stability test on the soya oil showed that TBHQ provides maximal protection at frying conditions (Fig. 13). Actual frying tests preparing potato chips with soya oil as a frying medium illustrate the "carry-through" effectiveness of TBHQ into fried foods—even at relatively low concentrations (Fig. 14). The low effectiveness of BHA and BHT antioxidants also is illustrated.

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Metabolic Aspects of Positional Monounsaturated Fatty Acids Isomers

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ABSTRACT

Absorption and distribution of positional *cis* and *trans* octadecenoic acid isomers in lipids from rat, egg and human tissues are reviewed. Selected data on enzyme, single-cell, and whole-animal studies with positional octadecenoic acid isomers are summarized and compared.

INTRODUCTION

Partial hydrogenation of soya oil is used to increase the flavor and odor stability by reducing the level of polyunsaturated fatty acids and, in particular, the linolenic acid content. Hydrogenation or hardening also imparts desirable physical qualities useful in the formulation of margarines and shortenings. Soya oil products are highly palatable and are widely consumed in the U.S. In fact, it can be estimated that hydrogenated soya oil contributes ca. 22% of the visible and nonvisible fat consumed in the American diet. Table I illustrates the effect of various levels of partial hydrogenation on the composition of soya oil (1).

In addition to the changes in fatty acid composition, the structure of a portion of the unsaturated fatty acids is rearranged to form *cis* and *trans* positional isomers (2-4). The distribution of these positional isomers in the monounsaturated fraction from hydrogenated soya oil is illustrated in Figure 1 for a commercial nickel hydrogenated soya oil.

The contribution of positional isomers to the daily American diet is estimated in Table II (5) to be ca. 9 g, which is ca. 0.6 of a tablespoon. Thus, the individual monomer isomers contribute ca. 80 calories/day, which is not particularly significant. The nutritional and biological impact of these isomers in the human diet is difficult to assess. We do know that due to biohydrogenation, ruminant

TABLE I

Fatty Acid Composition (%) of Soybean Oil (SBO) and Partially Hydrogenated Soybean Oils (1)

Fatty acid[a]	SBO	HSBO-1[b]	HSBO-2[b]	HSBO-3[b]
16:0	11	11	11	11
18:0	4.1	4.3	7	10.5
c-18:1[c]	22	29	33	18
t-18:1[c]	–	12	12	51
9*c*,12*c*-18:2	54	31	22	–
9*c*,12*t*- and 9*t*,12*t*-18:2	–	4	6	–
9*t*,12*t*-18:2	–	–	–	–
9*c*,13*t*- and 8*t*,12*t*-18:2	–	2	4	9
Conjugated isomers	–	2	0.5	
18:3 (all isomers)	7.5	2.3	2.0	–
>20:0	1	1	1	1

[a]Abbreviations used: Geneva numbering system used for fatty acids, where the first number indicates the position of the double bond; the *c* or *t* following the first number indicates *cis* or *trans* configuration, if known; and the second number indicates number of carbons in the acyl chain, with the number following the colon designating the number of double bonds.
[b]HSBO = partially hydrogenated soybean oil.
[c]Includes positional octadecenoic acid isomers.

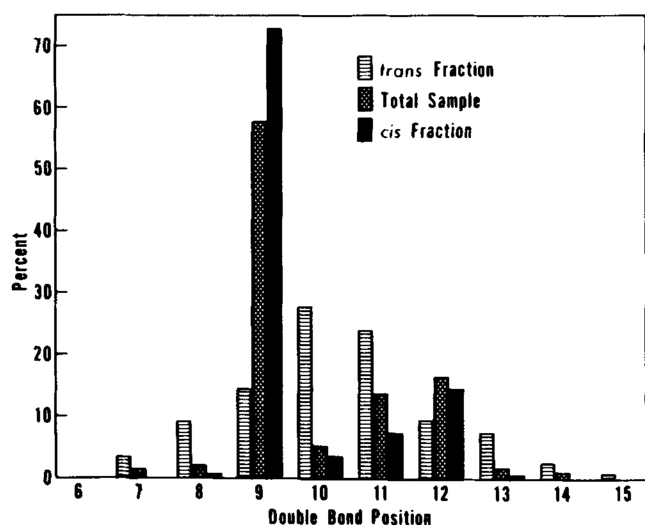

FIG. 1. Distribution of *cis-; trans-;* and total positional octadecenoic acid isomers in a salad oil containing commercially hydrogenated soya oil.

fats contain most of the isomers present in hydrogenated soya oil. In fact, 437 fatty acids have been identified in bovine milk fat (6). Distribution of the major monounsaturated isomers in butter is given in Figure 2 (7). These fatty acid isomers have been consumed by man for centuries, and appreciable levels of hydrogenated soya oil have been consumed since about 1940 with no obvious short-term deleterious effects. Long-term, multiple-generation feeding studies with rats have indicated no apparent effect on growth, size or reproduction, and pathological changes in vital organs were not detected (8).

Absorption of Hydrogenated Soya Oil and Specific Isomers

How well isomeric fats are absorbed has been fairly well established. The digestibility of various fats is shown in Table III and indicates that hydrogenated vegetable oils are well absorbed (9). The triglycerides associated with chylomicrons, which are the initial carriers of ingested fat, have been analyzed from humans fed deuterium-labeled 9*c*-; 9*t*-; 12*t*-; and 12*c*-18:1 (10). It is apparent from the curves in Figure 3 that 9*t*-; 12*t*-; and 12*c*-18:1 isomers are absorbed at a rate almost exactly equal to oleic acid.

Analysis of the positional isomers in human tissue confirms that all the positional isomers normally present in hydrogenated soya oil are absorbed and incorporated into the tissue lipids (11). Discrimination does occur against some of the isomers, particularly in the liver for 10*t*- and 10*c*-18:1 (see Fig. 4). Dietary histories on these subjects indicate that, despite large variation in the amount of hydrogenated fat consumed, a surprisingly constant level of positional isomers in the human adipose tissue was found which resembles the positional isomer distribution in hydrogenated soya oil. These data suggest that accumulation of isomers beyond a specific level in tissue is moderated by the body.

Distribution of Positional Isomers in Lipid Classes

Analysis of liver lipids from rats fed partially hydrogenated safflower oil demonstrates that the positional 18:1 isomers are both discriminated against and preferentially incorporated into rat liver phosphatidylcholine (12). The most striking feature of the data in Figure 5 is the strong discrimination against the 10*c*- and 10*t*-18:1 isomers and the strong selective incorporation of the 12-, 13-, and 14-18:1 isomers compared to 9*c*-18:1. The data in Figure 5 are plotted as selectivity values which are the log of the isomer/9*c*-18:1 ratio in the sample divided by the isomer/9*c*-18:1 ratio which was fed. Data for rat kidney, muscle, heart, lung, spleen and adipose phosphatidylcholine samples are similar to the liver data for the *cis* positional isomers (12). Selectivity values for *trans*-18:1 isomers showed greater variation among the various tissue lipid samples.

Radioisotope-labeled positional isomers have been fed to the laying hen and their incorporation into egg lipids compared to oleic acid (13). Selectivity values for egg triglycerides and phospholipids also are included in Figure 5 for comparison with rat liver phosphatidylcholine selectivities.

Selective incorporation of 8*t*- and 12*t*-18:1 vs 9*c*-18:1 in egg lipid classes is compared in Figure 6 (14). Incorporation of both the 8*t*- and 12*t*-18:1 isomers into egg lipids follows a very similar pattern, except for cholesteryl ester. Selectivities for the 12*t*-18:1 isomer also are larger than for the 8*t*-18:1 isomer. Large preferential incorporation of these isomers was found for the 1-acyl position of both phosphatidylethanolamine (PE) and phosphatidylcholine (PC), and negative selectivities were found for the 2-acyl position. The large difference in the selectivities of cholesteryl ester for 8*t*- and 12*t*-18:1 reflect the importance of double-bond position in determining the distribution of these isomers.

Selectivity values for various blood plasma lipid classes from human studies in which deuterium-labeled 9*t*-; 12*t*-; and 12*c*-18:1 isomers were fed are shown in Figure 7 (10,15,16). These values indicate that the 9*t*- and 12*t*-18:1 isomers both are preferentially incorporated into PC and sphingomyelin but to a much less extent than the 12*c*-18:1 isomer. In contrast to these positive selectivities, the cholesteryl ester fraction exhibited a very strong discrimination against the 9*t*- and 12*t*-18:1 isomers and a small negative selectivity for the 12*c*-18:1 isomer.

Selectivity values for the acyl positions of human plasma PC, using phospholipase-A_2 to remove the fatty acid in the 2-acyl position, are given in Table IV (10). The values in this table show that both the 9*t*- and 12*t*-18:1 isomers are

TABLE II

Estimated Daily Consumption of Specific Positional Octadecenoic Acid Isomers (5)[a]

	Positional octadecenoic acid isomer (g)									
	6	7	8	9	10	11	12	13	14	Total
c-18:1	0.01	0.14	0.24	6.41	0.37	0.54	0.68	0.14	0.03	8.6
t-18:1	0.01	0.27	0.65	1.56	1.53	1.26	0.82	0.48	0.29	6.8
	Total isomer content minus 9*c*-18:1 = 9.0 g									

[a]Based on estimated content of *c*-18:1 and *t*-18:1 in hydrogenated soybean oil of 25 and 20%, respectively, and on an estimated daily per capita consumption of 34 g hydrogenated vegetable oil.

selectively incorporated into the 1-acyl position of PC but not into the 2-acyl position, whereas 12*c*-18:1 is selectively incorporated into both the 1- and 2-acyl positions. The difference between the 12*c*/9*c* incorporation into the 1- and 2-acyl PC positions is 38.4-fold compared to 5.4-fold for 12*t*/9*c*. The large selectivity values for 12*c*-18:1 suggest that 12*c*-18:1 is being distributed similarly to linoleic acid because of the location and configuration of the 12*c* double bond. These data indicate that the 12*c* double bond may be of particular importance in the control of fatty acid distribution in lipid classes. However, the lack of accumulation of the 12*c*-18:1 isomer in human tissue lipids (as shown earlier in Fig. 4) indicates that lipid metabolism is controlled in order to prevent unusually high accumulation of this isomer.

Enzymatic Studies

In vitro studies have been used to determine the influence of double bond position and configuration on the activity of a variety of enzymes. These studies have utilized enzymes responsible for acyl transfer to phospholipids, cholesteryl ester hydrolysis and formation, acyl CoA activation, desaturation, elongation, triglyceride hydrolysis and β-oxidation. Space does not permit a complete review of the many excellent papers in this area; consequently, only cursory coverage is included in this paper. A more complete review of research involving isomeric fatty acid and specific enzyme systems has been published recently (17).

The enzyme acyl CoA:phospholipid acyl transferase (EC 2.3.1.23) is involved in the transfer of fatty acid CoA derivatives to lysophosphatidylcholine to form PC. The effect of double bond position and configuration on the rate of transfer to 1-acyl and 2-acyl PC is shown in Figure 8 (18). This figure shows a striking variation in the reaction rates, which are dependent on both the double bond configuration and position, and is useful in rationalizing the selectivity data from rat, chicken and human studies. In general, the various *cis* and *trans* 18:1 isomers in hydrogenated soya oil are transferred at a higher rate to the 1-acyl PC position than for 9*c*-18:1. In this respect, the fatty

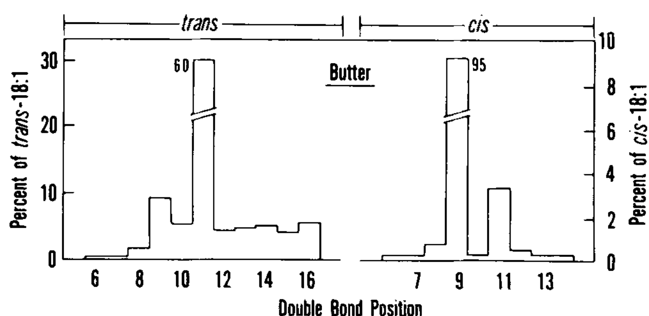

FIG. 2. Distribution of *cis* and *trans* positional octadecenoic acid isomers in butter (7).

TABLE III

Digestibility of Fats (9)

Fat or oil	Melting point (C)	Coefficient of digestibility (%) Human	Rat
Mutton	50	88.0	84.8
Hydrogenated cottonseed	54	–	68.7
Hydrogenated cottonseed	46	94.9	83.8
Hydrogenated peanut	50	92.0	–
Hydrogenated peanut	52.4	79.0	–
Hydrogenated corn	43	95.4	–
Margarine	35	96.7	97.0
Soybean	–	–	98.5
Lard	37	–	96.6
Butter	34.5	–	90.7
Tripalmitin	66.5	–	27.9
Tristearin	70	–	18.9

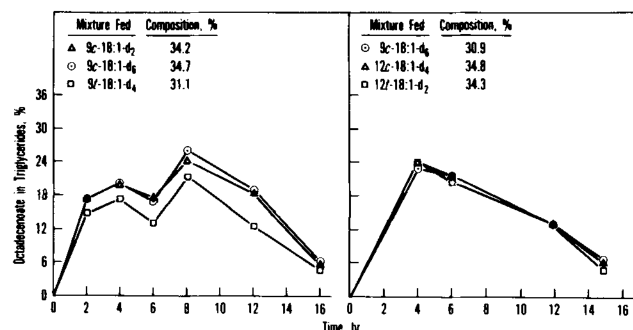

FIG. 3. Uptake of deuterated *trans*-9-; *trans*-12-; *cis*-12-; and *cis*-9-octadecenoic acids into human chylomicron triglycerides.

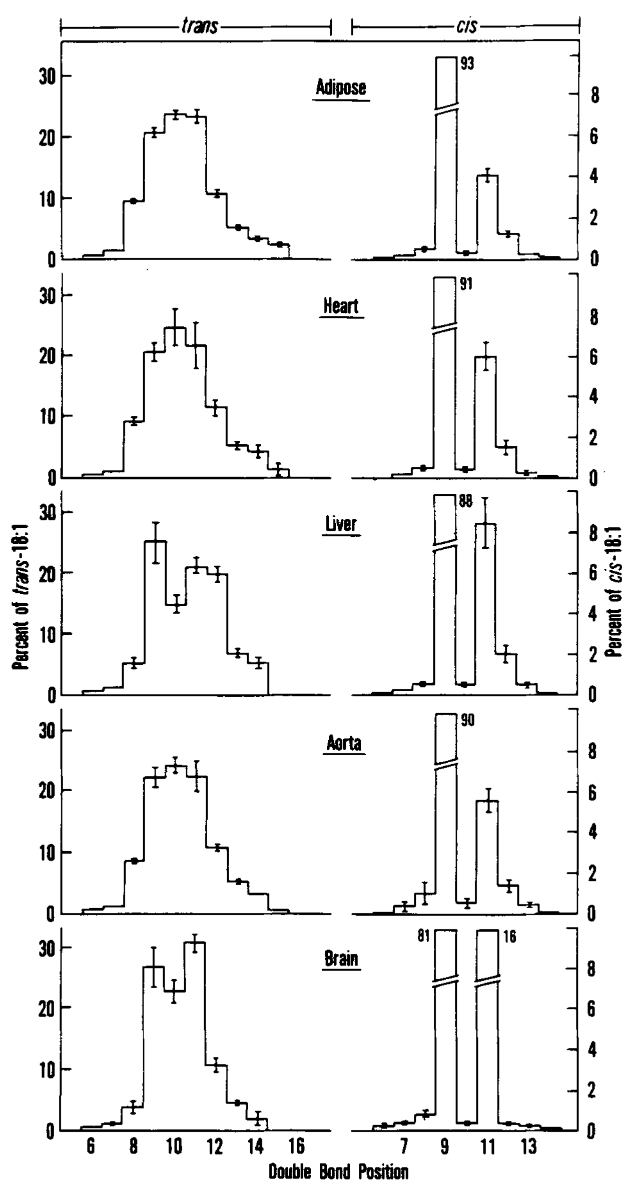

FIG. 4. Distribution of *cis* and *trans* positional octadecenoic acid isomers in various human tissue total lipids.

acid isomers resemble saturated fats. The relative reaction rates for transfer of fatty acids to the 2-acyl position of PC also show dramatic variations among different isomers but, in contrast to 1-acyl PC, the 2-acyl PC substrate has the highest rate of reaction with 9*c*-18:1. With both 1-acyl and 2-acyl PC as substrates, the 12*c*-18:1 gave relatively high reaction rates which suggests that the acyl CoA-phospholipid acyl transferase is involved in producing the high selectivities found for 12*c*-18:1 in human plasma lipids (Fig. 7). Also, the low 10*t*-18:1 transfer rates for the 1-position of PC indicate acyl CoA:phospholipid acyl transferase has a major role in the distribution of this isomer in rat liver phosphatidylcholine (Fig. 6) and in human liver tissue (Fig. 4).

The dependence of rat liver cholesteryl ester hydrolase (EC 3.1.1.13) activity on double bond position is shown in Figure 9 for a series of cholesteryl *cis*-octadecenoates (19). This enzyme is not as sensitive as the acyl transferases to double bond position. The rate of hydrolysis of the cholesteryl ester substrates gradually decreases the further the double bond is located from the center of the fatty acid chain, which suggests that once the cholesteryl ester of the fatty acid isomer is formed, its rate of turnover in tissue lipid should be slower.

The double bond position for the 7 through 16 *cis*-18:1 isomers has little effect on the rate of hydrolysis of triglycerides by pancreatic lipase (EC 3.1.1.3) (20). This lack of discrimination probably accounts for the lack of discrimination against absorption of triglycerides containing monounsaturated isomers.

The rate of formation of the acyl CoA esters of positional *trans* and *cis* fatty acid isomers by rat liver microsomes and mitochondria was found to be dependent on the double bond position and configuration. The reaction rates for the 4-15 *trans* positional isomers were determined and found to be lowest for the 9*t*-18:1 isomer (21). At similar fatty acid concentrations, there was no difference in the rate of activation for the *cis*-18:1 positional isomers (22). However, when the concentration of the *cis* positional isomers was tripled, two- to three-fold differences were found in the rate of CoA ester formation. Somewhat surprisingly, the 8*c*-, 9*c*-, and 10*c*-18:1 isomers had the lowest rates; the fatty acids containing double bonds farthest from the center of the fatty acid chain had the highest rates. Considerable variation in the maximal rate of acyl CoA ester formation for the various *cis* and *trans* positional isomers was also found to be dependent on pH, age of microsomal preparation, fatty acid concentration and temperature, and illustrates the difficulty of obtaining in vitro data comparable to in vivo data.

Nutritional Effectiveness in Single-Cell Organisms

Studies with single-cell organisms forced to survive on specific isomeric fatty acids have determined that nutritional effectiveness of the fatty acid is markedly reduced when the double bond is at either end of the fatty acid chain (23). Figure 10 summarizes the effectiveness of the *c*-18:1 positional isomers for supporting growth of *Saccharomyces cerevisiae* and *Escherichia coli*. The 12*c*-18:1 isomer, which was found to be selectively incorporated into human blood plasma phospholipids, has a very low nutritional effectiveness in these organisms. The data in Figure 10 also show no correlation between the melting point of the positional 18:1 isomers and their nutritional effectiveness.

In Vivo vs In Vitro Results

In considering the metabolism of *cis* and *trans*-octadecenoic acid positional isomers present in hydrogenated soya oil, the following features are apparent. Absorption or digestibility in vivo is not a problem because of the lack of pancreatic lipase specificity which has been demonstrated in vitro. Both in vitro and in vivo studies indicate that some enzymes responsible for lipid metabolism obviously are capable of recognizing differences in double bond position and configuration. Consequently, each isomer is distributed differently into the various lipid classes by factors which

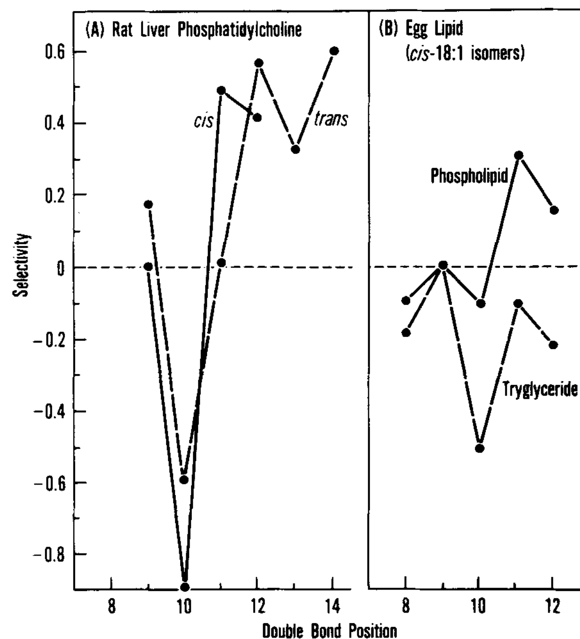

FIG. 5. Relative incorporation of octadecenoic acid isomers into rat liver phosphatidylcholine (A) and egg phospholipid and triglyceride (B). Data from references 12 (A) and 13 (B).

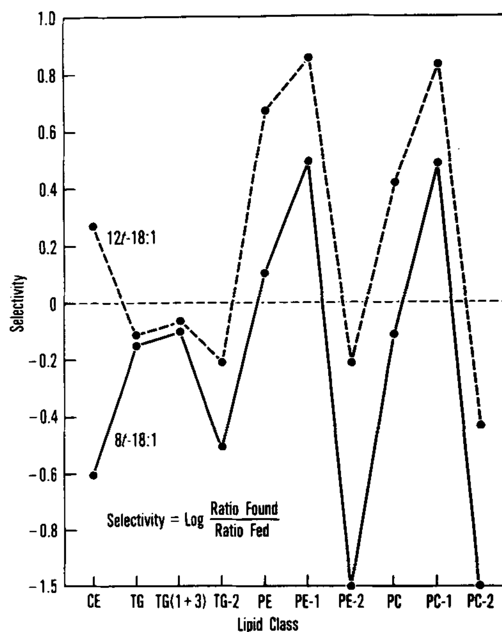

FIG. 6. Relative incorporation of *trans*-8- and *trans*-12-octadecenoic acid vs *cis*-9-octadecenoic acid into egg lipid classes (14). Abbreviations: CE = cholesteryl ester; TG = triglyceride; TG (1+3) = 1- and 3-positions of triglyceride; TG (2) = 2-position of triglyceride; PE = phosphatidylethanolamine; PE-1 = 1-position of phosphatidylethanolamine; PE-2 = 2-position of phosphatidylethanolamine; PC = phosphatidylcholine; PC-1 = 1-position of phosphatidylcholine; PC-2 = 2-position of phosphatidylcholine.

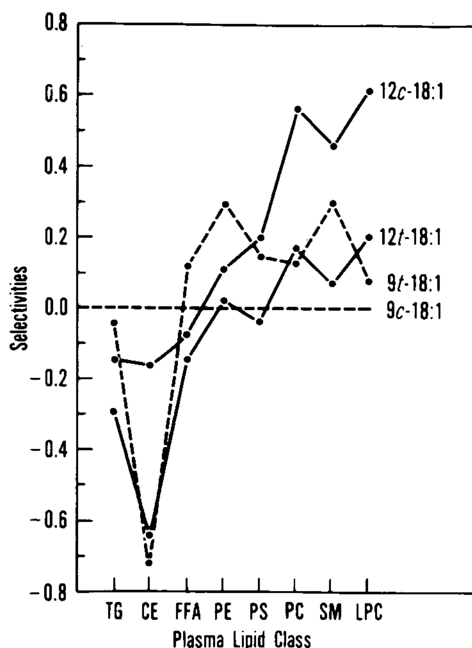

FIG. 7. Selectivity values for incorporation of *trans*-12-; *cis*-12-; and *trans*-9-octadecenoic acid relative to *cis*-9-octadecenoic acid in human plasma lipid classes (10,15,16). Abbreviations: (see Fig. 6 for CE, TG, PC and PE). FFA = free fatty acid; PS = phosphatidylserine; SM = sphingomyelin; LPC = lysophosphatidylcholine.

have yet to be identified. This preferential distribution is not unusual, because selective distribution of the "more common" dietary fatty acids such as stearate, oleate and linoleate is well known. For example, saturated fats are esterified at the 1-acyl position of phospholipids, and polyunsaturated fats are concentrated in the 2-acyl position of phospholipids and in the cholesteryl esters. In fact, this selective utilization of fatty acids may allow tissue membranes to achieve an optimal physical state suitable for cell function. Thus, the presence of a variety of fatty acids, including isomeric fatty acids, may help the body achieve this goal.

Numerous in vitro studies with specific enzymes have advanced our understanding of how the control of fatty acid distribution occurs. In particular, the phospholipid acyltransferases appear to have an important role in this process. However, determination of the octadecenoic acid isomer content of rat and human tissue lipids provides evidence that the selectivity of specific enzymes in in vitro studies are strongly modified in vivo. Analysis of human tissues from subjects consuming hydrogenated fats indicate that a threshold level exists, at which point the accumulation of the various isomers tends to be self-limiting. For example, if the strong selectivities observed for the 12*c*-18:1 isomer in human blood PC were not being modified, the tissue lipids, over a period of years, would contain

TABLE IV

Incorporation of 12*t*-18:1, 12*c*-18:1 and 9*t*-18:1 vs 9*c*18:1 into Plasma 1- and 2-Acyl Phosphatidylcholine (10,16)

Phosphatidylcholine	Selectivity 12*t*/9*c*	12*c*/9*c*	9*t*/9*c*
Total	0.297	0.562	0.127
1-Acyl	0.731	0.511	0.566
2-Acyl	-0.854	0.656	-0.167

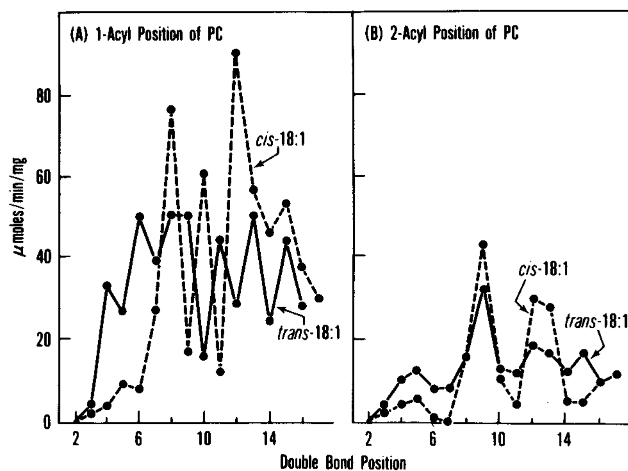

FIG. 8. Acyl transferase specificities of rat liver microsomes for incorporation of octadecenoic acid isomers into 1-acyl position of PC (A) and 2-acyl position of PC (B) (18).

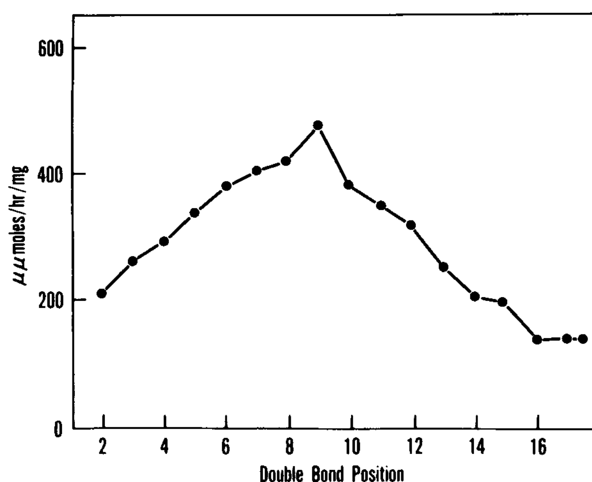

FIG. 9. Cholesteryl ester hydrolase specificities for cholesteryl *cis*-octadecenoate isomers (19).

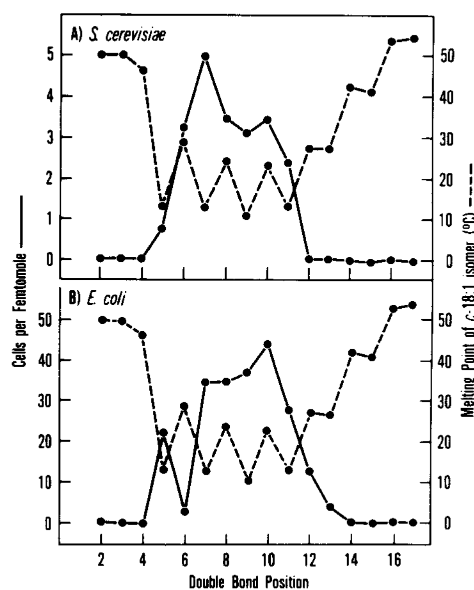

FIG. 10. Nutritional effectiveness of *cis*-octadecenoic acid isomers for supporting growth in single-cell organisms. (A) *Saccharomyces cerevisiae* and (B) *Escherichia coli* (23).

predominant levels of this isomer. An increase in the β-oxidation and turnover rates of the isomeric fatty acids at higher dietary levels may explain this observation.

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Nutritional Consequences of Processing Soybean Oil

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ABSTRACT

A major objective of commercial processing of soybean oil into edible products is to remove unwanted impurities from the oil with the least possible effect on nutritional quality of the oil. Soybean oil is an excellent dietary source of essential linoleic acid and also of tocopherols, which serve as sources of vitamin E and natural antioxidants. The data presented in this report indicate that the nutritional quality of soybean oil is largely retained after typical commercial processing conditions. Hydrogenation does reduce the level of essential fatty acids; however, typical commercial salad and cooking oils and shortenings made from partially hydrogenated soybean oil retain nutritionally significant levels of essential fatty acids. Tocopherols also are present at high levels in the finished oil. Among the unwanted components of crude soybean oil which are effectively removed by processing are pesticide residues, phosphatides, free fatty acids, color pigments, and compounds causing objectionable odors and flavors.

INTRODUCTION

Fats and oils have long been recognized as important nutrients for both humans and animals because they provide a concentrated source of energy, contain essential fatty acids and serve as carriers for fat-soluble vitamins. Soybean oil, in particular, is an excellent dietary source of linoleic acid, the primary dietary essential fatty acid. An essential fatty acid is one which the body cannot manufacture and which must be supplied by the diet. Soybean oil also contains significant levels of tocopherols. One of the tocopherols, referred to as vitamin E (or α-tocopherol), is an essential nutrient for higher animals, including man, and soybean oil is a good dietary source of vitamin E. Tocopherols provide vitamin E and serve as antioxidants that help protect the oil against rancidity.

In addition to desirable nutrients, such as linoleic acid and vitamin E, crude soybean oil contains varying, but relatively small, amounts of phosphatides, free fatty acids and color pigments that contribute unwanted properties to the oil such as color, flavor, odor, or instability. Furthermore, the crude oil may contain pesticide residues resulting from agricultural practices. These unwanted substances are removed through a series of processing steps which include degumming, alkali refining, bleaching and deodorization. Another processing step, hydrogenation, is frequently used to improve the stability of soybean oil by reducing the level of linolenic acid, which is highly susceptible to oxidation. Hydrogenation also is important for converting liquid oils to a semisolid form for greater utility in certain food uses.

One of the objectives of processing is to remove the objectionable impurities from the oil with the least possible effect on the glycerides (which contain linoleic acid) or the tocopherols, and with the least possible loss of oil. This report reviews how the various processing steps may affect the nutritional quality of soybean oil—in particular, how processing may affect the levels of linoleic acid and vitamin E. In addition, the effectiveness of processing in removing undesirable pesticide residues resulting from agricultural practices is discussed.

Principal Processing Steps

The major processing steps for converting crude soybean oil into edible products are reviewed in several publications (1-3). Crude oil usually is prepared by extracting soybean flakes with hexane and then removing the solvent. The crude oil contains significant quantities of phosphatides, which are largely removed by degumming, a process that