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

involves treating the crude oil with water to convert the phosphatides to hydrated gums which are insoluble in oil and readily removed by centrifugation.

Alkali refining removes free fatty acids and also largely eliminates residual phosphatides as well as traces of carbohydrate and protein which may be present in the crude oil. Alkali refining is done by treating the oil with sodium hydroxide or sodium carbonate (or a combination of these two) in order to convert the free fatty acids into water-soluble soaps. The soapstock is removed from the oil by centrifugation, and final traces of alkali are removed by water washing.

Bleaching removes color pigments, chiefly chlorophyll, but also some carotenoids, by means of adsorption onto a bleaching earth or clay. The bleached oil and clay usually are separated by filtration. Bleached oil flows directly to either the hydrogenator or to the deodorizer or to both in sequence for the preparation of edible products.

Hydrogenation is an important process for imparting the desired stability to many edible oil products, particularly those based on soybean oil. Soybean oil is more subject to rancidity than most other oils because it contains significant amounts of linolenic acid, a highly unsaturated and unstable fatty acid. Hydrogenation reduces the level of linolenic acid present, thereby increasing stability of the oil. It is also important to recognize that hydrogenation can be controlled to achieve the physical properties required in the finished food. For example, if the hydrogenation of soybean oil is stopped after only a small amount of hydrogenation has taken place, the oil remains liquid. Further hydrogenation produces a soft, but solid appearing, fat which may be used in the preparation of shortenings and margarines.

Winterization often is used in the preparation of salad and cooking oils to remove di- and trisaturated triglycerides which otherwise might precipitate if the oil were stored at refrigeration temperatures. The process involves chilling the oil, followed by filtration.

Deodorization is the final major step in processing soybean oil. Deodorization removes volatile trace constituents that cause undesirable flavors and odors. Some of the flavor and odor bodies removed by deodorization are produced during hydrogenation. Deodorization is done by treating the oil under vacuum with steam. The process is feasible because of the great differences in volatility between the substances in the oil that impart flavor and odor and the triglycerides in the oil. Among the compounds removed during deodorization are aldehydes, residual free fatty acids, carotenoids, pesticide residues, some sterols and some tocopherols.

Effects of Processing on Essential Fatty Acids

Effects of alkali refining, bleaching and deodorization on the fatty acid composition of soybean oil have been reported by Wilding and coworkers (4). These investigators processed soybean oil with laboratory-scale equipment using conditions simulating normal plant procedures. Alkali refining markedly reduced the level of free fatty acids, as expected. However there were no significant changes in fatty acid composition as a result of processing, even at 238 C, the higher temperature of deodorization.

In their paper (4), Wilding and coworkers identified the C_{18} dienes analyzed by gas chromatography (GC) as "linoleic acid." This is misleading, because the GC method they used is reliable only for determining the number of carbon atoms and the number of double bonds in the carbon chain. The GC method commonly used does not distinguish the geometric configurations of double bonds or their positions in the carbon chain. The essential fatty acid linoleic acid has both of its double bonds in the *cis* configuration and at specific positions (C_9 and C_{12}) along the carbon chain. On the other hand, isomers of linoleic acid which have their double bonds in configurations other than *cis,cis* (or in positions other than C_9 and C_{12}) do not act as essential fatty acids. However, except for essential fatty acid function, these isomers are absorbed and metabolized in a manner similar to the *cis,cis* fatty acid.

Reasonable estimates of essential fatty acids can be obtained by the lipoxygenase assay (5) which is specific for *cis,cis,* methylene-interrupted double bonds as found in linoleic acid. It should be noted that, in some recent analyses on a sample of refined, bleached, deodorized soybean oil performed at Procter & Gamble, the lipoxygenase assay gave essentially the same value as the sum of C_{18} dienes and trienes obtained by GC. Thus it seems reasonable to conclude from the data of Wilding and coworkers and from work in our laboratory that refining, bleaching and deodorization do not significantly affect the essential fatty acid level of soybean oil.

The processing step which is generally recognized as having the greatest effect on essential fatty acid levels of fats and oils is hydrogenation. Progressive hydrogenation results in reduction of dienes and trienes with accompanying increases in monoenes and a gradual increase in saturated fatty acids. Hydrogenation also results in the formation of some *trans* fatty acids and positional isomers. Formation of *trans* and positional isomers tends to increase as the hydrogenation temperature is increased.

The fatty acid compositions of unhydrogenated soybean oil and of several typical samples of hydrogenated soybean oil are shown in Table I. The unhydrogenated salad oil shown in this table was made from soybean oil which had been degummed, alkali refined, bleached and deodorized (2). Similar data for unhydrogenated soybean oil have been reported elsewhere in the literature (6,7). The hydrogenated soybean oil samples compared to the unhydrogenated oil have lower iodine values (IV), increased levels of monoenes (some of which are in the *trans* configuration) and decreased levels of dienes and trienes. Further progression of these changes is indicated by the data for the more highly hydrogenated shortening.

The data for *cis,cis* dienes in the hydrogenated soybean oil samples were determined enzymatically using lipoxygenase. This method is specific for *cis,cis,* methylene-interrupted double bonds. The *cis,cis* diene data for the soybean oil samples with IV in the 90-115 range would suggest a significant level of essential fatty acids (probably largely linoleic acid) in these samples. It should be pointed out that at another session of this conference, Dr. D'Alonzo of the Procter & Gamble Company reported a similar level of linoleic acid (ca. 30% of total fatty acids) in a commercial salad and cooking oil using a new GC method (8).

In terms of human nutrition, the Food and Nutrition Board of the U.S. National Academy of Sciences recommends an intake of essential fatty acids of at least 1-2% of total calories consumed (9). This minimum recommendation is equivalent to ca. 2.0-6.4 g of essential fatty acids/day for adult males and females. Considering a serving size of 1 tablespoon or 14 g, which is the serving size used by manufacturers of vegetable oil products in the U.S. for nutrition labeling purposes, a serving of the salad or cooking oil shown in Table I would contain ca. 4.1 g of linoleic acid. Furthermore, a 1-tablespoon (or 12-g) serving of the shortening would contain ca. 2.4 g of linoleic acid. These amounts of linoleic acid would largely satisfy the minimum levels recommended by the U.S. Food and Nutrition Board. Thus, it seems valid to conclude that while processing (principally hydrogenation) causes some reduction in the

TABLE I

Effect of Hydrogenation on Fatty Acid Composition of Soybean Oil (SBO)

Analysis	Unhydrogenated salad oil[a,b]	Hydrogenated, winterized SBO[a,c]	Typical salad and cooking oil	Typical shortening
Iodine value	131	109-115	114	90
$C_{16:0}$ (%)	10	8-10	8	15
$C_{18:0}$ (%)	5	3-4	4	12
$C_{18:3}$ (%)	26	46-47	47	44
cis monoenes (%)	–	39	35	30
trans monoenes (%)	–	12-15	12	14
$C_{18:2}$ (%)	52	36-39	37	27
cis,cis dienes (%)	–	29	29	20
cis,trans or *trans,cis* dienes (%)	–	–	3	2
$C_{18:3}$ (%)	8	2-4	3	2

[a]Data from Wolf and Cowan (2).
[b]Degummed, alkali refined, bleached, deodorized.
[c]Ranges where indicated are for 2 or 3 samples.

TABLE II

Effects of Processing on Total and α-Tocopherol Levels of Soybean Oil

Reference	Conditions	Total tocopherol (mg/100 g)	α-Tocopherol (vitamin E, mg/100 g)
13	Unrefined	168 (120-280)	20
	Refined	99 (92-140)	21 (9-21)
14	Crude	111 (53-167)	10
	Refined	94 (25-164)	11
	Hydrogenated	103	9.6
Procter & Gamble	Hydrogenated Winterized Deodorized	129	11

essential fatty acid level of soybean oil, commercial soybean oil products are excellent sources of essential fatty acids.

Effects of Processing on Tocopherols

Rawlings and coworkers (10) reported that continuous alkali refining in a plant resulted in a 3% loss of total tocopherols whereas batch refining caused a 12% loss. The greater loss during batch refining was attributed to longer contact time of the oil with air and alkali because tocopherols are unstable in the presence of air and alkali. Rawlings and coworkers noted further that laboratory-scale bleaching and deodorization resulted in tocopherol losses of up to 5 or 6% during each process, but that hydrogenation had no effect on tocopherols. (Specific data on tocopherol changes during bleaching, deodorization and hydrogenation, however, were not provided). Consistent with the data of Rawlings and coworkers, Mag (11) reported a slight reduction in total tocopherols after continuous alkali refining under typical plant conditions and a further slight reduction following bleaching.

According to the literature (1), a minor portion of tocopherols in oils is removed by deodorization. Typical distillates recovered from deodorization of soybean oil have been reported to contain 2-5% tocopherols (1). The amount of tocopherols removed from the oil during deodorization depends on the time, temperature, and stripping steam flow used. These parameters, of course, vary from one processor to another. In any event, a significant level of tocopherols is present in the finished oil after processing, including deodorization (94-100 mg tocopherols/100 g oil), as reported by Sherwin (12).

Additional data on effects of processing on tocopherols, including α-tocopherol, have been compiled from the literature by Lange (13) and also by McLaughlin and Weihrauch (14) (Table II). The data of McLaughlin and Weihrauch are included in the revised *USDA Handbook No. 8* (7). The data shown in this table, however, are not necessarily representative of changes in tocopherols occurring during the processing of a single batch of oil. Nevertheless, because industrial processing conditions are not expected to be highly variable, it seems valid to compare data from different sources. Thus, despite wide ranges reported for total tocopherols, the data indicate that losses of total tocopherol and of α-tocopherol under typical conditions of

TABLE III

Effect of Processing on Excessive Endrin Residues in Soybean Oil[a]

Oil condition	Endrin residue levels (ppb) Mean	Range
Crude	430	220-620
Refined	460	300-640
Bleached	410	290-510
Deodorized	ND[b]	–

[a]Data from Smith et al (16).
[b]ND = none detected.

TABLE IV

Pesticide Residues in Soybean Oil Samples at Different Stages of Processing[a]

Pesticide	Crude oil	Refined oil	Soapstock	Bleached oil	Bleaching earth sludge	Deodorized oil	Deodorizer distillate
Aldrin	10[b]	7.6	2.4	4.8	25	0.9	880
Dieldrin	220	180	41	140	96	ND[c]	8010
pp' DDT	19	21	3.1	13	15	ND	770
Heptachlor	8.8	15	2.7	3.2	17	ND	430
Heptachlorepoxide	40	60	15	67	30	1.6	3650

[a]Data from Chaudry et al. (17).
[b]All values expressed as ppb.
[c]ND = none detected.

refining and hydrogenation are not appreciable. Recent tocopherol analyses at Procter & Gamble of a sample of hydrogenated, winterized, deodorized soybean oil are consistent with these literature values.

In regard to human nutrition, the U.S. Food and Nutrition Board (9) recommends an intake of 8-10 mg of α-tocopherol equivalents/day for adults. Considering an α-tocopherol level of 11 mg/100 g in a typical salad and cooking oil made from soybean oil, a 1-tablespoon (14-g) serving of such a food would contain ca. 1.5 mg of α-tocopherol. Thus, a single serving of this product would provide ca. 15-20% of an adult's recommended intake as established by the U.S. Food and Nutrition Board. Other good dietary sources of vitamin E include other vegetable oils, margarines and shortenings.

Effects of Processing on Pesticide Removal

Chlorinated pesticides have long been used for increased agricultural production throughout the U.S. However, among the concerns about pesticides is their possible translocation from the soil to the edible portions of the plant. Bruce and coworkers (15) have correlated the translocation of some chlorinated pesticides from treated soils to oil-bearing plant seeds, such as soybeans and peanuts. The effectiveness of various processing steps in removing pesticide residues has been reported by several groups of investigators (16-20). Studies by Smith (16) and Chaudry (17) and their coworkers are representative.

Smith and coworkers (16) studied the effect of processing on four soybean oil samples containing excessive levels of endrin. The oil samples were processed in pilot plant equipment using conditions typical of commercial operations. To ensure that the processing conditions would not produce any unknown artifacts which could be interpreted as pesticide contamination, a crude sample which was analyzed as being free of chlorinated pesticides was included as a negative control. The samples were alkali refined, bleached and deodorized. Aliquots of oil from each processing step were submitted to three analytical laboratories for pesticide analysis.

The means and ranges of endrin levels at various stages of processing are shown in Table III. The data indicate that neither alkali refining nor subsequent bleaching reduced endrin contamination. However, deodorization (252 C, 1 hr) effectively removed the residue. The investigators noted that hydrogenation of two soybean oil samples prior to deodorization reduced the level of endrin to near or below the detectable limit.

Smith and coworkers, however, did not determine the pesticide residues in the processing by-products such as bleaching earth sludge and deodorizer distillate. In a recent study by Chaudry and coworkers (17), crude, refined, bleached and deodorized soybean oil, as well as the soapstock, bleaching earth sludge and deodorizer distillate were analyzed for several chlorinated pesticides. The oil was processed in the laboratory under conditions simulating commercial processing techniques. The results of these analyses for five of the pesticides studied are shown in Table IV. The data indicate that residue levels in the oil of aldrin, dieldrin, DDT, heptachlor and heptachlor epoxide decreased with refining, bleaching and deodorization. However, none of the processing steps except deodorization (250 C, 2 hr) was effective in completely removing the pesticide residues. The deodorized oil was essentially free of pesticide residues, whereas all of the residues were concentrated in the deodorizer distillate. Similar results were obtained for residues of other pesticides commonly occurring in soybeans, soybean oil and processing by-products, i.e., hexachlorobenzene, lindane, endrin, DDD and DDE.

These studies indicate that removal of chlorinated pesticides which may be present in crude oil is achieved largely through volatilization during deodorization. It should be pointed out, however, that the levels of certain pesticide residues reported in deodorizer distillates by Chaudry and coworkers (17) may be considerably higher than levels reported by more recent analyses. In the case of dieldrin, e.g., Chaudry and coworkers reported a level of ca. 8 ppm in deodorizer distillate. However, a recent analysis reported by the Institute of Shortening and Edible Oils of 60 lots of commercially produced deodorizer distillate showed that 65% of the lots contained dieldrin at less than 1.0 ppm (21). Only 5% exceeded 4.0 ppm. These data reflect the efforts of the U.S. Environmental Protection Agency to phase out usage of dieldrin in this country.

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Studies on Lipid Responses to Interesterified Soya Oil-Butterfat Mixture in Hypercholesterolemic Rats and Human Subjects

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ABSTRACT

Interesterified soya-butterfat feeding significantly decreased serum cholesterol in humans and in experimental rats. This decrease was more effective than when simple mixtures of the two fats were fed. Studies with the experimental rat indicate higher rates of side-chain degradation of cholesterol as well as 7-α-hydroxylation of cholesterol when interesterified fats replace a mixed fat regimen. The lowering of serum cholesterol parallels the decrease in concentration of fully saturated glycerides and redistribution of myristic acid from the 2-position in the glyceride to 1- and 3-positions of glycerides following interesterification.

INTRODUCTION

Soyabean oil is relatively high in polyunsaturated fatty acids (50-55%) and is likely to be a potential hypocholesterolemic agent if used as a dietary oil. In India, butterfat (ghee) is the most popular dietary fat and in spite of its proven hypercholesterolemic responses in humans and experimental animals, has occupied a unique place in the Indian diet. Substitution of butterfat in part by soyabean oil has not found much favor among the butterfat-consuming population, even though a significant lowering of serum cholesterol is attained. Also unpopular is the blending of Indian ghee with vegetable oil containing relatively high polyunsaturated fatty acids because of poor acceptability by the average Indian who prefers the hydrogenated vegetable oil.

Studies with laboratory animals have revealed cholesterol-lowering action of soyabean oil when mixed with butterfat in different proportions (1,2). Myristic acid, a predominant saturated fatty acid member of butterfat, has been singled out as a major factor contributing to increase in serum cholesterol in man (3) and this has been verified in the laboratory rat (4). In natural butterfat, myristic acid is present as trisaturated glycerides, occupying exclusively the 2-position in the glyceride moieties. Data presented elsewhere tend to show that increase in serum cholesterol is not merely dependent on the content of myristic acid in the dietary fat, but is probably related to its distribution among the various types of glycerides and the position it occupies therein (1). This study was designed to modify the glyceride structure of butterfat by interesterification using Eckey's procedure (5) and to study the changes in glyceride structure with corresponding changes in lipid responses when fed to rats and humans.

EXPERIMENTAL

Serum cholesterol and triglyceride estimations were done according to standard procedures (6,7). The separation of glycerides of interesterified fat mixture was attained on thin layer chromatographic plates coated with Silica Gel G according to the procedure of Wessels and Rajgopal (8) using a solvent system of benzene/di-isopropyl alcohol (85:15). Lipase hydrolysis was done according to the Weber procedure (9), scaled up to accomodate 50-mg samples. Products of hydrolysis were isolated by thin layer chromatography (TLC) on Silica Gel G plates developed in hexane/diethyl ether/acetic acid (65:35:1), converted to methyl esters with methanol BF_3 (10). The methyl esters were separated on a 700-12 F&M gas chromatograph equipped with dual flame ionization detectors using ethylene glycol adipate polyester, 10% of which was absorbed on 60-80 mesh Chromosorb P and packed into 4-mm bore aluminum column; they were identified according to the usual procedure. Oxidation of 26-^{14}C-cholesterol by rat liver mitochondria was performed according to Kritchevsky (11) with slight modifications. 7-α-Hydroxylation of 4-^{14}C-cholesterol was done following the procedure of Shefer et al. (12). Estimations of microsomal cytochrome P-450 was done according to Omura and Sato (13).

RESULTS AND DISCUSSION

Data in Table I show the effects on serum and liver cholesterol of feeding rats soyabean oil-butterfat mixtures as interesterified mixtures having identical amounts of various saturated and polyunsaturated acids. Replacement of 50% of saturated butterfat soya oil causes significant decreases in serum cholesterol at the end of one week, but when 1:1-interesterified fat mix is fed to the rats, greater lowering of serum level results and the fall is highly significant ($p<0.05$). A comparison of effects of mixed and interesterified fats in rats maintained on a 0.5% cholesterol diet (Table II) indicates that cholesterol lowering effect of the interesterified soya-butterfat mixtures were even more marked when cholesterol was present in the diet ($p<0.001$).