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The vitamin E content of margarine

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The destruction of vitamin E by chlorine dioxide, the agent at present most commonly used in Great Britain for 'improving' flour, is now well established (Moran, Pace & McDermott, 1953, 1954; Frazer, Hickman, Sammons & Sharratt, 1956; Moore, Sharman & Ward, 1957). If vitamin E is required by man, as seems probable from, *inter alia*, its presence in human blood plasma, it may be important to assess the amount of the loss. Information is therefore needed about the proportion of our total intake of vitamin E that could be provided by flour. As a basis for our calculations information must be available about the vitamin E contents of all the other dietary components. According to figures already published, which unfortunately have sometimes been based on obsolete methods of analysis, margarine and cooking fat can make by far the most substantial contributions to our vitamin E supplies. Accurate knowledge of their vitamin contents, of the relative proportions of the various tocopherols present and of the effect of biological differences associated with their different chemical structures, must therefore be obtained.

Up to the present only scanty information is available about margarine manufactured abroad, and none at all about margarine made in Britain. Lundborg (1945) reported from Sweden that margarine derived from sunflower-seed oil, coconut oil, rape-seed oil and melted lard contained 8·9 mg tocopherols/100 g. In clarified American margarine, however, Quaife & Harris (1948) found the much higher content of 28·4 mg α-tocopherol/100 g. Both α-tocopherol (28 mg/100 g) and γ- and δ-tocopherols (amounting together to 26 mg/100 g) were later reported by Harris, Quaife & Swanson

(1950). More recently Hellström & Andersson (1956) have examined specimens of Swedish margarine, collected from six producers over an 18-month period, by a one-dimensional chromatographic method. They found an average of 4·45 mg for the trimethyl tocol (α), 10·77 mg for the dimethyl tocols (β, γ and ζ) and 0·68 mg/100 g for the monomethyl tocols (δ, η and ε).

Although these findings differ among themselves, they agree in indicating a much higher vitamin E value for margarine than for butter, its natural counterpart. Thus the tocopherol content of butter reported by various workers ranges from 2·1 to 3·3 mg/100 g. None of the methods employed before 1953 measured α-tocopherol itself; it is likely, therefore, that many of the values given in the literature for both margarine and butter include irrelevant reducing substances and may be unduly high.

EXPERIMENTAL

Materials. Margarine was obtained, with the assistance of the Ministry of Food, from each of the eleven factories producing this food for retail sale in the latter half of 1953. Of the thirteen samples supplied, ten were labelled 'Special margarine' and three 'Kosher margarine'. The samples were made from four different oil mixtures, as indicated in Table 1. Some of the groundnut oil, most of the palm oil and all the whale oil was hydrogenated. The percentage of hydrogenated oils used, which was not known, was varied according to the seasonal temperature. Samples of five branded American margarines were also examined, but no details were available of their composition. For comparison three samples of butter from different areas of the world were purchased locally. At a later date typical samples of oils used in the margarines were also analysed for their tocopherol contents.

Table 1. *Percentage composition of controlled margarine (United Kingdom, 1953)*

Type	Groundnut oil	Coconut oil	Palm-kernel oil	Palm oil	Whale oil
1	27	23	20	8	22
2	27	23	20	30	—
3	34	45	—	—	21
4	20	80	—	—	—

Methods. The tocopherol content was estimated by the method of Brown (1952) as modified later by Eggitt & Ward (1953). The procedure consists of the removal of lipids by saponification with potassium hydroxide in the presence of pyrogallol (Tosic & Moore, 1945), elimination of sterols by crystallization from methanol at $-10°$, adsorption of vitamin A and carotenoids on activated Floridin earth (Emmerie & Engel, 1939) and separation of the tocopherols according to the number of methyl groups present in the molecule by means of reverse-phase one-dimensional chromatography. Difficulty in saponification of the large quantity of fat (10 g samples were taken) was overcome by using 50% more potassium hydroxide than did Tosic & Moore. Only a small amount of sterols crystallized out from the methanol, and this step was later omitted. The component oils were analysed by the method of Green, Marcinkiewicz & Watt (1955). The final stage of this procedure consists of two-dimensional chromatography, which separates the tocopherols into five zones, α, ζ, $\beta+\gamma$, $\epsilon+\eta$ and δ.

RESULTS

British margarine. The vitamin E contents of the British margarines are given in Table 2. With all the margarines four spots or zones were observed on the chromatogram; at the origin and in the α-, dimethyl- and monomethyl-positions. Upon spraying with diazotized *o*-dianisidine the presence of γ-tocopherol was confirmed, but the spot in the δ-position only gave a weak reaction. Analysis of the constituent oils indicated that ζ-tocopherol was present as well as γ- and that the spot in the δ-position was largely due to η-tocopherol.

Table 2. *Mean tocopherol content of British margarine* (*mg*/100 *g*)

Type	No. of samples	α-Tocopherol	$\beta+\gamma+\zeta$-Tocopherols	$\delta+\epsilon+\eta$-Tocopherols
1	6	2·69 (1·01–5·58)	2·24 (0·45–3·47)	0·93 (0·28–1·83)
2	4 (two Kosher)	4·20 (3·14–5·30)	3·49 (2·90–3·89)	2·32 (0·70–5·05)
3	2	1·56 (1·47–1·64)	1·20 (0·82–1·58)	0·19 (0·11–0·34)
4	1 (Kosher)	0·69	0·80	0·27

A bright red band was observed on the Floridin-earth column, below that due to vitamin A, from samples of margarine of types 1, 3 and 4. It was apparently caused by the use of aniline dye in those samples that did not depend for their yellow colour on the natural pigments of palm oil. Great difficulty was experienced in removing the dye from samples of type 3.

The α-tocopherol content ranged from 0·69 to 5·58 mg/100 g, with means of 2·69, 4·20, 1·56 and a single value of 0·69 mg/100 g respectively for the four different types of oil mixtures. Similarly, the dimethyl-tocopherol content ranged from 0·45 to 3·89 mg/100 g, with means of 2·24, 3·49, 1·20 and a single value of 0·80 mg/100 g. The monomethyl content, presumably largely η-tocopherol, ranged from 0·11 to 5·05 mg/100 g, with means for the four types of 0·93, 2·32, 0·19 and a single value of 0·27 mg/100 g, respectively.

American margarine. The tocopherol content of the American margarine (Table 3) was several times higher than that of the British margarines. The chromatograms had four spots; at the origin and in the α-, γ- and δ-tocopherol positions. Spraying with *o*-dianisidine clearly showed the presence of both γ- and δ-tocopherols. Only one of the samples (Nuc.) was coloured with carotene, the rest containing a dye similar to that used in the British margarines.

Butter. The α-tocopherol content of butter was, in mg/100 g, 1·36 for Dutch butter, 1·96 for Danish and 1·62 for New Zealand, with a mean of 1·65. Only two spots were observed on the chromatogram, at the origin and in the α-position.

Constituent oils. The tocopherols content of representative constituent oils is shown in Table 4. Palm-kernel, coconut and whale oils contain almost no vitamin E. The tocopherols of palm oil have been previously reported to be made up of 54% of α- and 46% of $\gamma+\delta$ (Harris *et al.* 1950). In the present work it has been found that palm oil contains α-, ζ- and η-tocopherols; no γ- or δ-tocopherol could be detected on the chromatograms. It was interesting to find that hydrogenation of the groundnut and palm oils resulted in considerable destruction of the tocopherols. It is evident from their stated constituent oils that the spot in the δ-position found in certain British margarines was in fact due to η-tocopherol.

Table 3. *Tocopherol content of American margarine (mg/100 g)*

Type	α-Tocopherol	$\beta+\gamma+\zeta$-Tocopherols	$\delta+\epsilon+\eta$-Tocopherols
Kr	3·3	13·0	7·3
Sw	12·7	28·3	10·1
Nuc.	10·8	27·0	8·7
B.B.	15·8	35·1	11·5
G.L.	8·6	27·1	20·1
Mean	10·2	28·1	11·5

Table 4. *Tocopherol content of constituent oils of margarines*

Oil	Total tocopherol (mg/100 g)	Percentage of total α	γ	δ	ζ	η
Coconut	0	—	—	—	—	—
Palm-kernel	0	—	—	—	—	—
Groundnut	20·3	47·6	52·4	—	—	—
Groundnut, hardened	3·3	54·6	45·4	—	—	—
Palm	32·7	49·8	—	—	34·1	16·1
Palm, hardened	9·7	57·5	—	—	26·0	16·5
Whale, hardened	0·58	100	—	—	—	—
Soya-bean	85·6	7·5	68·8	23·6	—	—
Cottonseed*	81·0	58·0	42·0	—	—	—

* Commercial sample, not purified for use in margarine.

DISCUSSION

From these results it is clear that the vitamin E content of margarine varies greatly, according to the oils from which it is made. A few oils are rich sources of the vitamin, whereas others are negligible sources. Thus the high vitamin E content of some American types of margarine is probably due to the use of cottonseed or soya-bean oils, whereas the lower content of British margarines results from using red palm oil and groundnut oil. Our information on the potencies of the various types may be summarized as follows:

Type of margarine	Tocopherol-containing oils used	Mean total tocopherol (mg/100 g)	Mean α-tocopherol (mg/100 g)
American	Cottonseed, soya-bean	49·8	10·2
British: type 1	Groundnut, whale (low percentage of palm)	5·86	2·69
type 2	Groundnut, whale (high percentage of palm)	10·01	4·20
type 3	Groundnut, whale	2·95	1·56
type 4	Groundnut	1·76	0·69

Even with margarines of the same type, large variations were observed in the α-tocopherol contents. Thus the α-tocopherol content of type 1 British margarine ranged from 1·01 to 5·58 mg/100 g. This wide range is probably related to variations in the amount of hardened oils used and to the treatment received by the oil during refining and deodorizing. In spite of this uncertainty, there was good agreement between the mean values found for the α-tocopherol contents of different margarines and the values calculated from the oils used. Thus if it is assumed that 30% of the groundnut and 80% of the palm oil of British margarine was hardened, the α-tocopherol contents of the four types would have been 2·77, 4·30, 2·62 and 1·46 mg/100 g, respectively.

The α-tocopherol content of the type 2 margarine agrees closely with those from Sweden, but the γ-tocopherol content is considerably less. This is possibly due to the use in Sweden of oils rich in γ-tocopherol, such as cottonseed.

The high value of 28·4 mg α-tocopherol/100 g, reported by Harris *et al.* (1950) may have been due to the technique employed. The difference between the total reducing substances and the sum of γ- plus δ-tocopherols was assumed to be solely due to α-tocopherol. This assumption seems questionable as examination by paper chromatography was not done.

For butter the observed mean value of 1·65 mg/100 g was lower than those found for all but four of the margarine samples. In contrast to what was found in margarine, the only tocopherol present was the α- form. The higher values reported previously (Emmerie & Engel, 1943; Kofler, 1943; Lieck & Willstaedt, 1945; Harris *et al.* 1950) were probably due to the presence in butter of reducing substances that are not tocopherols.

According to the figures published by the Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1955), the mean weekly intake of margarine in 1952–3 was 4·28 oz. (121 g). At that time 75% of the margarine consumed was of type 2, which has an average α-tocopherol content of 4·2 mg/100 g. Thus the α-tocopherol intake/head/week was of the order of 5·0 mg for the majority of the consumers of margarine. The mean α-tocopherol content of all thirteen samples of margarine, however, was 2·83 mg/100 g which would lower the mean intake to 3·4 mg/head/week. In contrast the highest published American figures would indicate an intake of 34 mg. Reliable information on the contribution of vitamin E made by margarine, in any country, can only be derived from accurate estimations on the particular types of margarine in use.

Calculations made in this laboratory have indicated the mean expected weekly intake from untreated bread and flour to be about 2·5 mg, which would be reduced to about 0·5 mg if the bread was made from flour treated with a destructive improver. The intake from margarine therefore can be over twice the loss expected from such treatment of the bread.

SUMMARY

1. Margarine was analysed for its tocopherol content in order to assess its possible contribution to the weekly human intake of vitamin E.

2. Samples of British margarine manufactured in late 1953 from four different oil mixtures, and typical constituent oils were examined for their tocopherol contents. Samples of five brands of American margarine and three butter samples purchased locally were examined for comparison.

3. The α-tocopherol content of the British margarine ranged from 0·69 to 5·58 mg/100 g, of the American margarine from 3·3 to 15·8 mg/100 g and of the butter from 1·36 to 1·96 mg/100 g.

4. Of the five oils used in the British margarine, only groundnut and palm oils contributed significant amounts of tocopherol.

5. The results indicate that the weekly intake of α-tocopherol from British margarine would vary with the type of oils used in its manufacture. The highest expected weekly intake in 1952–3 would have been about 5 mg/head.

6. The magnitudes of the amounts of vitamin E supplied by margarine and the amounts lost from bread from destruction by certain flour improvers are compared.

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