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Essential Fatty Acids and *Trans* Contents of Some Oils, Margarine and Other Food Fats

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ABSTRACT

Levels of polyunsaturated fatty acids (PUFA), cis, cis-*methylene interrupted polyunsaturated fatty acids (i.e. essential fatty acids—EFA) and isolated* trans *double bonds in six crude vegetable oils, seven commercial oils, eighteen margarines, two butter fats and fats from two types each of crisps and butter biscuits have been determined. The ratios between the PUFA or EFA and the saturated fatty acids of various samples have been tabulated. Normal (i.e. high linoleic) safflower seed oil gave the highest ratio. Margarines which were estimated to contain partially hydrogenated fish oils (45–90%, on fat basis) gave high values of* trans *double bonds. Butter fat having a similar EFA level to that present in a 'hydrogenated fish oil' margarine contained a much lower level of* trans *isomers. Almost all the PUFA in the oils of crisps, and about half the PUFA content of butter biscuits, existed as 'true' essential fatty acids.*

INTRODUCTION

The requirement of essential fatty acids (EFA), i.e. *cis*, *cis*-methylene interrupted polyunsaturated fatty acids in human diet, has been reviewed by several workers (Sinclair, 1958; Vergroesen, 1975; Holman, 1981;

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Tinoco, 1982). Essential fatty acid deficiency mainly results from a lack of linoleic acid (Galli & Avogaro, 1980). The symptoms of EFA deficiency are related to poor growth, scaly skin, loss of hair and other histological changes (FAO/WHO, 1980; Prout & Tring, 1981). Dietary linoleic (C18:2 *n*-6) and α-linolenic (C18:3 *n*-3) acids are metabolised by desaturation and chain elongation to long-chain essential fatty acids, which are involved in the structure and functions of all tissues (Holman, 1981). Linoleic, γ-linolenic (C18:3 *n*-6) and arachidonic (C20:4 *n*-6) acids are reported to be more effective than the other polyunsaturated fatty acids in preventing the deficiency symptoms (Hassam *et al.* 1977; Holman, 1979; Brisson, 1982). Söderhjelm *et al.* (1970) recommended a dietary requirement of 4–5 g linoleic acid per day; that is about 2% of the daily calorie intake for a 2000 kcal diet. FAO/WHO (1980) have revised these figures for EFA requirement to 3% of the total calorie intake for normal human adults and infants, to 4–5% during pregnancy and to 5–7% during lactation. An EFA intake of 10–12% of calories is recommended as a protection against cardiovascular disease (FAO/WHO, 1980). Linolenic acid deficiency occurs at a level of α-linolenic acid estimated to be 0·54% of the dietary energy (Holman *et al.*, 1982). The relationship between the polyunsaturated fatty acids (PUFA) and the incidence of cardiovascular disease has been discussed critically (McGandy & Hegsted, 1975; Ahrens, 1979; Brisson, 1982).

Almost all natural, unprocessed vegetable oils contain double bonds in the *cis* configuration. A small proportion of these *cis* isomers may, in some cases, be isomerised into the thermodynamically more stable *trans* forms during extraction and refining. *Trans* unsaturated fatty acids are natural components of beef fat and dairy products (Duncan & Gartan, 1967; Patton & Jensen, 1975), being formed during the hydrogenation that takes place in the rumen. Many foods, such as margarines and shortenings, contain vegetable oils that have been partially hydrogenated, resulting in the formation of variable amounts of *trans* and conjugated isomers.

There is an increasing interest in the biological utilisation and effects of *trans* and other isomeric unsaturated fatty acids on human health (Kummerow, 1974; 1979; Emken, 1979; Holman, 1981). Several conflicting reports on the effects of *trans* isomers on metabolic processes have been published (Anderson, 1980; Mahfouz *et al.*, 1980; Masson, 1981; Reiser, 1981; Brisson, 1982; De Schrijver & Privett, 1982). It has been pointed out, however, that *cis-trans* and *trans-trans* isomers of

PUFA do not act as true EFA (Houtsmuller, 1978). An advisory report to the Department of National Health and Welfare (1980) on the composition of Canadian margarines recommended that *trans-trans* isomers of linoleic acid should not exceed 1% of the total fat.

Despite the increased interest in these nutritional aspects of dietary fats, there is still a limited amount of data on the EFA, total PUFA and *trans* contents of processed foods, although it is known that processing of oils could have an adverse effect on their nutritional qualities (Rossell *et al.*, 1981). This study was undertaken to investigate the extent to which a correlation exists between EFA, as measured by the total level of *cis, cis*-PUFA determined by the lipoxidase technique, and the total PUFA content determined by packed column gas chromatography, in a variety of crude oils, processed oils and fats extracted from processed foods with relatively high fat content (e.g. margarines, butters, crisps, biscuits). Total *trans* double bond contents of the oil samples were also determined using infrared spectroscopy.

MATERIALS AND METHODS

Materials

A variety of oilseeds, of different origins was used for the extraction of crude oil in the laboratory. In the main these were 'tail ends' of samples supplied for contractual seed analysis by members of the Federation of Oils, Seeds and Fats Associations Ltd (FOSFA). The margarine samples, both of the soft (tub) and hard type, were obtained from retail outlets, as were samples of commercial oils, butters, potato crisps and butter biscuits.

Methods

Extraction of fats

The ISO method 659 (technically similar to BS Part IV) was used to extract oils from authentic seed samples.

Fats from the margarine, butter biscuit and crisp samples were extracted using light petroleum (boiling range, 40°–60°C). The solvent extracts were dried with anhydrous sodium sulphate and the bulk of the solvent was distilled off under reduced pressure. The last traces of solvent were then removed at about 40°C under a stream of nitrogen.

All the fat samples were then stored under nitrogen at −18 °C until analysis.

Fatty acid composition

Fatty acid compositions were determined by gas–liquid chromatography after transesterification of the fat samples to fatty acid methyl esters (FAMEs) using the BS method (BSI, 1980). The resulting FAME were analysed by a Sigma-2 chromatograph fitted with a glass column (1·8 m × 1·75 mm inside diameter) packed with 6 % Silar 5CP on 100–120 mesh Chromosorb HP. Operating conditions for isothermal separations were: column temperature, 200 °C; injector and detector temperature, 230 °C; carrier gas, nitrogen, at 20 ml/min. Fatty acids of the butter samples were separated using the following temperature programming conditions: initial oven temperature, 50 °C; initial time, 2 min; oven temperature programme rate, 5 °C/min from 50 ° to 180 °C, and hold at 180 °C until elution of all components (about 30 min). Data interpretation was by means of a Spectra Physics SP 4000 computer fitted with a printer plotter, and programmed for peak identification.

The apparatus was regularly calibrated by analysis of standard mixtures of FAMEs (obtained from Sigma Chemical Co. Ltd). These calibrations demonstrated that accuracy was ±0·1 %, ±0·2 % and ±0·5 % for components in the ranges 1 to 5 %, 10 to 20 % and 25 to 50 %, respectively. Reproducibility of the total PUFA content results reported here was ±0·2–0·3 %.

The above conditions led to chromatograms in which the geometric (i.e. *trans*) and positional isomers were not separated from corresponding *cis* isomers. The total PUFA contents (i.e. C18, C20 and C22 polyenes) were then calculated.

Essential fatty acids (EFA)

The EFA of the oils and the extracted fats were determined by the lipoxidase method according to MacGee (1959), and compared with the total PUFA determined as above. Lipoxidase enzyme No. L-7127 type 1 (Sigma Chemical Co. Ltd) was used in this study. Samples were diluted with acetone (BDH, AR grade). Extra care was taken as to the cleanliness of the glassware, and the cuvettes were washed several times with a mixture of ethanol and diethyl ether (1 + 1) and dried prior to the reading of the absorbance of each solution at 234 nm.

Trans *content*

The total isolated *trans* double bond contents (as % elaidate) of the samples were determined on the FAME, prepared as previously, by differential infra-red spectroscopy. A 1% (w/v) solution of the FAME in carbon disulphide (spectroscopic grade) was run against pure methyl stearate (1% solution w/v in carbon disulphide) as a reference. Measurement of the infra-red absorbance readings was carried out as recommended in the AOCS *Official Method* (1973). The results were calculated from the appropriate calibration curve for methyl elaidate.

The differential infra-red technique using 10% solution of FAME in carbon disulphide is reliable for *trans* acid contents of oils and fats over 0·2%. Good reproducible results are obtained when the baseline for the sample spectrum is drawn exactly in the same manner as the calibration baseline.

RESULTS AND DISCUSSION

Tables 1 and 2 give the total contents of saturated fatty acids, monounsaturated, PUFA, EFA and *trans* acids (expressed as total isolated *trans* double bonds by IR) in crude and fully refined oils, respectively. The levels of these fatty acids and *trans* double bonds in several margarine oils, and in some crisp, biscuit and butter fats are presented in Tables 3 and 4, respectively. The ratio between the concentration of PUFA and that of the saturated fatty acids is sometimes considered an important characteristic of dietary fats (Brisson, 1982). These ratios, calculated for various oils and fats, are listed in Tables 1 to 4. Short-chain fatty acids (C4 to C10), which are characteristic of butter oil, and stearic acid, have been reported to have little or no significant influence on blood cholesterol (McGandy & Hegsted, 1975; Brisson, 1982). Therefore, the ratios omitting these fatty acids were also determined (Tables 1 to 4). The ratios between the levels of EFA and the saturated fatty acids are also tabulated.

On the basis of 'typical' fatty acid data of pure oils (Vergroesen, 1975; Rossell *et al.*, 1983) and taking into account *trans* acids resulting from partial hydrogenation, possible compositions of oil blends used in the production of margarines, crisps and biscuits were estimated and given in Table 5.

TABLE 1
Content of Total Saturated, Total Monounsaturated, Total Polyunsaturated, Total Essential Fatty Acids and Total *Trans* Bonds Content in Crude Edible Oils

Oil	*Origin of seeds*	*Oil content* (%)	*TSFA* (%)	*TMUSFA* (%)	*PUFA* (%)	*EFA* (%)	*Total* trans* *content* (%)	*PUFA TSFA*	*EFA TSFA*
Cotton seed	Mali	20·2	27·6 [24·5]	21·5	50·9	49·0	1·0	1·84 (2·08)	1·77 (2·00)
Cotton seed	Unknown	20·1	29·3 [26·5]	19·7	51·0	50·1	0·6	1·74 (1·92)	1·71 (1·89)
Groundnut	Guinea Bissau	51·2	19·1 [11·2]	59·8	21·1	21·3	0·2	1·10 (1·88)	1·12 (1·90)
Groundnut	USA	44·6	19·6 [11·7]	49·8	30·6	30·8	0·7	1·56 (2·62)	1·57 (2·63)
Rapeseed[a]	Denmark	41·8	7·3 [4·7]	61·9	30·8	32·4	0·2	4·22 (6·55)	4·44 (6·89)

Rapeseed[b]	Sweden	40·8	5·7 [3·3]	64·7	29·6	25·8	0·4	5·19 (8·97)	4·53 (7·82)
Safflower seed[c]	Unknown	39·4	8·6 [5·5]	74·6	16·8	16·4	0·2	1·95 (3·05)	1·91 (2·98)
Sesame seed	Nicaragua	53·1	16·1 [10·9]	37·9	46·0	44·5	0·2	2·86 (4·22)	2·76 (4·08)
Sesame seed	Sudan	50·8	17·0 [10·4]	41·9	41·1	41·8	0·6	2·42 (3·95)	2·46 (4·02)
Soya bean	Brazil	21·6	16·4 [11·3]	23·7	59·9	57·9	0·9	3·65 (5·30)	3·53 (5·12)
Soya bean	Argentina	19·7	17·2 [12·8]	18·6	64·2	61·9	0·5	3·73 (5·02)	3·60 (4·48)

[a] Erucic acid content, 2·3%.
[b] Erucic acid content, 31·8%.
[c] Clearly a low linoleic acid content variety.
Notes: TSFA = total saturated fatty acids, TMUSFA = total monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, EFA = essential fatty acids.
[ ] figures based solely on lauric, myristic and palmitic acids as saturated fatty acids.
( ) figures based on ratio of PUFA or EFA to the sum of lauric, myristic and palmitic acids. (See Brisson, 1982.)
* 10% of solution of FAME in carbon disulphide used for *trans* values less than 2%.

TABLE 2
Content of Total Saturated, Total Monounsaturated, Total Polyunsaturated, Total Essential Fatty Acids and Total *Trans* Bonds Content in Some Fully Refined Edible Oils

Oil	*TSFA** (%)	*TMUSFA** (%)	*PUFA** (%)	*EFA** (%)	*Total* trans *content* (%)	*PUFA TSFA*	*EFA TSFA*
Frying oil F1	21·9 [13·0]*	42·0	36·1	36·2	0·5	1·65 (2·78)*	1·65 (2·78)*
Frying oil F2	16·6 [11·2]	33·2	50·2	46·8	6·7	3·02 (4·48)	2·82 (4·18)
Frying oil F3	14·1 [11·6]	25·6	60·3	62·0	0·2	4·28 (5·20)	4·40 (5·34)
Frying oil F4	12·6 [7·5]	17·2	70·2	71·2	0·5	5·57 (9·36)	5·65 (9·49)
Commercial soya bean	16·0 [11·2]	23·6	60·4	60·6	3·0	3·78 (5·39)	3·79 (5·41)
Commercial corn	13·4 [10·9]	25·3	61·3	62·7	0·2	4·57 (5·62)	4·68 (5·75)
Commercial safflower seed	13·1 [8·9]	20·5	66·4	67·2	0·2	5·07 (7·46)	5·13 (7·55)

* See notes to Table 1, and text.

TABLE 3

Content of Total Saturated, Total Monounsaturated, Total Polyunsaturated, Total Essential Fatty Acids and Total *Trans* Bonds Content in Margarines

Sample	*Oil content* (%)	*TSFA** (%)	*TMUSFA** (%)	*PUFA** (%)	*EFA** (%)	*Total* trans *content* (%)	*PUFA TSFA*	*EFA TSFA*
A	80·7	40·4 [31·5]*	46·8	12·8	1·6	42·3	0·32 (0·41)*	0·04 (0·05)*
B	81·8	37·3 [29·3]	48·9	13·8	3·8	40·5	0·37 (0·47)	0·10 (0·13)
C	nd	46·4 [29·1]	49·4	4·2	4·5	5·6	0·09 (0·14)	0·10 (0·15)
D	nd	42·0 [25·7]	49·8	8·2	5·8	5·0	0·20 (0·32)	0·14 (0·23)
E	80·7	32·4 [25·0]	50·5	17·1	6·5	39·1	0·53 (0·68)	0·20 (0·26)
F	80·2	44·2 [28·6]	47·2	8·6	6·8	4·1	0·19 (0·30)	0·15 (0·24)
G	80·4	34·5 [25·5]	52·0	13·5	8·8	29·0	0·39 (0·53)	0·26 (0·35)
H	81·3	32·7 [25·8]	48·4	18·9	11·1	33·4	0·58 (0·73)	0·34 (0·43)
I	80·2	25·5 [18·3]	55·3	19·2	13·7	34·7	0·75 (1·05)	0·54 (0·75)
J	81·8	32·1 [22·8]	44·5	23·4	15·1	35·7	0·73 (1·03)	0·47 (0·66)
K	80·9	31·3 [22·4]	44·5	24·2	18·1	31·8	0·77 (1·08)	0·58 (0·81)

(continued)

TABLE 3—*contd.*

Sample	*Oil content* (%)	*TSFA** (%)	*TMUSFA** (%)	*PUFA** (%)	*EFA** (%)	*Total* trans *content* (%)	*PUFA TSFA*	*EFA TSFA*
L	80·1	27·4 [19·6]	43·7	28·9	24·1	20·5	1·05 (1·47)	0·89 (1·23)
M	81·5	23·1 [15·5]	48·0	28·9	26·0	18·3	1·25 (1·86)	1·13 (1·68)
N	79·8	19·0 [10·9]	52·3	28·7	27·3	20·6	1·51 (2·63)	1·44 (2·50)
O	81·5	22·5 [14·8]	45·8	31·7	28·9	15·9	1·41 (2·14)	1·28 (1·95)
P	80·3	23·4 [17·5]	28·3	48·3	46·8	6·6	2·06 (2·76)	2·00 (2·67)
Q	81·9	23·4 [17·3]	27·1	49·5	48·1	5·6	2·12 (2·86)	2·06 (2·78)
R	79·7	17·7 [9·3]	28·1	54·2	53·8	10·9	3·06 (5·83)	3·04 (5·78)

* See notes to Table 1 and text.
nd = not quantitatively determined.
The levels of PUFA, EFA and *trans* bonds correspond to the lipid phase.

TABLE 4

Content of Total Saturated, Total Monounsaturated, Total Polyunsaturated, Total Essential Fatty Acids and Total *Trans* Bonds Content in the Lipid Phases of Three Fatty Foods

Sample	*Oil content* (%)	*TSFA** (%)	*TMUSFA** (%)	*PUFA** (%)	*EFA** (%)	*Total* trans* *content* (%)	*PUFA TSFA*	*EFA TSFA*
Potato crisps A_1	41·5	42·7 [37·8]*	43·9	13·4	13·3	3·4	0·31 (0·35)*	0·31 (0·35)*
Potato crisps A_2	37·4	38·8 [33·3]	34·6	26·6	25·8	1·1	0·69 (0·80)	0·66 (0·77)
Butter biscuits B_1	17·3	60·0 [45·3]	31·8	8·2	4·6	6·1	0·14 (0·18)	0·08 (0·10)
Butter biscuits B_2	15·8	58·1 [42·8]	33·4	8·5	4·9	6·3	0·15 (0·20)	0·08 (0·11)
Butter fat C_1	—	64·2 [38·0]	33·9	1·9	1·0	6·9	0·03 (0·05)	0·02 (0·03)
Butter fat C_2	—	63·1 [38·9]	34·8	2·1	1·5	6·6	0·03 (0·05)	0·02 (0 04)

* See notes to Table 1 and text.

TABLE 5
Possible Compositions* of Oil Blends Used in Margarines, Potato Crisps and Butter Biscuits

Sample	*Possible oil blend*
Margarine	
A	PHFO (90%) + PO (10%)
B	PHFO (75%) + PO (15%) + LERSO (10%)
C	BT (97%) + LRO (3%)
D	BT (95%) + SBO (5%)
E	PHFO (65%) + LERSO (25%) + PO (10%)
F	BT (95%) + SBO (5%)
G	PHFO (55%) + LERSO (25%) + PO (20%)
H	PHFO (50%) + LERSO (30%) + PO (20%)
I	PHFO (70%) + SBO (15%) + LERSO (15%)
J	PHFO (75%) + SBO (25%)
K	PHFO (70%) + SBO (30%)
L	PHFO (45%) + SBO (35%) + LERSO (15%) + PO (5%)
M	PHSBO (60%) + LERSO (25%) + PO (10%) + CNO (5%)
N	PHSBO (98%) + PKO (2%)
O	PHSBO (55%) + LERSO (30%) + PO (10%) + CNO (5%)
P	PHSBO (80%) + PO (20%)
Q	PHSBO (85%) + PO (15%)
R	SFO (55%) + PHSBO (40%) + PO (5%)
Potato crisps	
A_1	PO (80%) + PHSBO (20%) or PHLFPO (100%)
A_2	PO (65%) + SBO (35%)
Butter biscuits	
B_1	BF (45%) + PO (40%) + CNO (10%) + PHSBO (5%)
B_2	BF (45%) + PO (40%) + CNO (10%) + PHSBO (5%)

Notes: PH = partially hydrogenated, BF = butter fat, BT = beef tallow, CNO = coconut oil, FO = fish oils, LRO = lauric-rich oils (CNO or PKO), LFPO = liquid fraction palm oil (palm oleine), LERSO = low erucic rapeseed oil, PKO = palm kernel oil, PO = palm oil, SBO = soya bean oil, SFO = sunflower oil.

* Calculated from the 'typical' fatty acid composition data of pure oils given by Vergroesen (1975), see pages 22 to 27.

Oils

With a few exceptions, e.g. rapeseed and soya bean oils (Table 1), the total contents of PUFA and EFA in each of the various crude oils are very similar. Crude oils, and most of the commercial samples investigated, contained very low levels of *trans* double bonds, confirming the existence of PUFA in the *cis*, *cis*-configuration and showing that, in these cases, extraction/refining had caused insignificant change. The ratios of the PUFA and EFA to the saturated fatty acids are quite similar, the highest ratio of 5·1 being in a normal refined commercial safflower seed oil (high linoleic type) (Table 2). An oil extracted from safflower seeds of unknown origin (Table 1) contained a low level of both PUFA (determined by packed column GLC) and EFA (by the lipoxidase method). This is well within the range of linoleic and linolenic acids (11–19%) reported for the high oleic variety of safflower seeds (Swern, 1979). Commercial safflower seed oil is still primarily a 'high linoleic' type and FAO/WHO (1979) have published the ranges of linoleic and linolenic acids for 'high linoleic' type safflower oils. Our results for the normal safflower seed oil were within these ranges.

Margarines and butters

Eleven of the eighteen margarine samples (covering different brands, both hard and soft type) were found to have PUFA levels much higher than those of the EFA (Table 3). Obviously, in these eleven samples the calculated ratios of EFA to the total saturated fatty acids are significantly lower than the ratios of PUFA to saturated fatty acids. Margarine samples A, B, E, G, H, I, J, K and L were judged, from the fatty acid data, to contain partially hydrogenated fish oils (PHFO, Table 5). The *trans* contents of these samples were high (21–42%) and within the ranges reported by other workers (Zalewski & Kummerow, 1968; Carpenter & Slover, 1973; Smith *et al.*, 1978). Some high PUFA, high EFA, margarines (P, Q and R), and some low EFA samples (C, D and F) were noted to have low *trans* values. Butter fat samples (Table 4) have low EFA levels; these are similar to levels present in margarine A containing partially hydrogenated fish oils. In contrast, the butter fat samples contain much lower levels of *trans* double bonds than any of the margarines containing PHFO (Table 3 and 4).

Biscuits and crisps

Oil contents of the butter biscuits and potato crisps were in the region of 16–17% and 37–42%, respectively. The PUFA and EFA contents of crisp oil A_2 were about twice the amounts present in oil A_1. It appears that most of the PUFA in both exist as true essential fatty acids (Table 4). The two butter biscuit fats were estimated to comprise 45% butter oil, 40% palm oil, 10% coconut oil and 5% partially hydrogenated soya bean oil. Only 55% of the PUFA contents were found to be in the *cis*, *cis*-1,4-diene configuration. The *trans* contents of these samples were similar to those in butter fats.

CONCLUSIONS

The six crude vegetable oils and seven commercial oil samples had low levels of *trans* double bonds. The polyunsaturated fatty acids (PUFA) of most of these oils existed mainly in the *cis*-configuration, i.e. as true essential fatty acids (EFA). The safflower oil of the 'high linoleic' type had the highest ratio, of 5·1, between the PUFA or EFA and the saturated fatty acids.

Nine out of eighteen margarines were estimated to contain partially hydrogenated fish oils (45 to 90%, on a fat basis), and had high levels of *trans* double bonds. The EFA levels of these are considerably lower than those of the PUFA. The high EFA margarines containing soya bean oil or sunflower oil, and the low EFA margarines containing beef tallow (C, D and F) had low *trans* contents. Butter oils have much lower *trans* double bond levels in comparison with a sample of partially hydrogenated fish oil margarine having a similar amount of EFA.

Almost all the PUFA in the oils of two crisp samples were present as EFA. Only 55% of the PUFA in two biscuit fats existed as true essential fatty acids.

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