regulation [1] permits the use of this reagent. Other derivatisation techniques that could be used include formation of acetyl derivatives [27] and silylation using hexamethyl disilazane and trimethyl chlorosilane in the presence of pyridine [28]

Silylation of the alcoholic groups is required to improve the chromatographic properties of the analytes.

Step 9 (Section 5.4) Analysis of Individual Components in the Sterol and Aliphatic Alcohol Fractions by Capillary GLC

74. The conditions prescribed in the method are exactly the same as those in EC Regulation [1] and are satisfactory. In this study, on-column injection rather than split injection was used. Comparative analysis revealed that both injection systems gave similar results. On-column injection was used to a greater extent because, in theory, it has two advantages:

i) There is less analyte discrimination.

ii) There is greater sensitivity,

75. The injection technique is also critical. It is stated that the needle must be pre-heated for 1-2 seconds before the plunger is pressed. The same technique is prescribed in EC Regulation [1]. The needle should be heated for at least three seconds prior to injection. When the plunger is pressed the contact of the solution with the hot needle creates a minor explosion, during which the sample is ejected violently out of the needle. This minimises the amount of material left inside the needle, rapidly injects the sample on to the column, produces small droplets which move more easily with the carrier gas and volatilise more quickly, contributing to improved reproducibility and resolution The hot-needle technique can be used satisfactorily with both split and on-column injection systems.

76. The GLC conditions used for both sterol and aliphatic alcohol determinations are presented in Table 13.

TABLE 13

GLC Parameters for Sterol and Aliphatic Alcohol Analysis

Parameter	Sterol conditions	Sterol conditions	Aliphatic alcohol conditions	Aliphatic alcohol conditions
Stationary phase	CP SIL 19	CP SIL 8	CP SIL 19	CP SIL 8
Carrier gas	Hydrogen	Hydrogen	Hydrogen	Hydrogen
Initial Temp. (°C)	220	270	180	180
Hold time	0	0	4	5
Rate of increase (°C/min)	4	0	5	80
Final Temperature (°C)	260	270	240	260
Final Time (mins)	45	60	60*	60*

Note: * = Relevant peaks will have eluted within 30 mins.

However, other compounds such as triterpene diols will elute up to approximately 50-55 mins.

General Consideration of the Methodology for the Sterols and Aliphatic Alcohols

77. The non-availability of primary standards for the analysis of the 4-desmethyl sterols has been a consistent problem and although certain companies have claimed to produce sterols of high purity, examination has indicated that these products contain, on occasions, between 20-30% impurities.

78. It is generally accepted that cholesterol is the only desmethyl sterol that may be purchased in a highly pure form. In the current study, no attempt was therefore made to calculate response factors for the sterols. There would be an overlap between cholesterol and ß-cholestanol, the internal standard preventing reasonable measurements being made for the one sterol of suitable purity. However, the impure sterols could be used to establish retention times. A secondary standard is available which may be used to act as a guide to analytical repeatability, etc.

79. Three different GLC liquid stationary phases have been used in this study, namely:- CP SIL 19CB, CP SIL 8 and DB5. As would be expected, some minor differences in resolution were noted between the stationary phases. All three columns had insufficient resolution to separate 24-methylene cholesterol from campesterol, and sitostanol from ß-sitosterol. Both CP SIL 8 and DB5 were unable to resolve Δ-5,23-stigmastadienol from chlerosterol. However, it is the sum rather than the individual concentrations that is required for these compounds as they form part of the 'apparent ß-sitosterol' grouping.

80. On occasions, it is possible to employ simple ratios involving two or more analytical parameters to assist in the interpretation of the analytical data. Clearly, it is important that these are true differences and that analytical errors are not being enhanced. It became apparent, however, that it is not possible to use any of these ratios to distinguish between extra virgin and pure olive oils and although use of the ratio ß-sitosterol /Δ-5-avenasterol appeared to enhance the differences, it did not provide firm evidence of the difference between the two grades.

Aliphatic Alcohols

The same three GLC stationary phases were used for the aliphatic alcohol analyses as for the sterol analysis, namely CP SIL 8, CP SIL 19 and DB5. There were no apparent differences in resolving power of the three columns when used in the aliphatic alcohol analysis.

There are a number of difficulties in carrying out this analysis. These include the following.

a) There are few published data, or literature references.

b) There is no suitable reference material on which an analyst unfamiliar with the technique can practise.

81. The individual alcohols between C20 and C28 can be obtained. These were used to obtain relative retention times (RRT) and response factors (RF) (Table 14) which are very close to unity except for C26 and C27. The peaks associated with these compounds showed signs of tailing. This probably led to an increase in the corresponding response factors to 1.16 for C26 and 1.10 for C27.

82. A number of unknown compounds were observed in the chromatogram. Identification was attempted by examining the chromatographic properties of palmitic acid (RRT 0.02); uvaol (0.08); cholesterol (0.09); eicosanol (0.23); trilaurin (0.52); alpha-tocopherol and alpha-tocopherol acetate (0.56); squalene (0.72); beta-carotene (0.02 & 0.81). However, these compounds were not the cause and the identity of the interferences remains unknown.

83. It was found that the saponification must proceed for at least 1 hour. This was established following studies on the saponification of C32 and C33 wax esters, which are resistant to saponification. The EC Regulation is vague in that it states that the oil should be saponified (i.e. the solution becomes clear) and that saponification should continue for a further 20 mins once this has been observed.

TABLE 14

Response Factors and Retention Times for Aliphatic Alcohols

Aliphatic Alcohol	Typical Retention Time (min)	Relative Retention time	Response Factor
		(C26 = 1.00)	(C20 = 1.00)
Eicosanol (C20) - Internal Standard	11.13	0.50	1.00
Docosanol (C22)	15.36	0.69	1.00
Tricosanol (C23)	17.33	0.78	-
Tetracosanol (C24)	18.88	0.85	1.01
Pentacosanol (C25)	20.20	0.91	1.00
Hexacosanol (C26)	22.24	1.00	1.16
Heptacosanol (C27)	24.17	1.09	1.10
Octacosanol (C28)	27.01	1.21	0.99

(CP SIL 19 CB Stationary Phase)

(iv) Specific Extinction at 232nm and 270 nm

84. The specific extinction has been used as a simple, rapid method for establishing whether oils labelled as virgin contain refined oils.

85. In the initial MAFF publication [4] of this method, it was not made clear at what stage, or why, the alumina column should be used. The explanation is that the usual 1:4 distribution of double bonds as found in linoleic and linolenic acids may be

changed to a conjugated 1:3 system of dienes and trienes during oxidation or refining. Oxidation may give rise to $E^{1\%}_{1cm}$ 270 nm higher than the maximum 0.25 expected in a genuine virgin olive oil. If oxidation is suspected, the sample should be treated with alumina chromatographic column to remove oxidation products. Regulation no. 2568/91 [1] corrects this omission. The limit for the specific extinction at 270 nm after this alumina treatment is 0. 10[1].

86. Measurements have also been made at 232nm. to determine the presence of olive-pomace oils. It is considered that olive oils having a specific extinction greater than 5 are of poor quality.

87. There are two practical difficulties with the method as written. The dilution suggested (ca 1% oil) is satisfactory for measurements at or around 270 nm. However, a 5-fold dilution is required to enable measurement of the specific extinction at 232 nm which, to prevent underestimation, should be added to the text.

88. The second difficulty concerns the measurements at 270 nm. If the spectral curve is plotted between 260 and 280 nm, an actual maximum occurs at around 268-270 nm for most samples labelled pure olive oil. However, only a shoulder is seen for many of the samples labelled extra virgin olive oil. This makes the calculations for Δ E rather dubious. Δ E is defined as

$$\Delta E = E_m - \frac{E_{m-4} + E_{m+4}}{2}$$

where E_m is the specific extinction at wavelength m, the wavelength for maximum absorption around 270 nm.

89. The method is identical to BS 684 Section 1.15 [18], which was successfully ring tested before adoption. The repeatability and reliability are satisfactory.

(v) Free Fatty Acids (FFA)

90. The FFA of an oil is a guide to its quality. In general the greater the FFA the lower the oil quality. However, virgin olive oils cannot be classified in the same way as fully processed oils since their characteristic flavour is attributable, in part, to their FFA. The FFA can increase as a result of enzymic lipolysis of triglycerides but more often it occurs following the action of water on the ester linkage between the fatty acid and the glycerol moiety of the fat.

91. Methods for the titrimetric determination of FFA in oils fall into two categories. One uses aqueous alkali and is carried out in hot alcohol [30,31,32]. The other (used in this study and technically equivalent to ISO 660 [33]) uses a cold solvent mixture such as a 1:1 diethyl ether:ethyl alcohol mixture and titration with ethanolic potassium hydroxide solution [33,34].

The repeatability and reproducibility data quoted in the method indicate that the procedure is reliable. However, the following criticisms have been made of methods which use cold alcoholic alkali and diethyl ether

i) Diethyl ether has a high vapour pressure and low boiling point giving rise to high concentrations of the solvent in the locality. The inherent fire risk and disposal of the waste ether are more problematic than with alcohol alone.

ii) Alcoholic solutions of potassium hydroxide are unstable and readily evaporate. In addition, the solution absorbs atmospheric carbon dioxide more readily, necessitating more frequent standardisation of the titrant.

(iii) Ethanol has a greater thermal expansion than water. Therefore experimental error is higher when standard ethanolic solutions are used, especially under conditions where there are large laboratory temperature fluctuations. Partly for these reasons, ISO is about to withdraw the cold mixed solvent ISO 660 (1989) version and replace it with a hot ethanol version equivalent to the BS method. This new test will be ISO 660 (1993)[35].

(iv) It should be appreciated, however, that other factors such as the solubility of the oil and FFA in the solvent favour the use of diethyl ether, etc.

92. Extra virgin olive oil is defined as having less than 1% FFA calculated as oleic acid. If the prescribed procedure was followed for this concentration of FFA, 20 g of sample would have to be employed for each replicate analysis. There are many enforcement situations where such a large sample would not be available.

93. Many extra virgin olive oils have a strong green colour, due to chlorophyll and related pigments. In extreme cases the colour of the oil masks the phenolphthalein end point. This can be overcome by taking a smaller sample, as mentioned above, by further dilution, by use of a potentiometric end point detection, or by use of a different indicator such as thymolphthalein, alkali blue 6B or phenolphthalein masked with methylene blue.

Indicator	Colour change (acid > alkali)	Replicate results (FFA as % oleic acid)	Comments on end point
Thymolphthalein	Colourless to blue	0.73, 0.73	Easy to see
Alkali blue 6B	Blue to yellow	0.73, 0.74	More difficult to see
Phenolphthalein	Colourless to pink	0.70, 0.74	Easy to see, but CO_2 absorption a problem
Phenolphthalein masked with methylene blue	Turquoise to mauve	0.73, 0.73	Easy to see

On the basis of these results, all four indicators lead to titrations giving the same FFA value; however, thymolphthalein and phenolphthalein masked with methylene blue give end points that are readily observed. It is suggested that the official methods should give the analyst more scope to

choose from a variety of indicators, and that these should include thymolphthalein and phenolphthalein masked with methylene blue.

94. If mineral acids are present in the oil they should be removed with distilled water before the oil is titrated. This is not made clear in the Regulation.

It is important that the sample be gently warmed and filtered before analysis, as is described in the EC Regulation.

(vi) Peroxide Value

95. The peroxide value (PV) is a measure of the amount of lipid hydroperoxide present in the sample due to oxidation of the oil. The PV is, therefore, a good indicator of the quality of an oil. It would be expected that pure oils (which by definition are mixtures of virgin and refined oils) would have lower PV than those of virgin oils.

96. Chlorophyll-like pigments may interfere with detection of the end point. Dilution of the flask contents, use of a smaller test portion and measuring the end point potentiometrically are all suitable methods of overcoming this difficulty.

97. The technique involves the addition of potassium iodide following which the usual practice is to shake the contents of the flask for 1 minute, allow to stand for either 1 or 5 mins, then add water and immediately titrate the liberated iodine. In this study, a 5-min standing time was used as stated in the method. Experience suggests, the additional standing time is of limited benefit.

98. The method itself is well written, and there appear to be no omissions.

99. Chloroform is toxic. Alternative PV methods using less toxic solvents such as iso-octane and acetic acid are being developed.

100. The technique is identical to British Standard 684 Section 2.14 [36], which was successfully ring tested before adoption. The repeatability and reliability are satisfactory.

(vii) Overall Fatty Acid Composition

101. Edible oils are primarily composed of triglycerides (i.e. three fatty acids esterified to glycerol). Consequently, the determination of FAC is a most important measurement in that it provides identity, purity and nutritional information.

102. The method employed in this study is equivalent to BS 684:2.34/35 (ISO 5508/9) [10,11,21]. The technique is applicable to oils with FFA of less than 2%. It is not applicable to oils that contain short chain fatty acids (C10:0 and below). It involves the saponification of the oil following exposure to refluxing potassium hydroxide. The resulting soaps are methylated to produce fatty acid methyl esters (FAME) by the addition of a boron trifluoride-methanol complex. FAME are dried, diluted and determined by capillary column GLC. The repeatability and reliability of the method

are universally accepted. For example, it is recommended by IOOC for measurement of the fatty acid composition of olive oils. However, it is not one of the methods prescribed in the EC Regulation [1] (Annex 3).

103. EC Regulation [1] was recently amended. It is now necessary to identify a number of *cis* and *trans* isomers of C18:1, C18:2 and C18:3 EC [6]. However, to obtain this information requires a more complex analytical conditions than used in this study. It has not, therefore, been possible to obtain *cis* and *trans* values from the original data.

S.4

ANNEX 2

DESCRIPTIONS AND DEFINITIONS OF OLIVE OILS AND OLIVE-POMACE (RESIDUE) OILS

Taken from Council Regulation (EEC) No. 356/92 of 10 February 1992 amending Regulation No. 136/66/EEC

1. Virgin olive oils;

Oils derived solely from olives using mechanical or other physical means under conditions, and particularly thermal conditions, that do not lead to deterioration of the oil, and which have undergone no treatment other than washing, decantation, centrifugation or filtration, but excluding oils obtained by means of solvents or of re-esterification and mixtures with other oils.

These oils are classified as follows:

(a) Extra virgin olive oil

Virgin olive oil having an organoleptic grading of not less than 6.5, a free fatty acid content of not more than 1g per 100g and the other characteristics which comply with those laid down for this category:

(b) Virgin olive oil: (the expression 'fine' may be used at the production and wholesale stage):

Virgin olive oil having an organoleptic grading of not less than 5.5, a free acid content expressed as oleic acid of not more than 2g per 100g and the other characteristics which comply with those laid down for this category;

(c) Ordinary virgin olive oil.

Virgin olive oil having an organoleptic grading of not less than 3.5, a free fatty acid content expressed as oleic acid of not more than 3.3g per 100g and the other characteristics which comply with those laid down for this category;

(d) Lampante virgin olive oil.

Virgin olive oil having an organoleptic grading of less than 3.5, and/or a free acid content expressed as oleic acid greater than 3.5g per 100g and the other characteristics which comply within those laid down for this category.

2. Refined olive oil:

Olive oil obtained by refining virgin olive oil, having a free acid content expressed as oleic acid of not more than 0. 5g per 100g and the other characteristics which comply with those laid down for this category.

3. Olive oil:

Olive oil obtained by blending refined olive oil and virgin olive oil, other than lampante oil, having a free acid content expressed as oleic acid of not more than 1.5g per 100g and the other characteristics which comply with those laid down for this category.

4. Crude olive-residue oil:

Oil obtained by treating olive residues with solvents, excluding oil obtained means of reesterification and mixtures with other types of oil, and the other characteristics which comply with those laid down for this category.

5. Refined olive-residue oil

Oil obtained by refining crude olive-residue oil, having a free fatty acid content expressed as oleic acid of not more than 0.5g per 100g and the other characteristics which comply with those laid down for this category.

6. Olive-residue oil

Oil obtained by blending refined olive-residue oil and virgin olive oil other than lampante oil, having a free fatty acid content expressed as oleic acid of not more than 1.5g per 100g and the other characteristics which comply with those laid down for this category.

S.5

ANNEX 3

DIFFERENCES BETWEEN THE METHODOLOGY USED IN THIS STUDY AND THAT QUOTED IN EC REGULATION 2568/91

As will be seen from the following, there are a number of occasions where the methodology used in this study was different to that described in the EC Regulation 2568/91. However, in the vast majority of cases the differences are not significant.

1. Determination of the Fatty Acids at the 2-position of the Triglyceride

   Identical to the method quoted in EC 2568/91.

2. Determination of the Composition of Sterols by Capillary-column GLC

   Almost identical to that quoted in EC Regulation 2568/91. The only difference of any significance between the method used in this study and that quoted in the original MAFF publication was that the latter prescribed the use of methanolic solutions during saponification while the analysis was undertaken using ethanolic solutions. The EC Regulation also requires that ethanolic solutions are used. Further details on this point are provided at paragraph 65.

3. Determination of Aliphatic Alcohols Content by Capillary Column GLC

   The comments made regarding the determination of sterols are fully applicable here.

4. Spectrophotometric Analysis of Oils to Determine Specific Extinctions

   Very similar to that quoted in EC Regulation 2568/91.

5. Determination of Free Fatty Acid (FFA) Content and Acidity Value

   Very similar to the method quoted in EC Regulation 2568/91.

6. Determination of Peroxide Value (PV)

   Very similar to the method quoted in EC Regulation 2568/91.

7. Determination of Overall Fatty Acid Composition (FAC)

   The fatty acid composition of an oil is usually established by derivatising the substrate to produce fatty acid methyl esters which are then determined by capillary column GLC. In the study described in this paper, the oils were derivatised (methylated) by saponification with potassium hydroxide followed by addition of a boron trifluoride-methanol complex. This procedure produces fatty acid methyl esters (FAME) which are then analysed by capillary column GLC.

In the EC Regulation 2568/91, this method is not quoted but five different techniques are described. The main differences between the methodologies lie in the derivatising agents used for producing the FAME. The technique used in this study employed boron trifluoride-methanol complex; those referred to in the Regulation used the following:

(i) sodium methylate,

(ii) methanol-hydrochloric acid,

(iii) dimethyl sulphate and

(iv) methanol-hexane-sulphuric acid.

Nonetheless, the methodology used in this study for the determination of the FAC of oils is fully satisfactory.

REFERENCES

1. European Commission (1991a). Commission Regulation (EEC) No. 2568/91 of 11 July 1991 on the characteristics of olive oil and olive residue oil and on the relevant methods of analysis. Official Journal, 34, (248) 5 September. L248/3-L248/82.

2. EIU Retail Business No 400 June 1991

3. Firestone, D., Summers, J.L., Reina, R.J. & Adams, W.S. (1985). 'Detection of adulterated and misbranded olive oil products.' J. Am. Oil Chem. Soc., 62, 1558-62.

4. Ministry of Agriculture, Fisheries and Food (1990). MAFF Information Bulletin for Public Analysts on EC methods of analysis and sampling for foodstuffs. No. 110 Methods of Analysis for Olive Oil.

5. European Commission (1991b). Commission Regulation (EEC) No. 3682/91 Vol. 34 L349. Official Journal of 18 December 1991. Vol 34 L349 p. 36.

6. European Commission (1992). Commission Regulation (EEC) No. 1429/92 of 26 May 1992. [Amendment to Reg. no. 2568/91 (EC, 1991).]

7. European Commission (1992b). Commission Regulations (EEC) No. 1683/92. Official Journal of 30 June 1992. Vol. 35 L176 p. 27.

8. European Commission (1992c). Commission Regulations (EEC) No. 1996/92

9. European Commission (1993). Commission Regulation (EEC) No. 183/93 of 29 January Amendment to Reg. No. 2568/91. Official Journal, 22 (58-68), 30 January 1993.

10. International Standards Organisation ISO 5508, 1990. Analysis by gas chromatography of methyl esters of fatty acids.

11. International Standards Organisation ISO 5509, 1978. Preparation of methyl esters of fatty acids.

12. International Olive Oil Council (1990). Meeting of chemists to study methods of analysis for olive oils and olive-pomace oils. T.20/Doc. No. 18-1.30 October 1990.

13. Bonaga, G, Antonelli, A., Conte, L.S. & Lercker, G. (1986). 'Simultaneous determination of the absolute content of sterols and triterpenic dialcohols in edible oils.' Riv. ital. Sost. gras., 58, (11) 583-90.

14. Kochhar, S.P. (1983). 'Influence of processing on sterols of edible vegetable oils.' Prog. Lipid Res, 22, 161-88.

15. Kochhar, S.P., Jawad, I.M. & Rosseli, J.B. (1982). 'Studies on soyabean processing' Leatherhead Fd RA Res. Rep. No. 385.

16. Fedeli, E, Gasparoli, A. & Daghetta, A. (1983). 'Change in vegetable oils induced by heating in the presence of air. Note VI. Interaction between soyabean oil and olive oil triglycerides and starch.' Riv. ital. Sost. gras., 60 (5), 257-66.

17. Fedeli, E. & Tiscornia, E. (1990). Document CO1-WG 2-90. 'Collation of data for aliphatic alcohols, erythrodiol and waxes in olive oils.'

18. Joly, F. (1984). 'Donnés Caractéristiques des Principanx Corps Gras Alimentaries Consommés en B.' Institut des Corps Gras, Paris.

19. International Olive Oil Council (1987). International Trade Standard Applying to Olive Oils and Olive-pomace Oils. Document CO1/T. 15/NC no. I/Ref. 1.19. Feb. 1987.

20. Gattuso, A.M., Fazio, G. & Cilluffo, V. (1982). 'Investigation on the autoxidation of virgin olive

oils.' Riv. ital. Sost. gras, 59 (8), 383-93.

21. British Standards Institution (1980). British Standard 684 Section 2.34: 1980. Preparation of methyl esters of fatty acids. Section 2.35: 1980. Analysis by gas chromatography of methyl esters of fatty acids.

22. King, B. & Sibley, I. (1984). 'Authenticity of edible vegetable oils and fats. Part II. Palm oil and palm oil fractions.' Leatherhead Fd RA Res. Rep. No. 462.

23a. Paganuzzi, V. (1980). 'Distribution of alcoholic components of the unsaponifiable matter within the olive drupe. Note V. Aliphatic alcohols.' Riv. ital. Sost. gras., 57 (7), 341-5.

23b. Paganuzzi, V. (1981). 'Influence of origin and conservation state on the triterpene dialcohols relative content of untreated olive oils. II. Pressed oils from Tunisia.' Rev. frcps Gras., 28 (1), 9-12.

24. Paganuzzi, V. (1982). 'Influence of origin and conservation state on the triterpene dialcohols relative content of untreated olive oils. III. Pressed oils from Greece.' Fette Seifen Anstrichmittel, 84 (3), 115-20.

25. Passaloglou-Emmanouilidou, S. (1990). 'A comparative study of UV spectrophotometric methods for detection of olive oil adulteration by refined oils.' Z. Lebensm. Unters. Forsch, 191, 132-4.

26. Slover, H.T., Thompson, R.H. Jr., & Merola, G.V. (1983). 'Determination of tocopherols and sterols by capillary gas chromatography.' J. Am. Oil Chem. Soc., 60 (8), 1524-8.

27. Itoh, T., Tamura, T. & Matsumoto, T. (1973). 'Sterol composition of 19 vegetable oils.' J. Am. Oil Chem. Soc. 50 (4), 122-5.

28. Manzone, A.M., Boniforte, L. & Monacelli, R. (1971). 'On the sterol composition of olive oil.' 48 (8), 416-20

29. British Standards Institution (1990). British Standard 684 Section 1.15. Determination of ultraviolet absorbance.

30. British Standards Institution (1988). British Standard 684 Section 2.10: 1988. Determination of acidity.

31. American Oil Chemists Society (1989). AOCS Official Method Ca 5a-40 (1989). Free Fatty Acids. Official Methods and Recommended Practices of the American Oil Chemists Society. 4th Edition Vol. 1, 1990.

32. Slack, P.T. (1987). 'Analytical Methods Manual.' Leatherhead Food RA.

33. International Union of Pure and Applied Chemistry (1987a). IUPAC Method No. 2.201. 'Determination of the Acid Value and the Acidity.' Standard Methods for the Analysis Oils, Fats and Derivatives. 7th Ed. Blackwell.

34. International Standards Organisation ISO 660, 1992. Determination of acidity in oils and fats.

35a. International Standards Organisation ISO 660, 1983. Determination of acidity in oils and fats.

35b. International Standards Organisation ISO 660, 1989. Determination of acidity in oils and fats.

36. British Standards Institution (1987). British Standard 684 Section 2.14. Determination of peroxide value.

37. International Olive Oil Council (1985). Draft International Trade Standard applying to olive oils and olive residue oils. CCI/TIS/NC No. 1.15 February 1985.

38. Wessels, H. (1992) 'Olive oil types and their characteristics - Regulation of the EC' Fat. Sci. Technol. 94 (4), 130-7.

ANNEX 5 - Analytical Data

TABLE I

Two-Position Fatty Acid Profile of Olive Oils (See Note 1)

(%m/m)

Oil type:	EV	EV	EV	EV	O	EV	EV	EV	O	O	O	O	O	O	EV	EV
(Note 4)																
Sample no.:	B1	B2	B3	B4	B5	E1	E2	E3	E4	1	2	3	4	5	C1	C2
Fatty acids (Note 3)																
C16:0	0.5	0.5	0.6	0.5	0.8	0.5	0.5	0.7	0.7	1.3	0.8	0.8	0.9	0.7	0.4	0.5
C16:1	0.5	0.6	1.2	0.4	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.4	0.4	0.5
C17:1	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C18:0	NS	0.03	0.03	0.06	0.1	0.01	0.06	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.03	0.1
C18:1	83.8	91.2	76.9	89.2	87.4	87.3	83.3	85.3	85.9	86.6	87.8	86.1	87.4	87.9	90.2	89.3
C18:2	13.9	6.8	20.4	8.7	10.1	10.6	14.5	12.1	11.7	10.2	9.8	11.4	9.9	9.7	8.0	8.7
C18:3	1.1	0.7	0.8	1.0	0.8	0.9	0.90	0.9	0.9	0.9	0.9	1.0	0.9	0.8	0.8	0.8
others	0.1	0.07	0.0	0.04	0.2	0.0	0.04	0.1	0.0	0.2	0.0	0.0	0.1	0.2	0.1	0.0
Total																
(C16:0 + C18:0)	0.5	0.53	0.63	0.56	0.90	0.51	0.56	0.8	0.9	1.5	0.9	1.0	1.1	0.9	0.43	0.6
Enrichment factor																
C16:0	0.05	0.05	0.04	0.06	0.08	0.05	0.05	0.07	0.07	0.13	0.08	0.08	0.09	0.07	0.05	0.04
C18:0	-	<0.01	0.01	0.03	0.03	<0.01	0.04	0.03	0.06	0.06	0.03	0.06	0.06	0.06	0.01	0.03
C18:1	1.14	1.17	1.18	1.14	1.16	1.14	1.15	1.16	1.14	1.14	1.14	1.13	1.13	1.15	1.17	1.19
C18:2	1.35	1.42	1.46	1.40	1.26	1.43	1.37	1.33	1.33	1.31	1.32	1.41	1.36	1.29	1.25	1.23
C18:3	1.38	1.17	1.33	1.67	1.14	1.50	1.50	1.13	1.29	1.29	1.29	1.43	1.29	1.33	1.67	1.00

Notes

Note 1 Ranges and means have not been calculated.

Note 2 A and B refer to satisfactory replications of samples

Note 3 In view of the importance attached to the C18:0 peak (see text) values of less than 0.1% have been measured to the nearest 0.01%. Where resolution was not achieved, the term NS is used in the table. Where this has occurred, values below 0.1% would be expected. The value for (C16:0 + C18:0) does not include a C18:0 contribution in these cases.

Note 4 EV=oils labelled as extra virgin O=oils labelled as pure

TABLE I (contd.)
Two-Position Fatty Acid Profile of Olive Oils (See Note 1)
(%m/m)

Oil type:	EV	EV	EV	O	O	EV	EV	EV	EV	EV	O	EV	EV	EV	EV	EV	O	O	EV	EV	EV
(Note 4)																					
Sample no.:	C3a*	C3b*	C4	C5a*	C5b*	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	D1
Fatty acids (Note 3)																					
C16:0	0.6	0.5	0.4	0.8	0.5	0.7	0.7	0.6	0.5	0.5	0.8	0.5	0.5	0.6	0.6	0.5	0.6	0.9	0.5	0.5	2.1
C16:1	0.5	0.5	0.4	0.5	0.6	0.7	0.5	0.7	0.7	0.5	0.4	0.5	0.7	0.7	0.4	0.5	0.6	0.7	0.4	0.5	0.5
C17:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
C18:0	0.05	0.03	NS	0.2	0.05	0.05	0.06	0.03	0.04	NS	0.2	0.03	0.05	0.04	NS	NS	0.06	0.1	0.02	NS	0.6
C18:1	89.1	89.3	89.3	83.3	83.2	85.8	89.2	86.5	87.5	88.2	90.6	87.8	87.5	85.1	88.0	87.9	83.9	86.2	89.1	90.6	85.3
C18:2	8.6	8.7	8.8	14.0	14.4	11.9	8.5	11.1	10.3	9.7	7.0	10.2	10.4	12.6	9.9	10.1	13.9	10.9	9.1	7.4	10.1
C18:3	0.9	0.9	0.9	1.0	1.0	0.8	0.9	0.9	0.8	0.9	0.7	0.7	0.7	0.8	0.9	0.8	0.8	0.9	0.8	0.8	0.8
others	0.15	0.0	0.1	0.1	0.2	0.0	0.04	0.0	0.06	0.1	0.2	0.17	0.05	0.06	0.1	0.1	0.04	0	00.0	0.1	0.5
Total																					
(C16:0 + C18:0)	0.65	0.53	0.4	1.0	0.65	0.75	0.76	0.63	0.54	0.5	1.0	0.53	0.55	0.64	0.6	0.5	0.66	1.0	0.52	0.50	2.7
Enrichment factor																					
C16:0	0.05	0.04	0.04	0.07	0.04	0.06	0.07	0.05	0.05	0.05	0.09	0.05	0.04	0.05	0.06	0.05	0.05	0.08	0.05	0.05	0.18
C18:0	0.02	0.01	0.04	0.08	0.02	0.02	0.03	0.01	0.01	0.03	0.06	0.01	0.01	0.01	0.04	0.03	0.02	0.04	<0.01	0.03	0.2
C18:1	1.18	1.18	1.17	1.15	1.15	1.15	1.11	1.17	1.17	1.14	1.14	1.16	1.18	1.18	1.14	1.16	1.19	1.17	1.16	1.14	1.16
C18:2	1.23	1.24	1.33	1.31	1.35	1.38	1.39	1.32	1.37	1.39	1.40	1.36	1.37	1.31	1.38	1.33	1.28	1.3	1.32	1.42	1.2
C18:3	1.29	1.29	1.5	1.25	1.25	1.33	1.5	1.29	1.33	1.29	1.17	1.17	1.17	1.33	1.14	1.14	1.14	1.29	1.14	1.33	1.33

Notes

Note 1 Ranges and means have not been calculated.

Note 2 A and B refer to satisfactory replications of samples

Note 3 In view of the importance attached to the C18:0 peak (see text) values of less than 0.1% have been measured to the nearest 0.01%. Where resolution was not achieved, the term NS is used in the table. Where this has occurred, values below 0.1% would be expected. The value for (C16:0 + C18:0) does not include a C18:0 contribution in these cases.

Note 4 EV=oils labelled as extra virgin O=oils labelled as pure

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TABLE 2
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	A-1	A-1	A-1	A-2	A-2	A-2	A-2	A-2	A-3	A-3	A-3	A-4	A-4	A-5	A-5	A-5
Analyst	LH1	LH2	LH2	LH2	LH2	LH2	NOR	NOR	LH1	LH2	LH2	LH2	LH2	LH1	LH2	LH2
Replicate	A	B	C	B	C	D	E	F	A	B	C	B	C	A	B	C
Cholesterol	0.4	0.2	0.3	0.4	0.5	0.6	0.7	NR	0.5	0.2	0.3	0.2	0.4	0.4	0.5	0.7
Brassicasterol	0.1	0.4	0.1	ND	ND	ND	ND	ND	0.1	ND	ND	0.3	ND	0.2	0.4	0.7
2,4-methylene cholesterol	0.2	0.2	0.1	NR	NR	NR	NR	NR	NR	0.1	0.1	NR	0.1	0.1	NR	NR
Campesterol	3.4	3.3	3.4	2.8	3.0	3.2	4.1	4.1	3.7	3.6	3.9	4.1	3.8	4.4	4.4	4.2
Campestanol	0.5	1.7	1.3	ND	ND	ND	0.4	0.2	0.6	1.7	1.6	1.1	1.5	0.5	1.4	1.5
Stigmasterol	1.1	0.7	0.8	0.8	0.8	0.9	0.7	0.7	0.8	0.8	0.9	1.0	1.0	1.2	1.2	1.1
Delta-7-Campesterol	ND	ND	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND
Delta - 5,23-Stigmastadienol	0.1	0.1	0.1	ND	ND	ND	1.1	0.9	0.1	0.1	0.1	0.1	0.1	0.2	ND	0.1
Chlerosterol	0.8	0.8	0.8	0.6	0.8	0.7			0.7	0.6	0.5	0.9	0.8	0.8	0.7	0.8
Beta-Sitosterol	79.8	78.1	79.1	77.0	77.8	78.5	77.2	78.5	80.0	78.2	78.1	82.0	82.4	81.7	79.7	79.8
Sitostanol	NR	0.6	NR	1.2	0.6	NR	NR	NR	0.5	NR	0.6	NR	0.8	NR	0.7	NR
Delta-5-Avenasterol	12.1	12.3	12.5	15.5	15.1	13.7	14.3	13.9	11.6	11.8	12.0	7.5	7.3	9.3	9.9	9.2
Delta-5,24-Stigmastadienol	0.5	0.8	0.8	0.4	0.6	1.3	0.7	0.6	0.6	1.4	0.7	1.4	0.6	0.4	0.3	0.7
Delta-7-Stigmastenol	0.6	0.2	0.2	1.0	0.5	1.1	0.3	0.4	0.2	0.8	0.5	0.9	0.6	0.3	0.4	0.7
Delta-7-Avenasterol	0.4	0.6	0.4	0.3	0.3	ND	0.4	0.6	0.6	0.7	0.7	0.5	0.6	0.4	0.4	0.5
Apparent Beta-Sitosterol	93.4	92.7	93.4	94.7	95.0	94.2	93.1	93.9	93.6	92.0	92.0	91.9	92.1	92.4	91.4	90.7

TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	A-6	A-6	A-6	A-6	A-7	A-7	A-7	A-8	A-8	A-8	A-9	A-9	A-9	A-10	A-10	A-10
Analyst	LH1	LH3	LH3	LH2	LH1	LH2	LH2	LH1	LH3	LH3	LH1	LH3	LH3	LH1	LH3	LH3
Replicate	A	B	C	D	A	B	C	A	B	C	A	B	C	A	B	C
Cholesterol	0.8	0.1	0.1	0.4	0.3	0.2	0.2	0.5	0.2	0.1	1.4	0.2	0.2	0.5	0.1	0.1
Brassicasterol	0.6	0.1	0.1	0.5	0.3	ND	0.1	0.1	0.1	0.1	0.4	ND	ND	0.1	0.1	ND
2,4-methylene cholesterol	NR	0.1	0.2	0.1	0.1	NR	NR	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Campesterol	6.2	4.0	4.0	4.5	3.7	4.2	4.2	3.9	3.6	3.6	4.6	3.0	3.0	3.6	2.9	2.9
Campestanol	0.4	0.4	0.3	1.3	ND	1.1	1.1	0.5	0.3	0.4	0.4	0.3	0.4	0.6	0.4	0.4
Stigmasterol	1.0	1.1	1.0	1.1	1.0	1.0	1.0	1.1	0.9	1.0	0.8	0.8	0.8	1.6	0.9	1.0
Delta-7-Campesterol	ND	0.3	0.1	0.1	0.2	ND	ND	ND	0.1	0.4	ND	0.1	0.2	ND	0.1	ND
Delta - 5,23-Stigmastadienol	0.3	0.1	ND	ND	ND	ND	0.1	0.1	ND	ND	0.2	ND	ND	0.1	0.1	0.1
Chlerosterol	0.8	0.8	0.9	0.7	0.5	0.7	0.9	0.9	0.8	0.8	NR	0.9	0.8	0.8	0.8	0.7
Beta-Sitosterol	82.4	86.4	86.6	82.4	86.0	83.9	82.8	84.0	85.5	85.4	76.2	78.4	78.4	79.4	83.1	83.6
Sitostanol	0.3	0.2	0.1	0.4	NR	NR	0.5	0.4	0.2	0.1	NR	0.1	0.1	0.9	0.3	0.3
Delta-5-Avenasterol	6.6	5.8	5.9	6.6	7.2	7.7	7.9	6.9	6.8	6.8	14.5	14.7	14.7	10.8	10.0	9.4
Delta-5,24-Stigmastadienol	0.3	0.2	0.6	1.3	0.2	0.6	0.7	0.3	0.5	0.4	0.5	0.6	0.6	0.5	0.4	0.7
Delta-7-Stigmastenol	ND	0.2	ND	0.3	0.3	0.2	0.1	0.9	0.4	0.3	0.8	0.3	0.2	0.5	0.3	0.3
Delta-7-Avenasterol	0.3	0.2	0.1	0.3	0.2	0.4	0.4	0.3	0.5	0.4	0.2	0.5	0.5	0.5	0.4	0.4
Apparent Beta-Sitosterol	90.8	93.5	94.1	91.4	94.1	92.9	92.9	92.6	93.9	93.7	91.3	94.8	94.7	92.6	94.4	94.8

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TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	A-11	A-11	A-11	A-12	A-12	A-12	A-13	A-13	A-13	A-13	A-14	A-14	A-14	A-15	A-15
Analyst	LH2	LH2	NOR	LH1	LH3	LH3	LH1	LH2	LH2	LH2	LH1	LH3	LH3	LH2	LH2
Replicate	B	C	D	A	B	C	A	B	C	D	A	B	C	B	C
Cholesterol	0.3	0.4	0.2	0.3	0.4	0.3	1.3	0.5	0.4	0.4	0.3	0.2	0.2	ND	0.2
Brassicasterol	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.3	0.2	0.2	ND	ND	ND	0.1	0.2
24-methylene cholesterol	NR	NR	NR	0.3	0.2	0.1	NR	0.4	0.2	0.2	0.1	0.2	0.2	NR	NR
Campesterol	4.2	4.2	4.4	3.8	3.4	3.5	3.8	3.6	4.1	3.3	3.3	3.0	3.0	4.4	4.4
Campestanol	1.1	0.8	0.2	0.7	0.4	0.5	0.5	2.2	1.4	1.8	0.5	0.4	0.5	0.7	1.6
Stigmasterol	1.0	1.0	1.0	2.6	1.3	1.3	2.7	2.6	1.5	2.5	1.0	1.1	1.1	1.0	1.2
Delta-7-Campesterol	ND	ND	ND	0.2	0.2	ND	0.2	ND	0.4	ND	ND	ND	ND	ND	0.1
Delta - 5,23-Stigmastadienol	ND	ND	0.9	1.2	0.8	0.8	1.3	0.9	1.0	0.9	0.4	0.1	0.1	0.1	0.1
Chlerosterol	1.0	0.8		1.2	1.0	1.1	1.1	1.3	1.3	1.3	0.8	0.8	0.8	0.9	1.0
Beta-Sitosterol	82.8	82.8	84.0	80.2	85.6	85.3	80.2	79.0	80.3	81.4	83.4	84.7	85.2	84.3	82.5
Sitostanol	NR	0.5	NR	1.3	0.7	0.7	NR	1.6	1.5	1.5	NR	0.4	0.4	0.4	NR
Delta-5-Avenasterol	8.4	8.2	7.4	5.0	3.4	3.5	4.9	4.8	5.0	4.7	8.3	7.5	7.3	6.9	7.1
Delta-5,24-Stigmastadienol	0.5	0.6	0.8	2.0	1.6	1.8	1.8	1.8	1.9	1.5	0.8	0.8	0.6	0.7	0.8
Delta-7-Stigmastenol	0.2	0.2	0.4	0.9	0.5	0.5	1.5	0.6	0.4	0.2	0.9	0.3	0.2	0.2	0.4
Delta-7-Avenasterol	0.4	0.3	0.6	0.2	0.3	0.4	0.4	0.4	0.4	0.2	0.2	0.5	0.4	0.3	0.4
Apparent Beta-Sitosterol	92.7	92.9	93.1	90.9	93.1	93.1	89.3	89.4	91.0	91.1	93.7	94.4	94.4	93.2	91.4

TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	C-1	C-1	C-2	C-2	C-2	C-3	C-3	C-3	C-4	C-4	C-5	C-5	C-5
Analyst	LH2	LH2	LH2	NOR	NOR	LH1	LH2	LH2	LH1	LH2	LH1	LH2	LH2
Replicate	B	C	B	D	E	A	B	C	A	D	A	B	C
Cholesterol	0.3	0.4	0.3	0.2	0.3	0.5	0.3	0.3	0.1	0.6	0.5	0.3	0.5
Brassicasterol	0.1	0.1	ND	ND	NR	0.1	0.1	ND	0.5	0.6	ND	ND	0.3
24-methylene cholesterol	NR	0.1	0.1	NR	NR	NR	0.1	0.1	0.2	NR	NR	NR	NR
Campesterol	3.7	3.5	3.6	4.1	4.1	4.2	3.4	3.4	4.6	3.9	5.3	5.3	5.2
Campestanol	1.8	1.4	1.4	0.2	0.2	0.5	1.2	1.4	0.3	2.0	0.4	1.7	1.5
Stigmasterol	0.6	0.5	0.7	0.8	1.7	0.9	0.9	0.9	0.7	0.8	1.7	1.8	1.8
Delta-7-Campesterol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Delta - 5,23-Stigmastadienol	ND	ND	0.1	} 0.9	1.3	ND	0.1	ND	ND	ND	0.3	0.1	0.2
Chlerosterol	0.9	0.9	0.9			0.5	0.9	0.9	0.1	0.9	0.5	0.6	0.9
Beta-Sitosterol	70.0	70.0	74.8	74.4	76.1	77.1	76.2	73.8	72.1	71.7	79.0	78.0	78.1
Sitostanol	ND	0.5	NR	NR	NR	0.1	NR	NR	NR	0.8	1.4	1.6	1.2
Delta-5-Avenasterol	21.4	21.0	16.8	16.8	15.2	14.8	14.7	16.0	17.5	17.3	10.0	9.1	8.8
Delta-5,24-Stigmastadienol	0.6	0.9	0.8	0.9	0.5	0.5	1.0	1.7	2.0	0.9	0.6	0.4	0.5
Delta-7-Stigmastenol	0.2	0.4	0.2	0.7	0.2	0.3	0.6	0.9	1.6	0.2	0.2	0.6	0.5
Delta-7-Avenasterol	0.4	0.3	0.3	1.0	0.4	0.5	0.5	0.6	0.3	0.3	0.2	0.5	0.5
Apparent Beta-Sitosterol	92.9	93.3	93.4	93.0	93.1	93.0	92.9	92.4	91.7	91.6	91.6	89.8	89.6

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TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	D-1	D-1	D-1	Blend-1	Blend-1	Blend-1	Blend-1	Blend-2	Blend-2	Blend-2	Blend-2
Analyst	LH1	LH2	LH2	LH1	LH2	NOR	NOR	LH1	LH2	LH2	LH2
Replicate	A	B	C	A	D	E	F	A	B	C	D
Cholesterol	0.4	0.4	0.4	0.5	0.4	NR	NR	0.3	0.4	0.2	0.7
Brassicasterol	0.7	0.1	0.2	0.2	0.4	0.1	0.2	0.2	0.4	0.3	0.5
24-methylene cholesterol	NR	0.1	NR	NR	0.3	NR	NR	0.1	NR	NR	NR
Campesterol	4.8	3.6	3.9	4.1	4.2	4.5	5.0	4.9	4.8	4.8	4.4
Campestanol	0.1	1.4	1.5	0.5	1.5	0.1	0.2	0.6	1.3	1.0	1.6
Stigmasterol	0.7	1.0	1.0	1.0	1.6	1.4	2.0	1.5	1.5	1.5	1.5
Delta-7-Campesterol	ND	0.1	ND	0.2	ND	ND	ND	0.1	0.1	ND	1.0
Delta - 5,23-Stigmastadienol	ND	0.1	ND	0.5	0.9	} 2.2	2.6	1.1	0.2	ND	0.8
Chlerosterol	0.5	1.1	0.8	0.5	1.2			1.1	1.0	1.1	1.5
Beta-Sitosterol	76.6	74.1	75.0	86.0	84.0	85.3	85.1	83.8	85.6	86.0	83.1
Sitostanol	0.7	NR	0.7	NR	NR	NR	NR	0.6	NR	NR	0.7
Delta-5-Avenasterol	15.0	16.3	15.9	2.6	3.0	2.2	2.6	3.4	2.8	3.3	3.3
Delta-5,24-Stigmastadienol	0.3	0.8	0.4	1.1	2.0	2.6	1.2	1.3	1.4	1.5	NR
Delta-7-Stigmastenol	ND	0.5	0.4	2.7	0.3	0.7	0.4	0.8	0.3	0.2	0.7
Delta-7-Avenasterol	0.2	0.4	0.2	0.1	0.2	0.6	0.3	0.2	0.2	0.1	0.2
Apparent Beta-Sitosterol	93.1	92.3	92.8	90.7	91.1	92.4	91.8	91.2	91.1	91.8	89.3

TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	Blend-3	Blend-3	Blend-4	Blend-4	Blend-5	Blend-5	E-1	E-1	E-1	E-1	E-2	E-2	E-2	E-2
Analyst	LH2	LH2	LH2	LH2	LH2	LH2	LH1	LH2	LH2	LH2	LH1	LH2	LH2	LH2
Replicate	B	C	B	C	B	C	A	B	C	D	A	B	C	D
Cholesterol	0.4	0.9	0.4	0.4	0.5	0.5	0.7	0.4	0.3	0.3	0.4	0.2	0.2	0.3
Brassicasterol	0.5	ND	0.4	0.8	ND	0.2	0.1	0.2	0.1	ND	ND	0.3	0.5	ND
24-methylene cholesterol	NR	NR	NR	NR	NR	0.2	0.1	NR	0.1	NR	NR	NR	NR	NR
Campesterol	6.5	6.3	5.2	5.0	4.1	3.6	4.0	8.2	3.8	3.6	3.3	2.9	3.0	3.1
Campestanol	1.1	1.1	1.2	1.3	1.4	1.8	0.7	1.7	1.8	1.0	0.5	1.0	1.4	1.7
Stigmasterol	2.3	2.1	1.6	1.6	1.4	1.6	1.1	2.3	0.9	0.9	0.8	0.7	0.7	0.7
Delta-7-Campesterol	ND	0.3	ND	0.2	ND	ND	ND	ND	0.1	ND	ND	ND	ND	ND
Delta - 5,23-Stigmastadienol	0.3	0.9	0.7	1.1	0.7	0.8	0.1	ND	0.1	ND	0.1	ND	0.1	ND
Chlerosterol	1.1	1.1	1.0	1.1	1.1	1.0	0.7	0.7	0.9	0.9	0.7	ND	0.8	0.7
Beta-Sitosterol	83.1	79.3	84.1	82.2	84.8	85.0	82.2	76.2	82.1	84.1	88.0	88.2	85.7	86.4
Sitostanol	NR	NR	NR	NR	0.9	0.9	0.3	1.4	NR	NR	0.4	0.4	0.5	0.7
Delta-5-Avenasterol	3.0	3.1	3.3	3.4	2.9	2.8	8.1	7.3	8.2	8.1	4.1	3.9	4.0	4.1
Delta-5,24-Stigmastadienol	1.0	3.8	1.5	2.2	2.0	1.4	0.4	0.7	0.6	0.6	0.5	1.2	1.7	0.7
Delta-7-Stigmastenol	0.4	0.8	0.4	0.5	ND	0.2	0.8	0.4	0.4	0.2	0.4	1.0	0.8	0.8
Delta-7-Avenasterol	0.3	0.3	0.2	0.2	0.2	ND	0.7	0.5	0.6	0.3	0.8	0.2	0.6	0.8
Apparent Beta-Sitosterol	88.5	88.2	90.6	90.1	92.4	91.9	91.8	86.2	92.0	93.7	93.8	93.6	92.7	92.6

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TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	E-3	E-3	E-3	E-4	E-4	E-4	E-4	B-1	B-1	B-2	B-2	B-2
Analyst	LH1	LH2	LH2	LH2	LH2	NOR	NOR	LH2	LH2	LH1	LH2	LH2
Replicate	A	B	C	B	C	D	E	B	C	A	B	C
Cholesterol	0.3	0.4	0.3	0.1	0.3	ND	ND	0.5	0.6	0.6	0.4	0.3
Brassicasterol	0.6	0.2	ND	0.6	0.8	ND	ND	0.1	ND	0.1	0.1	ND
24-methylene cholesterol	NR	NR	NR	NR	NR	NR	NR	ND	0.1	0.2	0.1	NR
Campesterol	3.1	2.8	2.8	3.1	3.3	3.5	3.7	3.2	3.4	4.1	3.3	3.7
Campestanol	NR	1.1	1.1	1.1	1.4	0.1	0.2	0.9	1.2	0.4	0.9	1.7
Stigmasterol	0.4	1.1	1.0	0.9	1.0	0.9	0.9	1.3	1.4	1.0	1.0	0.8
Delta-7-Campesterol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	1.2
Delta - 5,23-Stigmastadienol	ND	ND	ND	1.4	1.4	} 3.1	3.1	0.1	0.1	ND	ND	ND
Chlerosterol	0.6	0.6	0.7	1.4	1.3			0.8	0.9	1.0	0.8	0.8
Beta-Sitosterol	85.0	86.2	85.4	85.5	84.3	86.8	86.9	83.8	83.4	84.1	85.0	83.6
Sitostanol	0.7	0.5	0.8	NR	NR	NR	NR	0.8	NR	0.8	0.6	0.8
Delta-5-Avenasterol	8.2	6.1	6.4	2.6	2.7	2.5	2.5	7.5	7.2	6.2	6.0	5.9
Delta-5,24-Stigmastadienol	0.2	0.4	} 1.1	3.1	3.2	2.4	2.2	0.5	0.5	0.3	0.4	0.4
Delta-7-Stigmastenol	0.8	0.3		ND	ND	0.4	0.3	0.5	0.6	0.8	0.9	0.4
Delta-7-Avenasterol	0.1	0.3	0.4	0.2	0.3	0.3	0.4	ND	0.6	0.4	0.4	0.4
Apparent Beta-Sitosterol	94.9	93.8	93.3	94.0	92.9	94.8	94.6	93.5	92.1	92.4	92.8	91.5

TABLE 2 (contd.)
Concentrations of individual desmethyl sterols
(% of total fraction)

Sample	B-3	B-3	B-3	B-3	B-3	B-4	B-4	B-4	B-4	B-4	B-4	B-5	B-5	B-5	B-5	B-5
Analyst	LH1	LH2	LH3	LH3	LH2	LH1	LH3	LH3	NOR	NOR	LH2	LH1	LH2	LH2	NOR	NOR
Replicate	A	B	C	D	E	A	B	C	D	E	H	A	B	C	D	E
Cholesterol	0.6	0.5	0.1	0.1	0.4	0.3	0.2	0.1	0.6	0.9	0.3	0.2	0.3	0.3	NR	NR
Brassicasterol	0.2	ND	ND	ND	ND	0.1	ND	ND	ND	ND	0.3	ND	ND	ND	ND	ND
24-methylene cholesterol	0.1	ND	0.1	0.1	NR	0.1	0.2	0.2	NR	NR	0.2	NR	NR	0.1	NR	NR
Campesterol	3.5	3.4	3.0	3.0	3.2	3.9	3.3	3.2	4.2	4.3	4.4	3.9	3.5	4.1	4.3	4.4
Campestanol	0.5	1.0	0.3	0.3	0.8	0.5	0.3	0.3	NR	0.1	1.0	0.4	2.3	2.0	0.3	0.3
Stigmasterol	0.8	0.7	0.6	0.6	0.6	0.6	0.5	0.5	0.7	0.7	0.6	1.3	1.5	1.6	1.8	1.4
Delta-7-Campesterol	ND	ND	ND	>0.1	ND	ND	0.1	ND	ND	ND	0.3	0.4	ND	ND	ND	ND
Delta - 5,23-Stigmastadienol	0.1	0.1	ND	ND	ND	0.1	ND	ND	} 1.1	0.8	ND	0.5	0.6	0.7	} 1.8	2.1
Chlerosterol	0.8	0.8	0.9	0.9	0.8	0.8	0.9	0.9			0.5	0.6	1.0	1.0		
Beta-Sitosterol	82.2	82.0	83.6	83.6	81.8	83.7	85.0	85.5	84.8	83.4	81.4	85.6	82.6	83.1	86.0	84.5
Sitostanol	NR	0.6	0.2	0.1	1.0	0.4	0.1	0.1	NR	NR	0.9	1.0	1.5	1.1	NR	NR
Delta-5-Avenasterol	9.8	9.3	9.6	9.6	9.9	8.4	8.2	8.2	7.3	7.8	8.5	4.3	4.3	4.1	3.4	3.3
Delta-5,24-Stigmastadienol	0.5	0.6	0.6	0.6	0.5	0.3	0.4	0.4	0.2	0.7	0.6	1.3	1.6	1.5	1.3	1.6
Delta-7-Stigmastenol	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.2	0.4	0.5	0.8	0.4	0.3	0.2	0.5	1.9
Delta-7-Avenasterol	0.6	0.6	0.7	0.7	0.6	0.4	0.5	0.4	0.7	0.8	0.2	0.1	0.5	0.2	0.6	0.5
Apparent Beta-Sitosterol	93.4	93.4	94.9	94.8	94.0	93.7	94.6	95.1	93.4	92.7	91.9	93.3	91.6	91.5	92.5	91.5

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TABLE 3
Aliphatic Alcohol content of Extra Virgin Olive Oil (mg/kg)

Sample	A-1	A-1	A-2	A-2	A-2	A-2	A-2	A-2	A-3	A-3	A-4	A-4	A-4	A-5	A-5	A-5
Analyst	LH2	LH2	LH1	LH2	LH2	LH2	NOR	NOR	LH2	LH2	LH1	LH2	LH2	LH1	LH2	LH2
Replicate	B	C	A	B	C	D	E	F	B	C	A	B	C	A	B	C
Carbon Number																
C22	42	34	43	44	34	42	35	35	29	28	39	32	35	37	32	35
C23	ND	ND	7	8	NR	16	4	4	1	2	4	2	ND	6	1	1
C24	79	53	78	91	65	88	72	70	48	48	58	55	63	58	53	61
C25	6	4	7	20	6	28	6	6	4	5	NR	4	5	5	4	4
C26	95	48	77	106	82	98	81	79	60	58	57	71	73	59	58	68
C27	5	ND	5	4	3	4	3	3	3	3	ND	3	ND	NR	2	4
C28	11	12	6	30	20	27	27	26	20	12	ND	10	20	7	7	9
Total	238	151	223	303	210	303	228	223	164	156	158	177	196	172	157	182
C26/C24	1.20	0.90	0.99	1.16	1.26	1.11	1.13	1.13	1.25	1.21	0.98	1.29	1.16	1.02	1.09	1.11

Sample	A-7	A-7	A-8	A-8	A-9	A-9	A-10	A-10	A-11	A-11	A-11	A-11	A-11	A-14	A-14	A-15	A-15
Analyst	LH2	LH2	LH3	LH3	LH3	LH3	LH3	LH3	LH2	LH2	NOR	NOR	NOR	LH2	LH2	LH2	LH2
Replicate	B	C	B	C	B	C	B	C	B	C	D	E	F	B	C	B	C
Carbon Number																	
C22	41	34	32	32	119	44	27	27	35	35	36	32	33	22	23	53	49
C23	1	1	3	3	4	4	ND	3	ND	ND	3	3	3	2	2	ND	ND
C24	76	55	53	54	79	78	44	43	60	60	66	69	58	36	38	80	73
C25	5	4	4	4	4	6	ND	5	4	5	5	4	4	4	4	5	4
C26	57	40	53	56	74	71	50	51	47	48	61	57	49	43	46	49	41
C27	3	2	3	2	3	3	ND	3	3	4	3	3	3	3	3	2	3
C28	23	14	15	19	23	25	20	20	15	15	13	18	16	17	18	20	15
Total	206	150	163	170	306	231	141	152	164	167	187	186	166	127	134	209	185
C26/C24	0.75	0.73	1.00	1.04	0.94	0.91	1.14	1.19	0.78	0.80	0.92	0.83	0.85	1.19	1.21	0.61	0.56

+ Interfering peak observed which was not fully resolved from internal standard

* Outlier result

TABLE 3 (contd.)
Aliphatic Alcohol content of Extra Virgin Olive Oil (mg/kg)

Sample	B-1	B-1	B-2	B-2	B-2	B-3	B-3	B-3	B-4	B-4	B-4	B-4	B-4	B-4
Analyst	LH2	LH2	LH1	LH2	LH2	LH1	LH3	LH2	LH1	LH3	LH3	NOR	NOR	LH2
Replicate	B	C	A	B	C	A	C	E	A	B	C	D	E	F
Carbon Number														
C22	33	30	46	25	30	37	30	33	39	25	25	23	27	26
C23	ND	2	NR	2	2	4	3	6	3	2	2	<2	2	3
C24	42	47	60	34	47	57	54	60	47	44	44	43	53	48
C25	3	4	NR	3	4	5	4	5	8	3	2	3	4	3
C26	58	61	72	39	61	47	45	52	30	27	28	31	38	34
C27	1	ND	NR	ND	ND	1	2	3	NR	2	2	<2	5	4
C28	14	6	4	8	6	19	17	8	15	11	13	13	17	10
Total	151	150	182	111	150	170	155	167	142	115	116	113	146	128
C26/C24	1.38	1.30	1.20	1.15	1.30	0.82	0.83	0.87	0.64	0.61	0.64	0.72	0.72	0.71

Sample	B-4	B-4	B-4	C-1	C-1	C-2	C-2	C-2	C-2	C-2	C-3	C-3	C-4	C-4
Analyst	LH2	LH2	LH2	LH2	LH2	LH2	LH2	NOR	NOR	NOR	LH2	LH2	LH2	LH2
Replicate	G	H	I	B	C	B	C	D	E	F	B	C	B	C
Carbon Number														
C22	26	28	24	46	48	41	49	43	42	41	35	38	32	35
C23	5	ND	3	ND	1	ND	5	5	4	5	ND	ND	ND	ND
C24	48	39	48	86	103	86	97	103	93	94	73	86	70	73
C25	2	4	NR	6	8	5	7	8	8	7	6	8	10	7
C26	35	25	34	76	105	77	88	121	103	110	78	107	80	87
C27	4	NR	12	4	4	4	4	4	4	3	4	5	5	5
C28	12	8	10	19	30	19	19	33	27	29	24	37	27	27
Total	132	104	131	273	299	232	269	317	281	289	220	281	224	234
C26/C24	0.73	0.64	0.71	0.88	1.02	0.90	0.91	1.17	1.11	1.17	1.07	1.24	1.14	1.19

+ Interfering peak observed which was not fully resolved from internal standard
* Outlier result

TABLE 3 (contd.)
Aliphatic Alcohol content of Extra Virgin Olive Oil (mg/kg)

Sample	D-1	D-1	D-1	E-1	E-1	E-1	E-2	E-2	E-2	E-3	E-3	E-3
Analyst	LH1	LH2	LH2	LH2	LH2	LH2	LH2	LH2	LH2	LH1	LH2	LH2
Replicate	A	B	C	B	C	D	B	C	D	A	B	C
Carbon Number												
C22	58	63	52	31	35	33	45	46	47	15	15	17
C23	NR	1	ND	2	ND	8	2	2	ND	2	1	1
C24	83	96	72	42	46	44	54	57	54	23	29	30
C25	NR	7	6	3	4	4	4	6	4	2	8	3
C26	87	90	75	44	50	44	44	48	48	24	30	37
C27	2	4	5	2	4	2	3	3	3	2	3	3
C28	34	33	28	18	22	19	7	12	18	17	10	25
Total	264	294	238	142	161	154	159	174	174	85	96	116
C26/C24	1.05	0.94	1.04	1.05	1.09	1.00	0.81	0.84	0.89	1.04	1.03	1.23

+ Interfering peak observed which was not fully resolved from internal standard
* Outlier result

TABLE 3 (contd.)
Aliphatic Alcohol content of Olive Oil (mg/kg)

Sample	A-6	A-6	A-12	A-12	A-13	A-13	A-13	A-13	B-5	B-5	B-5	B-5	B-5	C-5	C-5	C-5
Analyst	LH3	LH2	LH3	LH3	LH2	LH2	LH2	LH2	LH2	LH2	NOR	NOR	NOR	LH2	LH2	LH2
Replicate	B	C	B	C	A	B	C	D	B	C	D	E	F	A	B	C
Carbon Number																
C22	40	42	74	76	40	49	46	50	60	62	53	54	55	48	40	42
C23	4	2	7	7	NR	ND	ND	ND	5	6	6	6	6	NR	ND	ND
C24	62	65	141	147	85	98	78	111	100	108	100	105	104	85	89	95
C25	4	5	9	10	1	9	7	10	8	9	7	9	8	13	8	8
C26	47	50	141	149	96	116	90	130	106	119	109	119	122	93	98	106
C27	3	2	6	7	1	8	6	9	7	3	4	5	6	2	5	5
C28	15	17	54	59	57	58	47	70	46	51	47	52	55	43	38	47
Total	175	183	432	455	280	338	274	380	332	358	326	350	356	284	278	303
C26/C24	0.76	0.77	1.00	1.01	1.13	1.18	1.15	1.17	1.06	1.10	1.09	1.13	1.17	1.09	1.10	1.12

Sample	E-4	E-4	E-4	E-4	E-4	Blend-1	Blend-1	Blend-1	Blend-2	Blend-2	Blend-3	Blend-3	Blend-3	Blend-4	Blend-4	Blend-4	Blend-5	Blend-5
Analyst	LH1	LH2	LH2	NOR	NOR	LH2	NOR	NOR	LH1	LH2	LH2	LH2	LH2	LH1	LH2	LH2	LH2	LH2
Replicate	A	B	C	D	E	B	C	D	A	B	A	B	C+	A	B	C+	B	C
Carbon Number																		
C22	57	41	44	41	39	51	47	51	56	46	37	40	50	63	68	64	70	65
C23	2	1	1	4	4	ND	5	5	8	ND	NR	ND	2	NR	1	1	2	2
C24	80	78	80	82	78	85	82	89	88	70	64	68	86	58	110	93	99	101
C25	2	5	6	6	5	6	6	6	7	5	4	5	4	NR	8	6	7	7
C26	82	89	91	99	93	85	89	103	93	61	70	71	94	70	110	88	84	99
C27	5	4	5	6	7	6	4	6	NR	6	2	3	7	10	7	5	5	12
C28	39	9	11	37	29	33	37	47	11	20	31	26	42	40	46	34	28	36
Total	267	227	238	275	255	266	270	307	263	208	208	213	285	241	350	291	295	322
C26/C24	1.03	1.14	1.14	1.21	1.19	1.00	1.09	1.16	1.06	0.87	1.09	1.04	1.09	1.21	1.00	0.95	0.85	0.98

+ Interfering peak observed which was not fully resolved from internal standard

* Outlier result

TABLE 4
Extra Virgin Olive Oil - Specific Extinction data

Code	$E^{1\%}_{1cm}$ 232 nm	λmax at ca. 270 nm(a)	$E^{1\%}_{1cm}$ c,at λ max ca. 270 nm	ΔE
A-1	2.34	-	0.24	-
A-2	1.62	-	0.14	-
A-3	2.21	-	0.17	-
A-4	1.75	-	0.18	-
A-5	2.52	267.0	0.24	0.012
A-7	2.28	-	0.19	-
A-8	2.37	-	0.22	-
A-9	2.63	268.2	0.27	0.015
A-10	2.04	-	0.21	0.008
A-11	2.42	-	0.18	0.005
A-14	2.18	-	0.20	0.006
A-15	1.77	-	0.15	0.004
B-1	2.51	-	0.22	0.009
B-2	1.72	-	0.13	-
B-3	2.40	-	0.18	-
B-4	1.93	267.8	0.22	0.006
C-1	2.12	-	0.21	0.008
C-2	2.07	-	0.25	0.010
C-3	2.32	-	0.20	0.003
C-4	3.06	-	0.19	0.002
D-1	3.23	267.8	0.29	0.010
E-1	2.07	-	0.17	0.005
E-2	2.22	-	0.13	0.006
E-3	2.20	-	0.17	

(a) Where λ max not given, but λ E has been calculated, value at 270 nm used, because no maximum obtained.

TABLE 5
Olive Oil - Specific Extinction data

Code	$E^{1\%}_{1cm}$ 232 nm	λ max at ca. 270 nm(a)	$E^{1\%}_{1cm}$ at λ max ca. 270 nm	ΔE
A-6	1.87	-	0.15	0.002
A-12	3.87	267.6	0.34	0.039
A-13	2.89	267.4	0.77	0.070
B-5	3.47	267.2	0.60	0.044
C-5	2.50	268.0	0.73	0.078
E-4	3.54	267.0	0.53	0.043
Produce of more than one country				
1	2.31	267.2	0.53	0.054
2	2.35	266.8	0.49	0.038
3	2.44	267.8	0.61	0.073
4	2.58	267.6	0.49	0.043
5	2.44	267.5	0.41	0.032

TABLE 6
Specific Extinction data - after alumina treatment

Code	Oil Type	$E_{1cm}^{1\%}$ at 270 nm
D-1	Extra virgin	0.09
A-9	Extra virgin	0.09
A-13	Pure Olive oil	0.35
C-2	Extra virgin	0.09

TABLE 7
Extra Virgin Olive Oil - Oil Quality Tests

Code	Best before date	Acidity calc. as FFA (% as oleic)	Acidity calc. as acid value (mg KOH/g fat)	Peroxide Value (Meq/kg)
A-1	April 1991	0.58	1.15	11.4
A-2	May 1992	0.21	0.42	14.0
A-3	December 1991	0.34	0.68	11.3
A-4	July 1991	0.80	1.58	15.1
A-5	July 1991	0.66	1.32	16.1
A-7	July 1991	0.91	1.81	16.3
A-8	October 1991	0.81	1.61	15.3
A-9	January 1992	0.80	1.59	13.5
A-10	July 1991	0.50	0.99	12.4
A-11	September 1991	0.89	1.76	15.3
A-14	not declared	0.26	0.51	9.7
A-15	May 1991	0.93	1.85	10.1
B-1	August 1991	0.78	1.56	17.3
B-2	September 1991	0.89	1.76	14.7
B-3	not declared	0.79	1.57	14.4
B-4	June 1991	0.27	0.54	17.7
C-1	July 1992	0.58	1.15	13.0
C-2	not declared	0.68	1.34	16.0
C-3	April 1991	1.07	2.13	15.0
C-4	not declared	0.72	1.41	19.0
D-1	not declared	0.86	1.70	20.0
E-1	not declared	0.42	0.82	12.7
E-2	July 1991	0.35	0.68	12.8
E-3	July 1991	0.84	1.67	11.8

TABLE 8
Olive Oil - Oil Quality Tests

Code	Best before date	Acidity calc. as FFA (% as oleic)	Acidity calc. as acid value (mg KOH/g fat)	Peroxide Value (Meq/kg)
A-6	May 1991	0.81	1.59	13.4
A-12	December 1991	0.21	0.41	6.6
A-13	July 1991	0.27	0.53	5.7
B-5	not declared	0.54	1.07	6.4
C-5	not declared	0.52	1.04	8.8
E-4	December 1990	0.21	0.42	12.9
Produce of more than one country				
1	July 1991	0.18	0.36	6.2
2	July 1991	0.20	0.40	6.7
3	June 1991	0.42	0.85	7.1
4	October 1991	0.29	0.58	6.7
5	April 1991	0.31	0.62	6.0

TABLE 9
Overall Fatty Acid Composition of Olive Oil

Oil type[c]	EV	EV	EV	EV	O			EV	EV	EV	EV	O	EV		
Sample no.	B 1	B2	B3	B4	B5	Range	Mean	C-1	C-2	C-3	C-4	C-5	D-1	Range	Mean
Fatty acids															
C 12:0	trace[a]	0.1	trace	0.1	trace	trace-0.1		0.1	0.1	0.1	0.2	0.1	0.1	0.1-0.2	0.1
C 14:0	trace	0.1	0.1	0.1	trace	trace-0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C 16:0	9.9	10.3	14.8	8.8	10.4	8.8-14.8	10.8	10.5	11.3	11.4	10.7	11.2	11.9	10.5-11.9	11.2
C 16:1	0.8	0.7	1.7	0.5	0.7	0.5-1.7	0.9	0.6	0.8	0.8	0.7	0.7	0.8	0.6-0.8	0.7
C 17:0	0.1	trace	trace	0.1	0.1	trace -0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C 17:1	0.2	0.1	0.1	0.1	0.1	0.1-0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C 18:0	2.9	3.8	2.8	3.6	3.2	2.8-3.8	3.3	2.8	3.2	2.9	2.8	2.5	3.0	2.8-3.2	2.9
C 18:1	73.6	78.2	64.9	78.5	75.2	64.9-78.5	74.1	77.4	75.0	75.4	76.6	72.6	73.6	72.6-77.4	75.1
C 18:2	10.3	4.8	14.0	6.2	8.0	4.8-14.0	8.7	6.4	7.1	7.0	6.6	10.7	8.4	6.4-10.7	7.7
C 18:3	0.8	0.6	0.6	0.6	0.7	0.6-0.8	0.7	0.6	0.8	0.7	0.6	0.8	0.6	0.6-0.8	0.7
C 20:0	0.4	0.4	0.4	0.4	0.5	0.4-0.5	0.4	0.4	0.5	0.5	0.4	0.4	0.5	0.4-0.5	0.45
C 20:1	0.3	0.2	0.2	0.3	0.4	0.2-0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C 22:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1-0.2	0.15
C 24:0	0.5	0.5	0.3	0.4	0.3	0.3-0.5	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.2-0.4	0.4
Unknowns	0.1	0.1	0.0	0.2	0.3			0.0	0.0	0.0	0.3	0.1	0.0		
Calc. IV[b]	84.4	78.0	83.5	80.6	81.4	78.0-84.4	81.6	80.1	80.0	79.9	79.9	84.1	80.5	79.9-84.1	80.8
Saturates	13.9	15.3	18.5	13.6	14.6	13.6-18.5	15.2	14.6	15.9	15.7	14.8	14.7	16.2	14.6-16.2	15.3
Monoenes	74.9	79.2	66.9	79.4	76.4	66.9-79.4	75.4	78.4	76.2	76.6	77.7	73.7	74.8	73.7-78.4	76.2
Polyenes	11.1	5.4	14.6	6.8	8.7	5.4-14.6	9.3	7.0	7.9	7.7	7.2	11.5	9.0	7.0-11.5	8.4
P/S ratio(d)	0.8	0.35	0.79	0.50	0.60	0.35-0.80	0.61	0.48	0.5	0.49	0.49	0.78	0.56	0.48-0.78	0.55
Total C 18's	87.6	87.4	82.3	88.9	87.1	82.3-88.9	86.7	87.2	86.1	86.0	86.6	86.6	85.6	85.6-87.2	86.4

Notes

a) trace = less than 0.05% m/m

b) Iodine Values calculated from fatty acid composition by AOCS Official method Tz Ic-85 (AOCS 1991b)

c) EV = labelled as extra virgin oil O = labelled as pure olive oil

d) P/S = *cis, cis* - polyunsaturates (polyenes)/saturates

TABLE 9
Overall Fatty Acid Composition of Olive Oil

Blends of oil from more than one country.

Oil Type [c]	O	O	O	O	O			EV	EV	EV	O		
Sample no.	1	2	3	4	5	Range	Mean	E-1	E-2	E-3	E-4	Range	Mean
Fatty acids													
C 12:0	0.1	0.1	trace[a]	0.1	0.1	trace-0.1	0.1	trace[a]	0.1	0.1	0.1	trace-0.1	0.1
C 14:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	trace	0.2	0.1	0.1	trace-0.2	0.1
C 16:0	10.1	9.8	9.5	9.6	9.8	9.5-10.1	9.8	10.2	10.6	10.5	9.5	9.5-10.6	10.2
C 16:1	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.7	0.6	0.6-0.7	0.7
C 17:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C 17:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2
C 18:0	3.4	3.3	3.2	3.1	3.4	3.1-3.4	3.3	2.6	2.5	3.3	3.4	2.5-3.4	3.0
C 18:1	75.9	76.7	76.2	77.2	76.5	75.9-77.2	76.5	76.8	72.6	73.7	75.4	72.6-76.8	74.6
C 18:2	7.8	7.4	8.1	7.3	7.5	7.3-8.1	7.6	7.4	10.6	9.1	8.8	7.4-10.6	9.0
C 18:3	0.7	0.7	0.7	0.7	0.6	0.6-0.7	0.7	0.6	0.6	0.8	0.7	0.6-0.8	0.7
C 20:0	0.4	0.4	0.5	0.4	0.4	0.4-0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4
C 20:1	0.3	0.3	0.4	0.4	0.3	0.3-0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C 22:0	0.1	0.1	0.2	0.1	0.1	0.1-0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C 24:0	0.2	0.2	0.3	0.2	0.2	0.2-0.3	0.2	0.6	0.6	0.5	0.3	0.3-0.6	0.5
Unknowns	0.1	0.1	0.0	0.0	0.2			0.0	0.5	0.1	0.0		
Calc. IV[b]	81.5	81.5	82.4	81.8	81.2	81.2-82.4	81.7	81.5	83.4	82.3	82.9	81.5-83.4	82.5
Saturates	14.5	14.1	13.9	13.7	14.2	13.7-14.5	14.1	14.0	14.6	15.1	14.0	14.0-15.1	14.4
Monoenes	76.9	77.7	77.3	78.3	77.5	76.9-78.3	77.5	78.0	73.7	74.9	76.5	73.7-78.0	75.8
Polyenes	8.5	8.1	8.8	8.0	8.1	8.0-8.8	8.3	8.0	11.2	9.9	9.5	8.0-11.2	9.7
P/S ratio[d]	0.59	0.57	0.63	0.58	0.57	0.57-0.63	0.59	0.57	0.77	0.66	0.68	0.57-0.77	0.67
Total C 18's	87.8	88.1	88.2	88.3	88.0	87.3-88.3	88.1	87.4	86.3	86.9	88.3	86.3-88.3	87.2

Notes

a) trace = less than 0.05% m/m

b) Iodine Values calculated from fatty acid composition by AOCS Official method Tz Ic-85 (AOCS 1991b)

c) EV = labelled as extra virgin oil O = labelled as pure olive oil

d) P/S = *cis, cis* - polyunsaturates (polyenes)/saturates

TABLE 9
Overall Fatty Acid Composition of Olive Oil

Oil type [c]	EV	EV	EV	EV	EV	O	EV	EV	EV	EV	EV	O	O	EV	EV	Range	Mean
Sample no.	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9	A-10	A-11	A-12	A-13	A-14	A-15		
Fatty acids																	
C 12:0	trace[a]	trace	0.1	trace	trace	trace	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	trace-0.1	0.1
C 14:0	0.1	trace	0.2	0.1	trace	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	trace-0.2	0.1
C 16:0	11.4	10.6	11.8	11.1	9.8	9.0	10.2	11.5	12.3	10.4	10.4	11.6	11.6	10.5	9.3	9.0-12.3	10.8
C 16:1	0.9	0.7	0.8	1.0	0.6	0.6	0.8	0.9	1.0	0.5	0.8	0.8	1.0	0.6	0.6	0.5-1.0	0.8
C 17:0	trace	0.1	0.1	trace	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	trace	trace-0.1	0.1
C 17:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1-0.2	0.1
C 18:0	2.6	2.9	2.6	3.5	3.0	3.4	3.4	3.6	2.9	2.6	3.3	3.6	2.8	2.7	3.4	2.6-3.6	3.1
C 18:1	74.3	77.5	73.8	74.8	77.4	79.7	75.9	74.2	72.1	76.9	75.8	70.3	73.7	77.0	79.2	70 3-79.7	75.5
C 18:2	8.6	6.1	8.4	7.5	7.0	5.0	7.5	7.6	9.6	7.2	7.6	10.9	8.4	6.9	5.2	5.0-10.9	7.6
C 18:3	0.6	0.6	0.7	0.6	0.7	0.6	0.6	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.6-0.7	0.6
C 20:0	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4-0.5	0.4
C 20:1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.4	0.3	0.3	0.3	0.2-0.4	0.3
C 22:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.1-0.3	0.1
C 24:0	0.3	0.5	0.3	0.5	0.5	0.6	0.4	0.5	0.4	0.5	0.3	0.4	0.2	0.4	0.5	0.2-0.6	0.4
Unknowns	0.2	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1		
Calc. IV[b]	81.5	79.8	80.9	80.2	81.4	79.7	80.9	79.6	81.4	81.2	81.2	82.3	81.1	80.9	79.6	79.6-82.3	80.3
Saturates	15.0	14.7	15.6	15.7	13.9	13.7	14.8	16.4	16.4	14.3	14.8	16.8	15.5	14.4	13.9	13.7-16.4	15.1
Monoenes	75.6	78.6	75.0	76.2	78.4	80.7	77.1	75.4	73.4	77.8	76.9	71.6	75.2	78	80.2	71.0-80.7	76.7
Polyenes	9.2	6.7	9.1	8.1	7.7	5.6	8.1	8.2	10.2	7.9	8.3	11.6	9.1	7.6	5.8	5.6-11.6	8.2
P/S ratio[d]	0.61	0.46	0.58	0.52	0.55	0.41	0.55	0.50	0.62	0.55	0.56	0.69	0.59	0.53	0.42	0.41-0.69	0.54
Total C 18's	86.1	87.1	85.5	86.4	88.1	88.7	87.4	86.0	85.2	87.4	87.4	85.5	85.6	87.3	88.4	85.2-88.7	86.8

Notes

a) trace = less than 0.05% m/m

b) Iodine Values calculated from fatty acid composition by AOCS Official method Tz Ic-85 (AOCS 1991b)

c) EV = labelled as extra virgin oil O = labelled as pure olive oil

d) P/S = *cis, cis* - polyunsaturates (polyenes)/saturates

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Abbreviations Used in this Report

BCR	Community Bureau of Reference
BFMIRA	British Food Manufacturing Industries Research Association
BS	British Standard
BSI	British Standards Institute
EC	European Community
EF	Enrichment Factor
EU	European Union
FAC	Fatty Acid Composition
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
GC	Gas Chromatography
GLC	Gas Liquid Chromatography
IOOC	International Olive Oil Council
IS	Internal Standard
ISO	International Standards Organisation
kg	kilogram
KOH	Potassium Hydroxide
MAFF	Ministry of Agriculture, Fisheries and Food
mg	milligram
nm	nanometre
PV	Peroxide Value
RRT	Relative Retention Time
RT	Retention Time
SE	Specific Extinction
TLC	Thin Layer Chromatography
USM	Unsaponifiable Matter
CP SIL9	All trade names for GLC stationary phases
DB5	
OV17	
SE32	
SE52	

GLOSSARY OF TERMS

Fatty Acids: The types and relative concentrations of fatty acids in the triglycerides of oils profoundly influence their chemical, physical and nutritional properties. The fatty acid composition (FAC) is, therefore, the most important chemical determination undertaken on oils. Fatty acids fall into three main categories.

Saturated fatty acids (saturates): The hydrocarbon chain of this group does not contain any unsaturation (i.e. double carbon-carbon bonds). The saturated fatty acids of major importance include butyric acid (C4:0), which is found in milkfat; caproic, caprylic and capric (C6:0, C8:0 and C10:0) which are found in milkfat, palm kernel and coconut oils; lauric acid (C12:0) the major constituent of palm kernel and coconut oils; palmitic and stearic acids (C16:0 and C18:0) which are found in all

vegetable oils and animal fats. Arachidic, behenic and lignoceric acids (C20:0, C22:0 and C24:0) are also observed in many oils and fats, generally in low concentrations (< 1.0%).

Monounsaturated fatty acids (monounsaturates or MUFA): The hydrocarbon chain of this group of compounds contains one double carbon-carbon bond, generally in the *cis* configuration. Although a large variety of such acids exist, the monounsaturated fatty acid of major importance is oleic acid (C18:1). It is present in all oils and fats of commercial importance and, on occasions, in high concentrations (i.e. 60-80%).

Polyunsaturated fatty acids (polyunsaturates or PUFA): These have two or more double bonds in the hydrocarbon chain. In vegetable oils that have not been hydrogenated, these bonds are configured in the *cis, cis*-1,4-methylene interrupted form and can be considered as essential fatty acids (EFA). The most important polyunsaturated fatty acids in vegetable oils are briefly discussed below.

(a) Linoleic acid (C18:2 n-6c): This is the principle fatty acid in sunflower, safflower, soyabean and corn (maize) oils. However, it occurs in all oils and fats of commercial importance and is considered to be the most significant EFA in the diet.

(b) Alpha-(a)-Linolenic acid (C18:3 n-3c): Soyabean and rapeseed oils contain significant quantities of this acid (approximately 8-12%). Gamma-(γ)-linolenic acid (GLA) (C18:3 n-6c) also exists but is much less common, being found in blackcurrent, evening primrose and borage oils. GLA is used to treat eczema, pre-menstrual tension and multiple sclerosis.

Fatty Acid Composition (FAC): Fatty acids constitute approximately 95-97% of most oils. Therefore, the FAC is a very important determination since it acts as a guide to oil purity, and provides information on the oil's nutritional, physical and chemical properties. To determine the FAC of an oil, it is first saponified with refluxing alkali (i.e. sodium hydroxide), followed by reaction with a methylating agent, typically a boron trifluoride-methanol complex. This leads to the formation of fatty acid methyl esters (FAME) which are identified and quantified by GLC.

Free Fatty Acids: The presence of free fatty acids (FFA) in an oil indicates that hydrolysis of previously esterified fatty acids has taken place. This can occur as a result of lipolytic action (i.e. hydrolytic enzyme), the action of water and/or oxidation. Elevated concentrations of FFA will cause taints and generally reduce the ability of the oil to function in the required manner.

Iodine Value: The iodine value (IV) is a measure of the degree of unsaturation of an oil and is a useful purity criteria. The greater an oil's IV the greater its degree of unsaturation. For example, coconut oil has an IV in the range 7 to 13 and, of all the major oils, contains the lowest quantity of unsaturated fatty acids. In contrast, sunflowerseed oils are highly unsaturated and have IVs in the range 117-140.

Peroxide Value: Unsaturated fatty acids are prone to reaction with oxygen. Oxidation of an oil takes place via the formation of hydroperoxides which rapidly decompose to produce secondary oxidation products such as unsaturated aldehydes, ketones and alcohols. The presence of these compounds gives rise to the unpleasant odour and flavour associated with rancid oils. The reaction between fatty acids and oxygen is catalysed by iron, copper, heat and light. The peroxide value of a fat is an indication of its content of hydroperoxides and, therefore, its oxidative state.

Repeatability: A measure of intra-laboratory variation.

Reproducibility: A measure of inter-laboratory variation.

Sterols: The sterols of major interest in vegetable oils are the desmethylsterols, although both mono- and dimethyl sterols are also present but generally at comparatively low concentrations. Sterols occur in the unsaponifiable fraction of oils. The most well known sterol is cholesterol (a zoosterol) which is found in significant concentrations in animal fats, fish oils and egg yolks. It is found at low levels in vegetable oils. Plant sterols (phytosterols) include stigmasterol, brassicasterol, campesterol, etc. The sterol composition can act as a useful guide to an oil's purity.

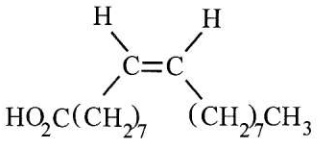
Specific Extinction in the Ultraviolet: Oxidation of an unsaturated oil leads to the formation of conjugated dienes which have a characteristic ultraviolet absorption spectra. However, different absorption spectra associated with conjugated dienes and trienes are generated during oil bleaching and deodorisation. Consequently, the use of specific extinction can indicate whether the oil under investigation has oxidised or whether it contains bleached and/or deodorised oils.

Tocopherols: Tocopherols are naturally occurring antioxidants and are found in most vegetable oils and fats. There are four isomers (α,β,γ and δ-tocopherol) all of which express Vitamin E activity, although to differing extents. Tocotrienols are compounds of similar structure and are found in vegetable oils, particularly soyabean oil (δ-tocotrienol) and palm oil (α, γ and δ-tocotrienols).

Trans Fatty Acids: Unsaturated fatty acids (MUFA and PUFA) in non-hydrogenated vegetable oils will almost exclusively be present in the *cis* form. In PUFA this will be in the *cis, cis*-1,4-methylene interrupted configuration. However, the *trans* configuration is more thermodynamically stable than the *cis.* Therefore, oils that have either been industrially chemically hydrogenated or those fats obtained from ruminants will contain MUFA and PUFA a portion of which will be present in the *trans* form. To illustrate this further, the structures of the *cis* and *trans* isomers of C 18:1 (oleic and elaidic acids respectively) are illustrated below:-

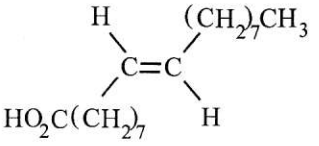
Oleic acid (C18:1n-9c)

or 9-*cis*-octadecenoic acid



Elaidic acid (Cl8:1n-9t)

or 9-*trans*-octadecenoic acid



Triglycerides: Oils and fats are almost totally (95-97%) composed of triglycerides (triacylglycerols). These are made up of a glycerol 'backbone' esterified to three fatty acids. The structure of glycerol, a trihydric alcohol, is shown below. The variety of different triglycerides in an oil is great. For example, it is possible to form six chemically different triglycerides from glycerol and only two fatty acids; bearing in mind that most oils contain at least 8-10 different fatty acids, it will be seen that the number of possible triglycerides is very large.

Glycerol: $CH_2.(OH)CH(OH)CH_2(OH)$

Glycerol moieties esterifed to either one or two fatty acids are known as monoglycerides and diglycerides respectively.

Unsaponifiable Matter: Non-saponifiable or unsaponifiable matter (USM) are synonymous terms. They refer to that material in oils which is not volatile at 100°C (i.e. water) and which, following reaction of the oil with sodium hydroxide, remains undissolved and insoluble in alkali. The USM of an oil contains sterols, tocopherols, higher alcohols, hydrocarbons and pigments (i.e. Vitamin A, carotene and chlorophyll). In general, the USM accounts for less than 2% of the oil.

Uvaol and Erythrodiol: These are triterpene dialcohols, similar in structure to the desmethyl sterols. Uvaol and erythrodiol are found in high concentrations in olive-pomace (residues) oils.

Acknowledgements

The bulk of the analyses were conducted by the Leatherhead Food RA and the Central Science Laboratory (Norwich) kindly assisted in the validation procedure.

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FAT IN MINCED BEEF

A Review by the Association of Public Analysts

History / Background

Since the earliest appointments of Public Analysts, which were designed to control the composition of food, they have exercised their prerogative of expressing opinions on the nature, substance and quality of foods.

The need to control food quality has led to the introduction of Regulations covering the composition of food. However, these Regulations do not cover all aspects of food quality and it is under such circumstances that the Public analyst has, through the Association of Public Analysts (APA), evaluated data derived from samples and surveys to establish compositional parameters which demonstrate both the composition reasonably to be demanded and expected by consumers.

Based on detailed evaluations the APA proposed a standard for Fat in Minced Beef. This was first proposed and adopted in 1976. The standard then used was a maximum of 25% fat in England and 20% in Scotland (See Table 1). Since that time breeding has produced leaner animals and there has also been a demand by the more health conscious consumer for a less fatty product (See Graph 1).

The current survey takes this into account along with data collated up to 1989 and from 1990 to 1994.

Minced Beef - Definition

The Meat Products & Spreadable Fish Products Regulations 1984, SI No 1566 define meat as: "Meat" means the flesh, including fat and the skin, rind, gristle and similar in amounts naturally associated with the flesh used, of any animal or bird which is normally used for human consumption etc. The Regulations do not define the term "minced beef", so the APA has adopted the following definition.

A product produced wholly from one or more cuts of beef carcass meat, without the addition of other ingredients, so that the resultant fat content shall be not more than 20%.

Working Practices

It is recognised that normal butchery practice is to use trimmings (e.g. clod/sticking) and some carcass meats not usually sold as such (e.g. diaphragm (skirt)), together with some fattier cuts (e.g. flank), shin, leg, masseter, neck and chuck in the preparation of minced beef.

All of these forms of beef are expected to be characterised by their natural fat contents and as animals become leaner so these cuts must also become leaner.

If minced beef were simply minced flank or minced skirt the question of composition would be relatively simple but because minced beef is an article often prepared by blending different cuts of beef the composition calls for further consideration.

0004-5780/95 +8 $20.00

It is not unreasonable to expect that the fat content of a minced beef produced from various cuts should equate to the weighted average fat content of the representative cuts/trimmings used, with a maximum value not exceeding the maximum natural fat content of any part of the carcass meat used.

Data from McCance & Widdowson suggests that this would equate to 25.1% if fore-rib alone were used. It is, however, reasonable to assume and accept that smaller traders are much less likely to use a single cut of meat (though large producers are not likely to use trimmings in the same way as butchers and may use only 2-3 types of carcass meat) and so produce a significantly lower fat product. If a processor wishes to produce a minced product from a fattier single cut of beef, then there is nothing wrong with calling it by the name of the cut, e.g. minced flank.

It is also likely that large producers would try to satisfy consumer demand for a lower fat product and would be unlikely to use only high fat carcass material. Consequently it is highly unlikely that minced beef would be produced with a high fat content by either category of producer. This is borne out by the statistics of the survey which show mean values of 16.47% before 1990 and 15.73% in 1994. These mean values can and should be regarded a "target values" and they almost certainly reflect the use of a mixture of meat cuts consistent with good manufacturing practice.

This demonstrates that values of less than the 25% fat content, recommended in 1976, can be easily achieved.

Physiological Data

A variety of cuts of meat are used for the preparation of minced beef, mince is generally prepared from cheaper cuts of meat and from trimmings from all parts of the carcass resulting from the preparation of meat for sale.

The following cuts of meat may be used:

Flank	Clod	Chuck (shoulder)
Shin	Neck	Skirt (diaphragm)
Leg	Heart	Masseter (head meat)

Studies of the composition of various cuts of beef are listed in Table II and III.

It can be clearly seen that only flank and diaphragm contain fat at the 20% level, other cuts contain considerably less.

This reinforces the argument that minced beef can and should have a fat content of less than 20% when made from a mixture of cuts - no matter whether prepared by Butchers or Factory Processing.

Statistics

Statistics have been used to determine the mean and the standard deviations from both surveys and these have been used for comparison purposes.

As samples have been taken in the same manner for both surveys (pre 1990 and post 1990), comparison between the two sets of data is legitimate. Only samples which were described as minced beef were included in the survey (see Table IV and V).

Comments from the Statistics

1. There is a small but significant reduction in mean values obtained for pre 1990 and post 1990 samples, respectively.

2. There is no significant change in the Standard Deviations of the data for the two periods

3. The very broad spread of results clearly demonstrates that the designation "minced beef" is a generic term and does not indicate to the intending purchaser, the true nature of the product, with respect to the fat content,

4. By simple inspection of the data, an upper limit for the fat content of minced beef, not described more specifically, can be defined by using the mean as a nominal target value and adding to this value a suitable tolerance. A tolerance of 25% on the target value is considered generous for a major constituent and this equates to a maximum fat content of 19.7%. Such a value can be easily supported by the physiological data.

5. Data from Scottish Public Analysts' Laboratories show that in Scotland minced beef has a fat content consistently below 20% and in fact regularly achieve the 15% "target value".

Regional Variations

There is no doubt that regional variations do occur, the further south, the fattier the product becomes. But as factory processing increases and movement of food across regions also increases, the regional variation argument should diminish such that a UK Standard can be applied.

European Legislation

Whilst the APA Sub Committee considers this project as one which is local to the UK, it would be wrong to ignore the European dimension. The EC 88/657 of 14 December 1988 specifies a maximum fat value of 20% for minced pure beef. Thus the current proposals do not conflict with the European Standard.

Legal Decisions

In the case of Goldup v Manson (John) it was held that where no standard had been prescribed by statute or regulation, a Public Analyst could not make good the deficiency by himself determining the Standard. In these circumstances the standard of quality to be applied must be defined in terms of the purchaser's demand, which is a question of fact. The prosecution has to prove that a purchaser of minced beef is demanding meat of a commercial quality superior to that sold to them.

Consequently, in proposing a standard for the fat content of minced meat the Association of Public Analysts must have regard to the public's perception of the expected fat content of minced meat and not necessarily other considerations based on the analyst's professional experience or opinions.

However, what is important, without re-drafting the statute, is to establish the quality demanded by the purchaser and whether or not, at a particular price, the commercial quality so demanded does not contain significantly more fat than is usual.

In real terms this means that the Association of Public Analysts must establish a compositional definition for minced beef so that a purchaser, when asking for that product, knows that it refers to a food containing a maximum proportion of fat.

Price

As the survey is ongoing it would be completely misleading to compare prices in say 1987 with those in 1994. Also fat is not the only criteria used to judge the quality of minced beef. Consequently prices have not been included in the data.

Comments

1. The data shows that a "target value" for fat of 15.7% can be achieved.

2. Physiological data on a mixture of cuts of meat also reinforce the 15.7% "target value".

3. Minced beef sold in Scotland is consistently below the 20% level and is regularly at 15%.

4. Trends in the two sets of data show that a slightly lower mean fat content is being produced in the 1990s.

5. Compounded minced beef plus fat (presumably at a lower cost) produced with fat levels in excess of 20% are in fact compound products and should be labelled accordingly i.e. "Minced beef with added fat".

An indication of the fat content should also be declared along with a list of ingredients, this would take into account "economy products"

Conclusions and Recommendations

The Council of the APA in September 1995, recommended the following Guidelevel for fat in Minced Beef sold throughout the UK:

Minced Beef - Fat content not to exceed 20%.

The APA Sub Committee recommend that the definition of Minced Beef is:

Minced Beef shall be defined as: A product produced wholly from one or more cuts of beef carcass meat, without the addition of other ingredients, so that the resultant fat content shall be not more than 20%.

TABLE I

Mean percentage of fat in minced beef

Date	1976	1989	1994
Number of samples	1324	508	1307
Mean Fat Values (%)	17.5	16.47	15.73

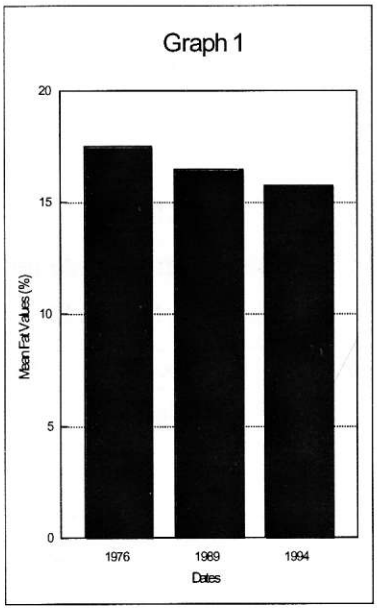


TABLE II

Fat Contents of beef cuts

(JAPA: 1986,24,123)

Cut	No of Samples	Range %	Mean %	Standard Deviation (%)
Chuck	30	1.6-13.5	6.6	3.1
Clod	27	1.2-10.6	4.8	2.7
Neck	26	2.7-10.6	5.8	2.6
Masseter	27	2.4-14.9	5.2	3.2
Shin	26	1.0-3.7	2.5	0.7
Diaphragm	27	4.6-20.9	10.5	4.3
Flank	27	1.2-20.4	7.0	4.0

TABLE IIIA

Fat Contents of beef cuts - Lean Tissue

(Analyst: 1993, 118, 1217)

Cut	Clean beef	Clean beef	Cull Cow	Cull Cow
	mean	Std. Deviation	Mean	Std. Deviation
Samples	43	43	30	30
Chuck	5.9	0.22	6.2	0.39
Clod & Sticking	3.8	0.16	4.0	0.25
Shin & Leg	2.5	0.15	2.6	..52
Flank (thin)	6.9	0.31	3.8	.50
Forequarter	5.9	0.19	6.1	0.27

TABLE IIIB

Fat Contents of beef cuts - Lean and Intermuscular Tissue

(Analyst: 1993, 118, 1217)

Cut	Clean beef	Clean beef	Cull Cow	Cull Cow
	mean	Std. Deviation	Mean	Std. Deviation
Samples		43		30
Chuck	13.1	0.4	13.2	0.63
Clod & Sticking	14.7	0.48	15.1	0.83
Shin & Leg	6.2	0.27	6.2	0.71
Flank (thin)	21.1	0.67	19.5	1.11
Forequarter	17.9	0.51	17.5	0.77

TABLE IV

	Up to 1989	1990 - 1994	All
Number of samples	508	1307	1815
Mean	16.47	15.73	15.93
Median	16.2	15.5	15.8
Sample SD	5.97	5.84	5.88
SKEWNESS	0.31	0.29	
Ftest	0.54		
S.E.	0.26	0.16	

TABLE V

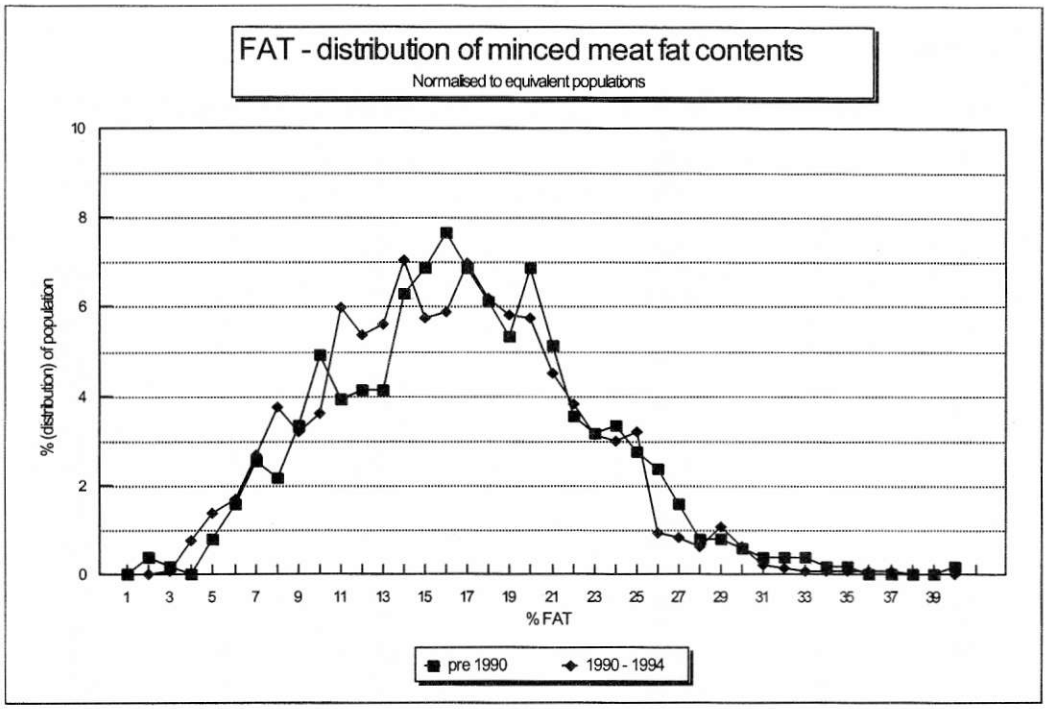
Pre 1990			Post 1990		
Range	Frequency	% of total	Range	Frequency	% of total
1	0	0	1	0	0
2	2	0.39	2	0	0
3	1	0.2	3	1	0.08
4	0	0	4	10	0.77
5	4	0.79	5	18	1.38
6	8	1.57	6	22	1.68
7	13	2.56	7	35	2.68
8	11	2.17	8	49	3.75
9	17	3.35	9	42	3.22
10	25	4.92	10	47	3.60
11	20	3.94	11	78	5.97
12	21	4.13	12	70	5.36
13	21	4.13	13	73	5.59
14	32	6.30	14	92	7.04
15	35	6.89	15	75	5.74
16	39	7.68	16	77	5.90
17	35	6.89	17	91	6.97
18	31	6.10	18	81	6.20
19	27	5.31	19	76	5.82
20	35	6.89	20	75	5.74
21	26	5.12	21	59	4.52
22	18	3.54	22	50	3.83
23	16	3.15	23	41	3.14
24	17	3.35	24	39	2.99
25	14	2.76	25	42	3.22
26	12	2.36	26	12	0.92
27	8	1.57	27	11	0.84
28	4	0.79	28	8	0.61
29	4	0.79	29	14	1.07
30	3	0.59	30	8	0.61
31	2	0.39	31	3	0.23
32	2	0.39	32	2	0.15
33	2	0.39	33	1	0.08
34	1	0.20	34	1	0.08
35	1	0.20	35	1	0.08
36	0	0	36	1	0.08
37	0	0	37	1	0.08
38	0	0	38	0	0
39	0	0	39	0	0
40	1	0.20	40	0	0
	0		>40	1	
	508	100		1306	100

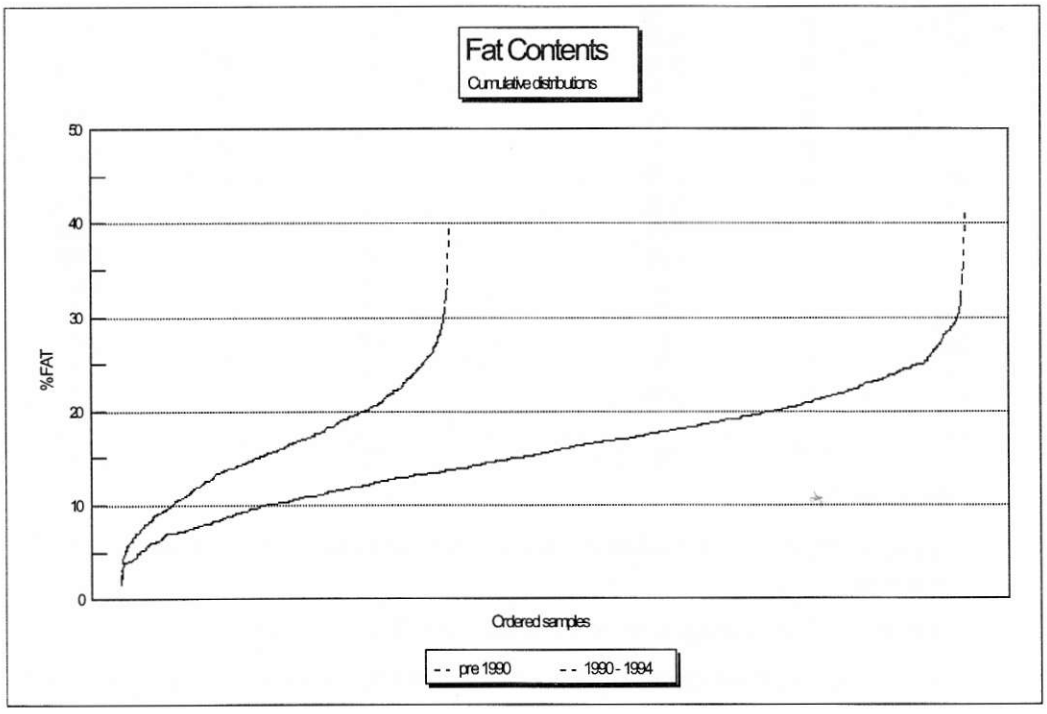
Comments (General)

1) There is a small but significant reduction in the mean values obtained for pre-1990 and post-1990 samples respectively.

2) There is no significant change in the standard deviations of the data for the two periods.

This may reflect the move to leaner livestock over the period rather than to changes in butchery practices to meet consumer demand.


FAT - distribution of minced meat fat contents
Normalised to equivalent populations
% (distribution) of population
10
8
6
4
2
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
% FAT
pre 1990
1990 - 1994


Fat Contents
Cumulative distributions
%FAT
50
40
30
20
10
0
Ordered samples
pre 1990
1990 - 1994