THE DERIVATION, STABILITY AND FUNCTIONALITY OF A POURABLE FRYING OIL FROM BEEF TALLOW

By

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A DISSERTATION

Submitted to

Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1985

ABSTRACT

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Beef tallow was investigated as a source of pourable frying oils using aqueous detergent fractionation. Tallow rendered at 120°F possessed optimal fractionation characteristics at 40°C. Subsequent fractionation at 33, 27 and 22°C produced an olein fraction with desirable deep-fat frying characteristics.

Optimization studies of the fractionation process indicated that surfactant (sodium dodecyl sulfate, 0.6% w/w) and a 5% aqueous electrolyte solution (sodium citrate) were required for optimal fractionation.

Cholesterol in triglyceride model systems held at 180°C was investigated to ascertain the effects of aeration and unsaturation on cholesterol oxidation. At frying temperatures, unsaturated fatty acyl groups were more susceptible to oxidation than cholesterol. The initiation of cholesterol oxidation probably occurred due to the presence of

free radicals generated in the autoxidation of fatty acids. Aeration enhanced cholesterol oxidation in all model systems, regardless of the degree of unsaturation.

Stability studies with the 22°C olein revealed that palm oil and hydrogenated cottonseed oil (HCSO) added at 5-15% levels, as well as TBHQ and ascorbyl palmitate-mixed tocopherol, inhibited oxidative deterioration. Cholesterol in these systems oxidized as a consequence of unsaturated triglyceride oxidation, while compounds which inhibited peroxide development also inhibited cholesterol oxidation.

The 22°C olein fraction produced for deep-fat frying was assessed in the processing of potato chips. Potato chips produced in this fraction were found to exhibit superior quality attributes when compared to chips processed in commercial HCSO and tallow:HCSO (1:1, w/w). The potato chips processed in 22°C olein were as stable to oxidation as commercially produced chips. These chips contained 53.8±4.2 mg cholesterol/100 g chips. Cholesterol oxidation products were not detected over the five week storage period.

Packed and capillary GLC and GC-MS analyses of fresh and five week old chips indicated that off-flavor development in the latter was due to the oxidation of oleic and linoleic fatty acyl moieties in the absorbed 22°C olein. Saturated aldehydes (C_6-C_{10}), 2-enals (C_6 and C_8), 2-enones (C_6-C_9) and 2,4-dienals (C_7 and C_{10}) were identified in a

pentane extract of potato chips described as rancid by sensory panelists.

To my wife, Nancy

ACKNOWLEDGEMENTS

My sincere gratitude is extended to Dr. J. I Gray for his genuine concern, professional guidance and friendship in the execution of his duties as research chairman and major professor. The input and influence of Dr. Gray was very much appreciated.

The members of the guidance committee, Drs. A. M. Pearson, B. R. Harte, E. R. Grulke, and J. W. Allen also have my appreciation for their critical review of this dissertation. A special note of thanks to Dr. L. R. Dugan for his accessability and the lengthy discussions which ensued over the course of this work.

Further acknowledgements are also due to the Ralston Purina Company, the Institute of Food Technologists and the American Oil Chemists Society for their recognition and financial assistance during this study.

Special thanks are also extended to Louise Partridge for her assistance in the preparation of this dissertation.

I would like to also acknowledge those persons who made my experiences over the course of this work special. The friendship of Dan Skrypec, Bill Ikins, Arun Mandagere, Julie Luby Orabone and Loraine Stephens is now and will be always appreciated.

I am especially grateful to my parents, Mr. and Mrs. Thomas J. Ryan, for the love, encouragement and understanding they have shown me in all of my endeavors. I also thank Dr. and Mrs. Thomas G. Varbedian for their love, friendship and confidence in my efforts.

Lastly, my heartfelt appreciation is extended to my wife, Nancy, for her love, patience and sacrifices made during the course of my work at Michigan State University.

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INTRODUCTION

Edible beef tallow, the fat rendered from cattle during the processing of beef, is produced in large quantities in the United States. Although approximately 5.5 billion pounds of tallow are produced in this country per annum, only 500 million pounds are utilized for human consumption (Kromer and Gazelle, 1974). Figures indicate that half of all tallow rendered in the United States is exported (Taylor et al., 1976). Meanwhile, approximately 1.8 billion pounds of fat and oil such as those derived from cocoa, coconut, palm and palm kernal are imported each year. Traditionally, tallow has been priced at 15-40% of the market cost of other fats such as cocoa butter (Taylor et al., 1976). Therefore, the fractionation of edible tallows merits investigation since tallow fractions may be used as cost-effective, domestic alternatives to imported fats and oils of vegetable origin.

Fractionation refers to the chemical and/or physical separation of fats and oils into solid (stearin) and liquid (olein) phases. In recent years, three methods have been employed industrially in the fractionation of fats such as tallow. These include dry, solvent and aqueous fractionation techniques. Of these, aqueous fractionation has been reported to optimize olein yields and is more labor and cost effective (Braae, 1976).

Application of aqueous fractionation techniques to tallow may be used to procure tallow fractions which would prove suitable for the processing of deep-fat fried foods. It is known that tallow imparts aesthetically desirable flavors to foods fried therein (Baeuerlen et al., 1968; Chang et al., 1978; Chang, 1979). To date, the use of tallow as a frying medium is limited to foods consumed while hot due to the high melting point of tallow. However, the application of aqueous fractionation to tallow can be used to sequentially remove higher melting glyceride types, resulting in fractions suitable for the processing of fried foods traditionally consumed at ambient temperatures, such as potato chips. The development of such a fraction from tallow may provide finished products with high marketability due to flavor development typical of tallow-fried foods.

Of equal concern in such a tallow fractionation scheme is the distribution and oxidative stability of cholesterol in fractionated tallow and products fried therein. Beef tallow contains 0.15-0.20% cholesterol by weight (Punwar and Derse, 1978). Recent studies indicate that cholesterol, heated and/or exposed to air, readily oxidizes to form compounds which possess angiotoxic (Imai et al., 1976; Peng et al., 1978), carcinogenic (Bischoff, 1969; Black and Douglas, 1973; Smith and Kulig, 1975) and mutagenic properties (Smith et al., 1979). Cholesterol in tallow utilized as a deep fat frying medium was also found to undergo oxidation (Ryan et al., 1981) and the oxidative products were found to be preferentially absorbed by the fried food (Ryan, 1982). Thus, storage of foods processed in fractionated tallow may also be conducive to the oxidation of their inherent cholesterol and subsequent formation of possibly delerterious compounds.

The objectives of this study were as follows:

1. To investigate the chemical and physical properties of tallow which may influence their subsequent fractionation.
2. To develop and optimize an aqueous detergent fractionation scheme by which a pourable, stable frying oil could be derived.
3. To assess the distribution properties of cholesterol in the fractionation process as well as characterize the fractions derived in this process.
4. To investigate the influence of unsaturation and aeration on the oxidative stability of cholesterol in model triglyceride systems.
5. To investigate the oxidative stability of a frying oil fraction from tallow, as well as factors influencing stability.
6. To assess the attributes of the tallow-derived frying oil in the production of potato chips, and the subsequent stability of these chips during storage at ambient temperature.

REVIEW OF LITERATURE

The review of literature pertinent to the development of a pourable frying fat derived from refined edible beef tallow will address areas of key concern in the development process as well as those areas which are critical in the successful application of such a product. Primarily, the review of fractionation processes as they pertain to tallow will be discussed. Areas of concern which also merit review in this study include methods of assessing lipid oxidation, the oxidative stability of cholesterol and the biological activity and preponderance of cholesterol oxidation products in foods, since cholesterol is inherently present in beef tallow. Lastly, this review will also address the processing and stability of potato chips.

Description of Beef Tallow

Beef tallow is the hard fat rendered from cattle during the processing of beef and is produced in large quantities in the United States. Approximately 1.3 billion pounds of edible tallow are produced per annum, representing only 10% of the total tallow production (Figure 1). Figures indicate that half of all tallow rendered in this country is exported (Taylor et al., 1976). Figure 2 indicates that as per capita consumption of food fats has been increasing, consumption of

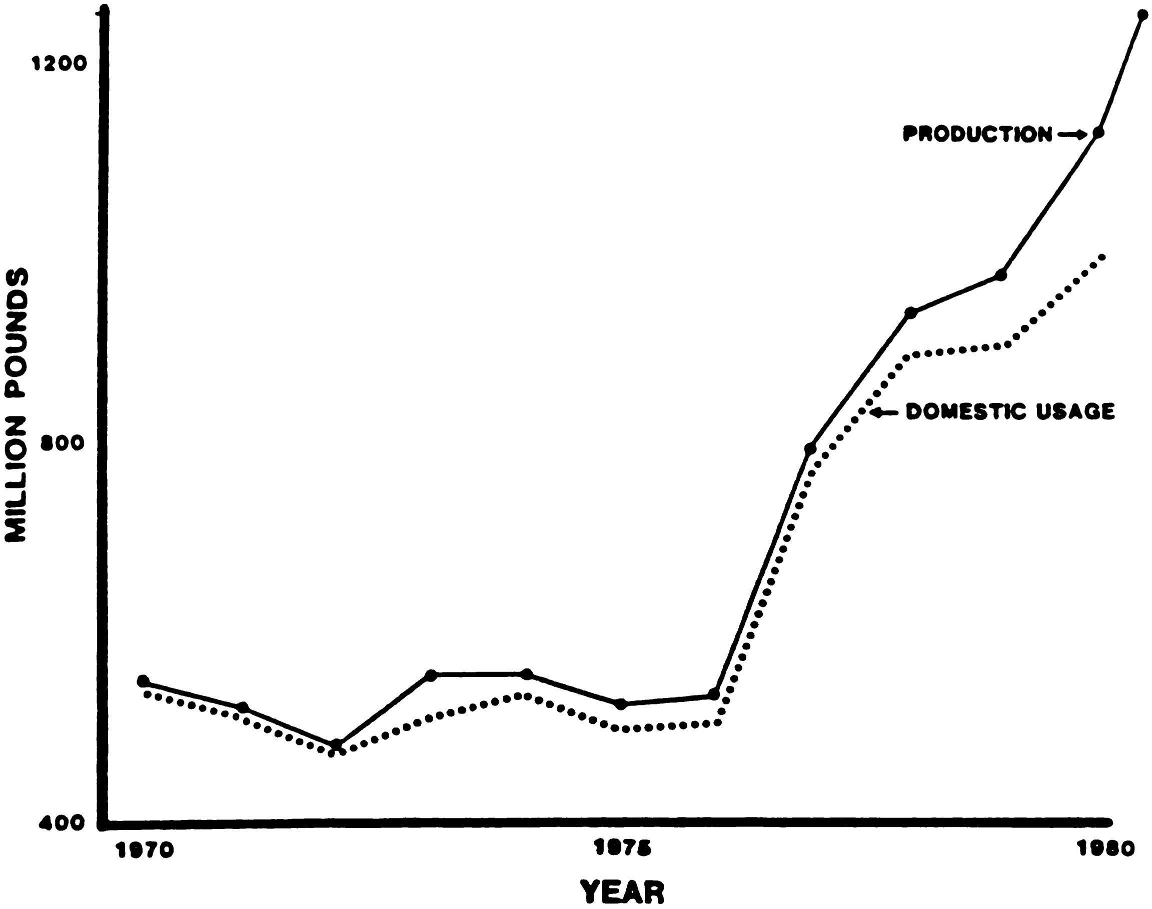


Figure 1. Edible tallow production and domestic usage in million pounds from 1970-1981 (Dillery, 1982).

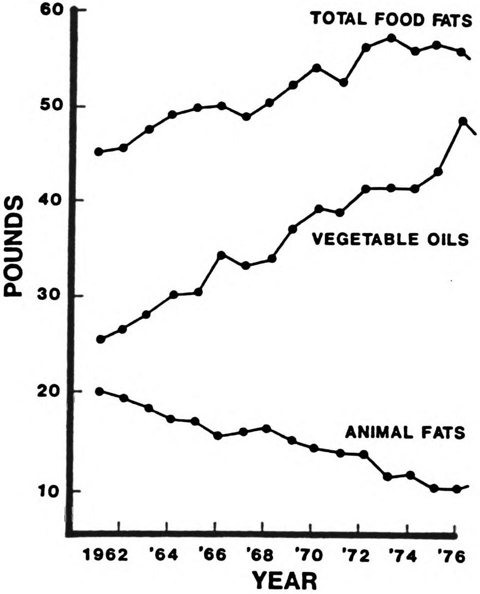


Figure 2. U.S. per capita consumption of total food fats, vegetable oils and animal fats, 1960-1977 (Dillery, 1982).

fats from animal sources have declined. Meanwhile, approximately 1.8 billion pounds of fats and oils such as those derived from cocoa, coconut, palm and palm kernal were imported in 1973. Traditionally, tallow has been priced at 15-40% of the market cost of other fats such as cocoa butter (Taylor et al., 1976). Tallow prices are found in Figure 3.

The average characteristics and composition of beef tallow are found in Table 1. As can be seen from this table, the composition of tallow is not invariant. Generally, the fatty acid composition of tallow consists of nine or ten predominant fatty acids, although approximately 180-200 individual fatty acids occur in tallow, mostly in small or trace quantities (Swern et al., 1979). Average packing house tallows in the United States have been reported as having iodine values of 42, although much variation has been noted yielding a range in values of 34-47.

Several studies have indicated that environmental factors may influence the composition and characteristics of beef tallow. Swern et al. (1979) reported that the body fat of cattle is minimally affected by feed, especially with respect to saturated acids. Edmondson et al. (1974), however, reported that feeding sodium caseinate-encapsulated safflower oil effectively increased the linoleic acid content of veal fat from 3% to 12% with a simultaneous decrease in the palmitic acid content. Kimoto et al. (1974) observed that the decreased stability of beef fat

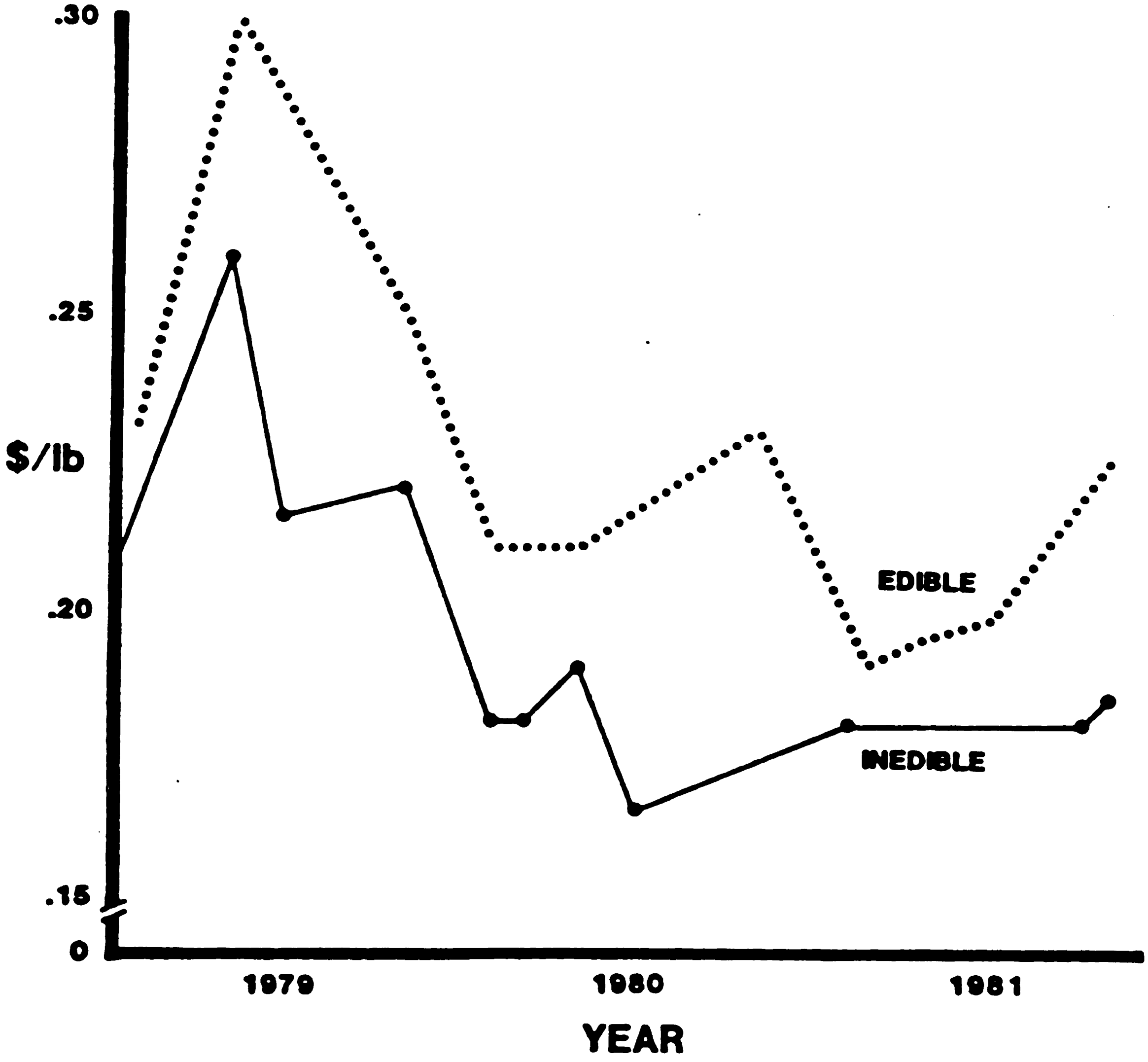


Figure 3. Edible and inedible tallow prices from 1979-1981 (Dillery, 1982).

Table 1. Characteristics and compositional ranges of beef tallow (adapted from Swern et al., 1979).

Characteristics	Range of values
Iodine value	34 - 47
Saponification number	193 - 202
Titer, °C	40 - 46
Specific gravity at 99/15.5°C	0.860 - 0.870
Refractive index at 40°C (Zeiss)	46 - 49
Unsaponifiable matter, %	<0.8
Composition, fatty acids	**weight percent**
C12:0	tr - 0.2
C14:0	2 - 8
C16:0	24 - 37
C18:0	14 - 29
C20:0	tr - 1.2
C14:1	0.4 - 0.6
C16:1	1.9 - 2.7
C18:1	40 - 50
C18:2	1 - 5
Composition, glyceride type	**mole percent**
GS_3	15 - 28
GS_2U	46 - 52
GSU_2	20 - 37
GU_3	0 - 2

produced in this manner could be alleviated via tocopherol supplementation in the feed.

Climate has been found to influence the levels of unsaturation in beef fat. Warmer temperatures tend to decrease the concentration of unsaturated fatty acyl groups in tallow. Achaya and Banerjee (1946) stated that tallows from Indian cattle consistently had iodine values as low as 26-31. Factors such as trimming procedures at the time of rendering can also influence the composition of refined edible tallows, since fat is more unsaturated near the skin, and more saturated near the internal organs. This variation in distribution of fatty acids and fat composition with respect to body site location has been well established (Chacko and Perkins, 1965). Beef tallow triglycerides types and ranges, dependent on differing fatty acid concentrations, are also given in Table 1.

Fractionation Techniques

The variability in the constituents of tallow demonstrated in Table 1 indicates the complex chemical nature of tallow. These variations may cause differing properties in tallows having similar melting points and iodine values, specifically due to the stereospecific distribution of the many fatty acyl moieties on the glycerol molecule. These differences manifest themselves via large changes in crystallization properties and yields at specific temperatures. Individual triglycerides in the same lipid system may vary in melting point characteristics, due to their stereospecific positioning of saturated and unsaturated fatty acids. Triunsaturated triglycerides (GU_3) have melting points 60-70^{o}C lower than trisaturated triglycerides (GS_3), yet these effects are diminished in biological systems due to random distribution of fatty acids as well as mutual solubility effects (Glassner, 1983). The different melting points of the mixed triglycerides in fats and oils are the basis for fractionation processes. Tallow can be fractionated to yield fractions with vastly different melting ranges.

Fractionation refers to the partial crystallization of a fat or oil at a specific temperature. The lipid system subjected to such a fractionation is tempered at the crystallization temperature for periods of time to allow equilibrium or near equilibrium to occur between crystallizing and non-crystallizing triglycerides. Once partial crystallization is complete, the actual fractionation proceeds by effecting the liquid-solid separation.

Dry Fractionation

The main objective of fractionation processes is to optimize the separation of liquid (olein) and solid (stearin) fractions. An effective early method of separating olein and stearin phases is known as winterization. This technique involves the removal of higher-melting glycerides by subsequent decreases in the temperatures of storage. This technique originally utilized winter temperatures to crystallize the saturated glyceride types from oils. This technique is slow and results in large crystals which are removed from the resultant olein by crude skimming or filtration processes.

Winterization techniques were utilized for the first large scale fractionations, which require crystallization times of 72-96 hours. This process retards nucleation and allows large crystal growth, which is necessary for subsequent filtering of the crystals from the oil. Plate types presses with canvas cloths are employed in the process. Yields of 55% olein have been reported via this technique for fats yielding 75% by aqueous fractionation techniques (Braae, 1976).

Another dry fractionation process is referred to as Tirtiaux fractionation in which directed crystallization on a suitable fat is directly followed by filtration (Tirtiaux, 1983). In this modern dry process, a continuous vacuum filter equipped with a stainless steel perforated belt as a filtration support is utilized. Olein yields of 70% are achieved when fractionating tallow at temperatures where aqueous fractionations yield 80% (Tirtiaux, 1983).

Solvent Fractionation

Solvent fractionation involves the use of large amounts of organic solvents, such as acetone, to completely dissolve fats and oils. The lipid-solvent solution is then exposed to temperatures of crystallization and held at such temperatures to allow partial crystallization of the higher-melting glycerides. This technique uses solvent to decrease the viscosity of the lipid phase, which inherently optimizes the separation of the dissolved, diluted olein phase from the solid crystals (Luddy et al., 1977). Although this technique is efficient, the solvent fractionation process mandates a costly explosion and flame-proof operating system, as well as an expensive solvent recovery system (Braae, 1976).

Aqueous Fractionation

Aqueous fractionation was first described and patented by Lanza in 1905. Aqueous detergent fractionation processes have been employed industrially for twenty years by Alfa-Laval (1977). The process is that described by Haraldsson (1974) and is used to fractionate both tallow and palm oil. In this process, the lipid system is melted and cooled to partially crystallize in large scraped-wall vessels. To the partially crystallized oil, an aqueous solution containing sodium dodecyl sulfate and magnesium or sodium sulfate is added. After addition, the solutions are mixed causing rearrangement of the phase equilibrium, resulting in a continuous phase aqueous solution, containing entrapped crystalline stearin. The dispersed crystalline stearin is separated from the olein via a centrifugal separator.

In this system, the olein produced has a lower density than the spontaneously formed aqueous-stearin dispersion. Thus, centrifugation is utilized to affect the olein-stearin separation. The residual stearin phase is recovered from this process by heating to temperatures above the melting point of the higher melting glycerides followed by subsequent centrifugation. This process thus yields liquid stearin and an aqueous solution which can be recycled.

Rek (1977) patented yet another aqueous fractionation process. This technique proports to be superior to other aqueous fractionations due to the use of a surface active agent. These surfactants were described as being unsaturated fatty acid salts of carbon chains ranging from C_{12}-C_{28} in length. One such salt which was noted to work well in this fractionation scheme was potassium oleate. It was proposed that these soaps were preferentially solubilized in the triglyceride matrix prior to the cooling of the oil. The fractionation procedure after the use of the unsaturated soap was the same as that specified by Haraldsson (1974), although the use of the unsaturated fatty acyl salts were noted to improve olein yields, especially at lower fractionation temperatures.

Research conducted by Bussey et al. (1981) focused on the optimization of aqueous detergent fractionation techniques to yield fractions with specific characteristics for food applications. Modification of the aqueous fractionation techniques previously described was found to increase the yield of olein significantly. These modifications included the addition of a water soluble surface active agent directly to a partially crystallized fat. Specifically, sodium dodecyl sulfate (SDS) was added to softened tallow which was tempered to 40°C from a storage temperature of 4°C. The SDS was mixed with the partially crystallized tallow and allowed to equilibrate for 18 hours, followed by the addition of an electrolyte solution (5% sodium sulfate) and mixing. The separation of olein from stearin was carried out by centrifugation after the formation of the aqueous dispersion. This process yielded olein at 40 °C of greater than 80% of the original unfractionated tallow.

Of the three fractionation processes described, aqueous techniques appear to be the most compatible with the derivation of fractions from beef tallow (Bussey et al., 1981). However, very little information regarding the engineering parameters for specific processes exist. The major parameters have been defined and reviewed by Glassner (1983), and are stated as being: mixing requirements during the crystallization and aqueous dispersion, required surfactant and electrolyte concentrations and requirements regarding centrifugal separation of olein and stearin phases.

Crystallization

The kinetics of crystallization for fats are difficult to define due to the large number of triglyceride types present in fats. This multiplicity of triglyceride types give rise to mutual solubility effects causing changes in the crystallization temperature of single triglyceride types present in a heterogenous system (Glassner, 1983). Crystallization phenomena are dependent on two factors: nucleation of crystals and crystal growth rate. Nucleation is induced by agitation, mechanical shock, friction and extreme pressure. Agitated solutions nucleate spontaneously at lower degrees of super-cooling than non-agitated solutions. However, increases in agitation intensity may not increase rates of nucleation. The crystal growth rate is usually proportional to crystal size (Mullin, 1972).

To date, kinetic data for the crystallization of tallow can not be found in published literature. Aqueous fractionation techniques, as applied to tallow, require the formation of the proper types of crystals, as wetting of crystallized stearin is necessary for phase separation (Glassner, 1983). In this regard, crystal size is relatively unimportant. If an oil is exposed to rapid cooling, unstable amorphous crystals are formed which are not wetted by the aqueous solution (Haraldsson, 1974). Alfa-Laval (1977) reported that crystals in the order of 10-25 microns or larger are of sufficient size for aqueous detergent fractionation to be successful. Glassner (1983) found that control of the formation of emulsified olein is a critical factor for the successful fractionation of tallow, since the emulsion is formed at the expense of olein. The formation of emulsified olein was found to be a function of surfactant concentration, as well as a function of the crystal size formed, with small crystal formation enhancing the emulsification of olein. The same study also indicated that agitation of the tallow during the partial crystallization causes the formation of extremely small crystals. These crystals, studied under photomicroscopy, were found to be cylindrical in shape, approximately 20 microns in length and one micron or less in diameter. These crystals were formed under a stirring rate of 260 rpm in a turbine impeller vessel. The fractionation of these small crystals in the subsequent process resulted in emulsified olein, even at low surfactant concentrations.

Aqueous Dispersion

Aqueous fractionation, as stated earlier, involves the use of a surfactant and an aqueous solution of electrolyte. Directly following partial crystallization, aqueous solutions must be dispersed into the partially crystallized tallow. Glassner (1983) speculated that the addition of surfactant causes adherence to solid crystalline surfaces which aids in the wetting of the stearin crystals by the aqueous solutions. The electrolyte was theorized as functioning to help distribute the surfactant evenly by overcoming electrostatic forces occurring between crystals.

Thus, the levels of surfactant, electrolyte and aqueous volume are important to the fractionation process. Haraldsson (1974) did not reveal the chemical concentrations utilized in his process. Rek (1977) specified the amount of oil soluble surface active agent or soap to be above 0.6% (by weight of the oil) to the solubility limit of 2.0% depending on the oil. The level of water soluble surface active agent should be above 0.3% (by weight of fat) although the amounts used were not constant. The electrolyte level in the aqueous solution was stated as being optimal at amounts of 2.5-5.0% based on the weight of the solution. Volume ratios of aqueous solution to the fat is in an optimal range between 0.2:1.0 to 0.8:1.0. The procedure developed by Bussey et al. (1981) specified the amount of water soluble surface active agent to be 0.6% by weight of the tallow. The electrolyte concentration was found to be optimum at 4.0% by weight of the aqueous solution and the volume of water used was specified at 60% of the volume of tallow fractionated.

Glassner (1983) studied the process of aqueous detergent fractionation specified by Bussey et al. (1981) and found that the yields of olein derived via fractionation were contingent on the levels of reagents used in the process. This study indicated that for crystals of approximately 100 microns in size, increasing the concentration of the surfactant increased the olein yield up to approximately 1.0% SDS by weight of the tallow. At levels of 1.3% or higher, SDS was found to emulsify olein to a great extent, resulting in significant product loss. This study also noted the observation that increasing the electrolyte concentration in the aqueous fractionation process increased olein yield until the electrolyte concentration reached an optimal 5.0%, above which olein yield was not significantly improved.

Functionality of Tallow and Tallow Fractions in Food Systems

The functionality of beef tallow and tallow fractions has been investigated in a variety of food systems. Morris et al. (1956) investigated the use of tallow, rearranged tallow, hydrogenated tallow and tallow-vegetable oil blends for shortening applications. The tallow samples studied compared well with standard vegetable oil shortenings, especially those samples which were partially hydrogenated or had been treated with antioxidants. Bundy et al. (1981) also investigated the shortening application of beef tallow. This study utilized solid and olein fractions from tallow, with and without emulsifiers, in the preparation of white layer cakes. Results indicated that optimal products were prepared using a stearin fraction derived at 25°C in conjunction with an emulsifier. Dillery (1982) also prepared white layer cakes utilizing original tallow, a 35°C olein fraction and a 25°C stearin fraction in conjunction with an emulsifier. Analysis of the physical and sensory characteristics of the products revealed that cakes prepared with original tallow and both tallow fractions compared favorably with the control cakes produced with commercially available shortenings. De Fouw (1981) investigated unfractionated tallow for use as a shortening for the production of chocolate chip cookies. This study indicated that panel test subjects slightly preferred cookies produced with vegetable shortening over those produced with tallow, however approximately 70% of the respondents liked to some degree the cookies prepared with the tallow.

Holsinger et al. (1978) studied the use of solvent fractionated tallow as a fat component in a whey-soy nutritious beverage mix. The results of this study showed that when used in the formulation, beverages made with tallow fractions were as stable to flavor changes as beverages made with soybean oil after six months of storage at 37°C. In addition, derived beef oils were less susceptible to oxidation than soybean oil determined by peroxide values, indicating that the use of such tallow fractions may increase the oxidative stability of finished products.

Luddy et al. (1973) and Taylor et al. (1976) studied tallow fractions as cost-effective substitutes or extenders for cocoa butter. Luddy et al. (1973) utilized acetone fractionation to derive five fractions from tallow. These fractions were identified as having characteristics consistent with fats and oils used for shortening and margarine formulations, salad oils, cooking oils and cocoa butter substitutes and extenders for use in confectionary coatings.

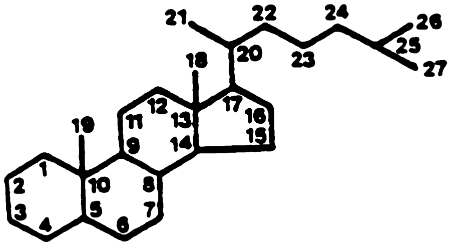
Tallow and tallow fractions have also been investigated for possible application as deep-fat frying media. Tallow has been used extensively by restaurants for frying because of the desirable flavor it imparts to foods fried therein (Baeuerlen et al., 1968; Chang et al., 1978; Chang, 1979). De Fouw et al. (1981) reported that tallow as well as olein fractions derived at 25, 35, 40 and 45^{o}C and blends of unfractionated tallow with soybean and corn oil (50%) were used to process french fried potatoes. Results of this study indicated that tallow, tallow fractions and tallow blends performed well in producing french fried potatoes, scoring equal to or better than potatoes processed in commercial frying oil. It was also noted that tallow fractions were more stable to oxidative, thermal and polymeric reactions than were unfractionated tallow, blended tallow and commercial vegetable frying oil. Dillery (1982) utilized tallow and tallow olein derived at 20 and 30^{o}C to process doughnuts and batter-coated chicken wings. When compared to products fried in a commercial soybean oil, both doughnuts and chicken wings scored better when fried in the tallow and tallow-derived oleins. Also noted was the lower fat absorption and higher sensory scores for the tallow processed products.

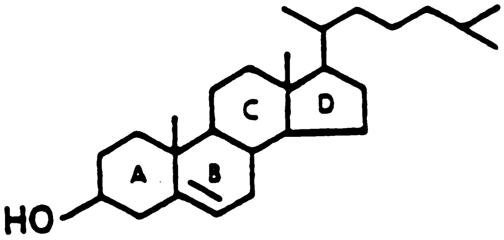
Ryan (1982) investigated tallow stability under conditions representative of frying and reported that tallow is an excellent medium for deep-fat frying. He also pointed out that intermittent heating was more detrimental than continuous heating in regard to frying stability and that frying product in tallow had a suppressive effect on the degradation of tallow. However, this study also indicated that tallow utilized as a deep-fat frying medium contains compounds, formed via the oxidation of cholesterol, which may pose hazards to human health.

Cholesterol and Its Oxidation

Description of Cholesterol

Cholesterol (5-cholesten-3β-ol) is a relatively non-polar neutral lipid with a chemical formula of $C_{27}H_{46}O$. The compound as shown below consists of a cyclopentanophenanthrene ring structure with an eight carbon side chain at C_{17} and angular methyl groups at the C_{10} and C_{13} positions. The alcoholic function is located at the C_3 position and an unsaturated bond exists at the C_5-C_6 position.





Cholesterol is distributed in all cells of animal tissues, with the highest concentrations found in brain and nervous tissue. Cholesterol functions in biological systems as a structural component in membranes, in the transport of blood lipids, and as a precursor to fecal sterols, bile acids and steroid hormones (Smith, 1981).

Cholesterol Autoxidation

Cholesterol is an unsaturated lipid and is therefore susceptible to oxidation by free radical processes via reaction mechanisms analogous to those involved in the autoxidation of unsaturated fatty acids. The sensitivity of cholesterol to oxygen has been noted in various reviews. Smith (1980) reported that Schulze and Winterstein investigated the sensitivity of crystalline cholesterol to air as early as 1902. Bladon (1958) mentioned early studies by Lifschultz who used acetic and sulfuric acids to detect sterols other than cholesterol in wool fat. Lifschultz, in 1907, recognized the deterioration of cholesterol as an oxidative process, the product of which he termed "oxycholesterol". This compound was believed to be a single chemical species which could be derived by the action of oxidizing agents such as benzoyl peroxide, or isolated as a putative metabolite from some animal tissues. Bergstrom and Samuelsson (1961) cited the work of Lamb who in 1914 implicated oxygen as a necessary reactant for oxidative changes in cholesterol colloidally dispersed in an aqueous system. Bischoff (1963) confirmed these findings by reporting that no "oxycholesterol" formed from cholesterol in the absence of air.

Blix and Lowenhielm (1928) expressed doubts about the purity of Lifschultz's "oxycholesterol". They emphasized that oxygen was necessary for its formation and alkali soaps capable of dispersing cholesterol catalyzed this oxidation. Heilbron and Sexton (1928) dry distilled cholesterol at atmospheric pressure to form what they described as cholestenone and ψ-cholestene. All of this work was carried out prior to the assignment of the correct chemical structure to cholesterol in 1932.

Bergstrom and Wintersteiner (1941, 1942a, 1942b) studied the autoxidation of cholesterol in aerated colloidal aqueous solutions at 85°C and 37°C. Molecular oxygen was utilized as the oxidizing species in these series of experiments. Crystallization techniques revealed that aeration of cholesterol for five hours caused an eighty percent conversion of cholesterol to oxidized species. Chromatographic analysis indicated that 7-hydroxy-α cholesterol, 7β-hydroxy-cholesterol, 7-ketocholesterol and 7-keto-$\Delta^{3,5}$-cholestadiene were formed during oxidation, and were believed to be analogous to Lifschultz's "oxycholesterol" with the diols causing color formation in the Lifschultz reaction. Prooxidant metal ions, both ferrous and zinc, were found to accelerate the reaction, but did not increase the yields of the various cholesterol oxidation products. Susceptibility to oxygen attack was greatly diminished when cholesteryl esters, such as cholesterol acetate, palmitate and oleate esters, were exposed to the same system at 85°C (Bergstrom and Wintersteiner, 1942c).

Bergstrom and Wintersteiner (1942a) postulated an oxidative mechanism for cholesterol based on free radical processes whereby molecular oxygen attack at the C_7 position yields an unstable 7-hydroperoxy cholesterol. They attributed the presence of 7-ketocholesterol to the dehydration of the 7-hydroperoxide, and the epimeric 3β7-diols to form directly from the simple decomposition of the 7-hydroperoxide. The epimeric nature of these compounds was believed to be from either two stereoisomeric hydroperoxides, or due to rearrangement from a common precursor during decomposition. Although the 7-hydroperoxy cholesterol was not isolated, trace amounts of cholestan-3β, 5α, 6β-triol and 7-keto-$\Delta^{3,5}$-cholestadiene reinforced the validity of this proposed scheme.

Bladon (1958) also recognized the free radical induced nature of the commonly formed oxidation products. A similar mechanism was thus proposed with a 7-hydroperoxycholesterol as the primary oxidation intermediate. However, Bladon theorized that the epimeric 3,7-diols commonly formed were the product of the reaction of a 7-hydroperoxide with a propagating species such as an unoxidized cholesterol molecule. Dehydration and oxidation of these compounds accounted for the various secondary oxidation products found in autoxidized cholesterol.

The elucidation of cholesterol oxidation mechanisms depended on the development and application of thin-layer chromatography (TLC) to the complex mixture of products formed (Claude, 1966; Claude and Beaumont, 1966; Horvath, 1966; Fioriti and Sims, 1967; Smith et al., 1967).

Gas-liquid chromatography (GLC) has also aided cholesterol autoxidation studies by affecting adequate separation and identification of some cholesterol oxides (Claude, 1966; Van Lier and Smith, 1968a), preparation of pure cholesterol oxides (Van Lier and Smith, 1968b), and investigations of the thermal decomposition products and patterns of these compounds (Van Lier and Smith, 1970b; Teng et al., 1973a; Smith et al., 1973a).

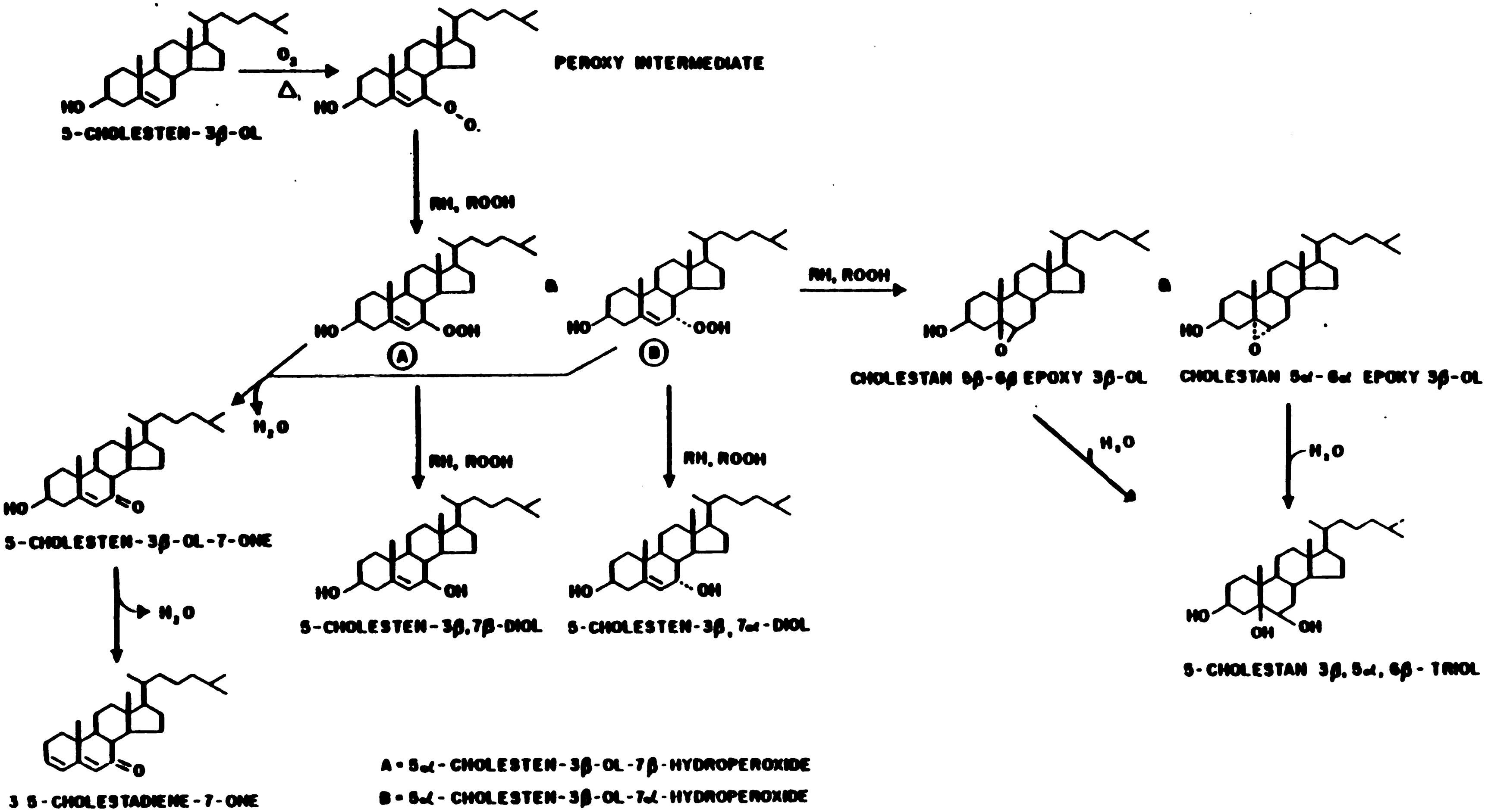
The development of the overall autoxidation scheme for cholesterol, utilizing these techniques, was reviewed by Smith (1980; 1981). There are two generally recognized distinct oxygen dependent processes in the autoxidation of cholesterol. The major process (Eq. 1) involves formation of sterol hydroperoxides while the minor process (Eq. 2) represents a formal alcohol dehydrogenation. This latter process is believed to be responsible for formation of small amounts of cholest-5-en-3-one (Ansari and Smith, 1978).

$$\mathrm{RH} + \mathrm{O_2} \text{ --------------- } \mathrm{ROOH} \qquad (1)$$

$$\mathrm{RCH(OH)} + \mathrm{O_2} \text{ --------------- } \mathrm{RC = O} + \mathrm{H_2O_2} \qquad (2)$$

The major reactions and products formed from Eq. 1 are shown in Figure 4. The initial reaction in this mechanism involves the abstraction of the allylic C_7 hydrogen and reaction with ground state dioxygen to form a C_7 peroxy radicals. These peroxy radicals are stabilized by hydrogen abstraction to form the epimeric 7α and 7β-hydroperoxy cholesterols. The initial formation of these compounds was

Figure 4. Major autoxidative pathways of cholesterol.



A = 5α-CHOLESTEN-3β-OL-7β-HYDROPEROXIDE
B = 5α-CHOLESTEN-3β-OL-7α-HYDROPEROXIDE

established by Smith and Hill (1972) utilizing N,N-dimethyl-p-phenylenediamine and N,N,N^1,N^1-tetramethyl-p-phenylene diamine dehydrochlorides as visualizing agents in the TLC analysis. Smith et al. (1973a) identified these compounds as the initial products of cholesterol autoxidation.

Both 7α and 7β-hydroperoxy cholesterol are formed initially, with the 7α-hydroperoxide predominating due to greater thermodynamic stability. The 7α-epimer was found to be capable of undergoing isomerization to the 7β species in solution, solid state or vapor state (Teng et al., 1973a and 1973b; Smith et al., 1973a). Formation of secondary oxidation products was reported to proceed by formal reductions and dehydrations (Smith, 1980). The 7α- and β-hydroperoxides are reduced to 5-cholesten-3β, 7α-diol and 5-cholesten-3β, 7β-diol respectively. Direct dehydration of either hydroperoxide epimer yields 5-cholesten-3β-ol-7-one (Van Lier and Smith, 1970a; Smith et al., 1973b). The 7α-diol may epimerize to the 7β-diol, and the 7-ketone may undergo dehydration to form 3,5-cholestadien-7-one (Van Lier and Smith, 1980).

The formation of epimeric cholesterol 5,6-epoxides occurs during the secondary autoxidation of cholesterol (Fig. 4). These reactions are facilitated by direct attack by cholesterol hydroperoxides or H_2O_2 on a molecule of unoxidized cholesterol, yielding both cholestan-5β, 6β-epoxy-3β-ol and cholestan-5α, 6α-epoxy-3β-ol, the former compound predominating. Hydration of these epimers yields the triol species, cholestan-3β, 5α, 6β-triol (Smith and Kulig, 1975).

Less common transformations of cholesterol oxidation products occurring on the A and B rings have been extensively reviewed by Smith (1981). These reactions in part explain the formation of such oxidative derivatives of cholesterol as cholest-5-en-7α-ol-3-one, cholest-4-en-7α-ol-3-one, 4,6-cholestadiene-3-one and cholestan-3β, 5α, 6β, 7α-tetraol.

Cholesterol present in crystalline form is susceptible to side chain oxidation due to the geometry of its structure. The reactions involved in side-chain oxidation have not been noted in aqueous or organic solutions of cholesterol (Beckwith, 1958). These reactions proceed via hydrogen abstraction by compounds such as 7-peroxycholesterol, followed by reaction with ground state oxygen yielding cholesterol hydroperoxides at the C_{20}, C_{24}, C_{25} and C_{26} positions. The tertiary carbons at C_{20} and C_{25} were observed to be most susceptible to peroxy radical attack (Van Lier and Smith, 1970a, 1970b, 1971; Van Lier and Kan, 1972). The resultant hydroperoxycholesterols may then undergo reductions and dehydrations described for 7-hydroperoxycholesterols. In addition, β-scissioning of carbon-carbon linkages in the side chain may take place.

This mechanism may be responsible for the formation of short chain volatile compounds during cholesterol autoxidation (Van Lier et al., 1975).

The results of many studies investigating cholesterol oxidation under various conditions supports the preceding proposed mechanisms. The instability of crystalline cholesterol exposed to air over periods up to 32 years have been reported by Mosbach et al. (1963), Fioriti and Sims (1967), Smith et al. (1967) and Van Lier and Smith (1970a). Over 30 autoxidation products have been detected in 12 year old cholesterol samples (Smith et al., 1967). Engel and Brooks (1971) however found only negligible amounts of oxidation products in highly purified cholesterol exposed to air for over 30 years.

Aerated colloidally dispersed cholesterol systems have also been studied extensively (Norcia, 1958; Horvath, 1966; Fioriti and Sims, 1967; Chicoye et al., 1968b; Weiner et al., 1972, 1973; Norcia and Mahadevan, 1973; Kimura et al, 1976, 1979). The oxidation of cholesterol in monomolecular films have also been investigated to assess the occurrence in cell membranes (Norcia, 1961; Kamel et al., 1971; Weiner et al., 1972). Products identified in these studies included 5-cholesten-3β-ol-7-one and the epimeric 5-cholesten-3β, 7-diols. Sevanian (1984) studied membrane cholesterol oxidation by using model membranes (liposomes) consisting of 75 mol percent bovine liver phosphatidylcholine and cholesterol. These liposomes were then subjected to peroxidizing conditions. Results indicated that the extent of fatty acid oxidation, cholesterol oxidation and cholesterol epoxide formation was well correlated. Moreover, 5-cholestan-5,6β-epoxy-3β-ol was found to form under propagation phases of lipid oxidation while cholestan-5,6α-epoxy-3β-ol was formed at the initiation phase of lipid oxidation.

The effect of esterification on the stability of cholesterol has also been studied. Esterification can increase or decrease the susceptibility of the cholesteryl moiety to oxidative attack dependent on the level of unsaturation of the fatty acyl moiety. Colloidally dispersed systems containing acetate, palmitate and oleate esters showed less susceptibility to oxidation than did unesterified cholesterol (Bergstrom and Wintersteiner, 1942c; Norcia and Janusz, 1965). This protective effect of esterification was proposed to be due to steric hindrance which interferes with the C_7 sterol position. Cholesteryl linoleate however, was found to be more prone to sterol moiety oxidation than unesterified cholesterol (Norcia and Janusz, 1965).

Linoleate, linolenate and arachidonate cholesterol esters were reported to be susceptible to oxidative attack in aqueous colloidal suspensions (Norcia and Mahadevan, 1973). The rate of oxidation was found to be greater in the linoleate system, followed by linolenate and then the arachidonate system, attributed to the extent and time course of free radical formation in the fatty acyl chain with resultant intramolecular propagation of sterol oxidation. Also, changes in the fatty acyl chain conformation, contingent on the introduction of oxygen containing functional groups, may have affected the oxidation rate.

Kamel et al. (1971) reported that cholesteryl acetate esters spread as a monomolecular film on water rapidly underwent hydrolysis, followed by oxidation of the sterol moiety. Thus in aqueous systems, esterification was less effective in inhibiting cholesterol oxidation.

Biological Consequences of Cholesterol Autoxidation

Atherosclerosis and Angiotoxicosis

The atherosclerotic effects of diets containing cholesterol have been well documented since Anitschkow first reported the induction of atherosclerosis in rabbits by cholesterol in 1912 (Smith, 1981). In many of these studies, USP-grade cholesterol added to the diet was found to have a profound effect on the amount and rate of deposition of atherosclerotic materials. Smith et al. (1967), however, reported the presence of 32 autoxidation products in USP-grade cholesterol stored at room temperature for 12 years. The possibility that contamination by these oxidative derivatives of cholesterol may have influenced the atherosclerotic characteristics of USP-grade cholesterol challenged the validity of previous studies (Taylor et al., 1979).

Studies of atheromatous tissue from human aortic sections indicated conclusively that in addition to cholesterol, trace amounts of autoxidation products were also present. Among the hcompounds identified were 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol (Henderson, 1954; Brooks et al., 1971), 5-cholesten-3β-ol-7-one (Brooks et al., 1966; Van Lier and Smith, 1967), 3,5-cholestadiene-7-one and 5-cholestan-3β, 5α, 6β-triol (Henderson and MacDougall, 1954; Henderson, 1956). In addition, cholestan-5, 6α-epoxy-3β-ol, 4-cholesten-3-one and 4, 6-cholesten-3-one were isolated from human serum (Gray et al., 1971). In this light, studies were designed to elucidate the roles of cholesterol oxidation products, USP-grade cholesterol contaminants and highly purified cholesterol in eliciting arterial damage.

MacDougall et al. (1965) investigated the toxicity of 103 steroids closely associated with cholesterol. Utilizing rabbit aortic cell cultures, 36 compounds were found to be toxic, and thus possible lesion inducers. Severe cell toxicity, resulting in cell death, regarded as the primary event in atherosclerotic lesioning, was exhibited by cholestan-3β, 5α, 6β-triol and 5-cholesten-3β, 26-diol.

Cholesterol, 5-cholesten-3β, 7β-diol and 5-cholesten-3β, 25 diol were moderately toxic, while 5-cholesten-3β, 7α-diol demonstrated no toxic effects.

Two studies cited by Peng et al. (1978) indicated an inherent difference between hypercholesteremia induced endogenously and exogenously. In these studies, endogenously-induced hypercholesteremia caused only mild amounts of spontaneous atherosclerosis in chickens whereas hypercholesteremia induced by a 2% cholesterol diet produced numerous, grossly visible atheromata. The possibility existed that compounds present in USP-grade cholesterol or in foodstuffs containing large amounts of cholesterol in the diet may have caused this difference. Taylor et al. (1979) implied the exogenous origin of autoxidation products of cholesterol in atheromata, referring to studies in which in vivo antioxidant systems, such as glutathione systems, effectively prevented endogenous formation of cholesterol oxidation products.

As a result of these findings, studies of the contaminants in USP-grade cholesterol regarding their angiotoxic and athersclerotic effects were undertaken. Imai and co-workers (1976) fed concentrated contaminants from USP-grade cholesterol to rabbits. Results indicated an increase in frequency of dead or dying aortic smooth muscle cells (angiotoxicity) and induced focal edema 24 hours after administration of the contaminants at a gavage of 250 mg/kg body weight. Administration of 5 year old and new samples of USP-grade cholesterol at dosages of 1 g/kg for seven weeks produced similar effects, with the older sample eliciting the greater atherosclerotic response. This was attributed to the greater concentration of autoxidation products in the 5 year old sample. Purified cholesterol administered at equivalent levels caused no increase in the frequency of dead or dying cells, exhibiting only slight increases in arterial aggregate debris. Administration of cholesterol-contaminant concentrates over the seven week period induced intimal diffuse fibrous lesions, while purified cholesterol showed no such effect. These findings reinforced the theory that initial atherosclerotic lesioning may be caused by exogenously produced cholesterol oxidation products rather than their cholesterol precursors.

In a similar experiment (Iami et al., 1980), impurities from USP-grade cholesterol were separated in three fractions and injected into rabbits via saline solutions. The use of injections over feeding was to overcome uncertainties associated with biotransformations, retention, excretion and absorption variances occurring in oral administration. Aortic sections examined after 24 hours showed that cholesterol hydroperoxides exhibited no angiotoxicity. The concentrate of impurities from USP-grade cholesterol, as well as several synthesized cholesterol oxidation products were also administered via injection. Two weeks after administration, cell death and inflammation were observed with intimal fibromuscular thickening apparent after 10 weeks. Freshly purified cholesterol induced no changes in major or minor pulmonary artery branches while cholestan-3β, 5α, 6β-triol and 5-cholesten-3β, 25-diol caused cell death within 24 hours, followed by necrosis, inflammation and fibromuscular thickening after 10 weeks. Cholestan-5, 6α-epoxy-3β-ol and 5-cholesten-3β-ol-7-one produced lesions in minor pulmonary artery branches.

Peng et al. (1978) identified the contaminants in USP-grade cholesterol as autoxidation products of crystalline cholesterol by thin-layer and gas-liquid chromatographic techniques. Compounds identified included 5-cholestan-3β, 5α, 6β-triol, 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol, 5-cholesten-3β, 25-diol, 5-cholesten-3β-ol-7-one, 3,5-cholestadiene-7-one and 7-hydroperoxycholesterol. A mixture of these compounds showed extensive cytotoxicity to cultured rabbit aortic smooth muscle cells, indicating potent angiotoxicity. Purified cholesterol exhibited no effect on the cell cultures. Separation and purification of these compounds in the concentrate by preparative thin-layer chromatography and subsequent administration of the pure compounds to cell cultures indicated that 5-cholesten-3β, 25-diol and 5-cholestan-3β, 5α, 6β-triol were responsible for the observed angiotoxic effect. The other compounds produced effects ranging from mild toxicity to no measurable toxicity in the cell cultures.

The mechanism by which cholesterol autoxidation products may cause intimal aortic cell death has only recently been investigated. Chen et al. (1974) noted that certain oxygenated sterols influenced cholesterol biosynthesis by inhibiting the enzyme, 3-hydroxy-3-methyl-glutaryl-Co-enzyme A reductase (HMG-CoA reductase). Subsequent investigations indicated a definite structural requirement for compounds having this capacity. It was shown that the prerequisite structure for inhibitors of the enzyme included a side chain of at least 6 carbons, a ketone or hydroxyl function at the C_3 position and a second ketone or hydroxyl function at positions 6, 7, 15, 20, 22, 24, 25 or 26 (Chen et al., 1974; Kandutsch et al., 1978; Kandutsch and Chen, 1978). Many products of cholesterol autoxidation have structures which were proven to inhibit this enzyme. The presence of these compounds in exogenous cholesterol has been associated with the suppression of hepatic cholesterol synthesis as well as inhibition of cholesterol biosynthesis in cell cultures (Kandutsch et al., 1978). Chen et al. (1974) observed 5-cholesten-3β,25-diol to be a potent inhibitor of HMG-CoA reductase, with a lesser inhibitory effect exhibited by 5-cholesten-3β,20α-diol, 5-cholesten-3β,ol-7-one, 5-cholesten-3β,7α-diol and

5-cholesten-3β,7β-diol as well as other recognized cholesterol autoxidation products.

The results of these studies have led to theories which implicated the autoxidation products of cholesterol as initiators of atherosclerosis via HMG-CoA reductase inhibition. Chen et al. (1974) concluded that the inhibition of cholesterol synthesis leads to membrane fragility, which results in improper membrane growth and subsequent cell death. Alteration of sterol concentrations in membranes also changes cell fluidity thus affecting transport, as well as membrane localized enzymes, which could lead to cellular disfunction. Kandutsch et al. (1978) stated that low cholesterol concentrations may also affect the cell's ability to reproduce.

Peng et al. (1979) supported these theories and postulated an alternative mechanism. Replacement of cholesterol in membranes by autoxidation products could easily occur due to the presence of both polar and apolar functional groups on the same molecule, leading to cellular disfunction. This theory is supported in that cholestan-3β,5α,6β-triol has a greater toxic effect than 5-cholesten-3β,25-diol, despite its lower ability to inhibit HMG-CoA reductase.

Carcinogenicity and Mutagenicity

Certain steroidal compounds have been suspected of possessing carcinogenic properties. As cited in the review by Lane et al. (1950), Roffo (1938) suspected that "oxycholesterol" may exhibit carcinogenic effects. Subsequent studies by Veldstra (1939) and Larsen and Barret (1944) showed that 3,5-cholestadiene induced papillomas in mice and rats. Conflicting data were presented by Kirby (1943, 1944) when both 3,5-cholestadiene and cholesterol heated to 270-300°C showed no papilloma induction when fed to rats over a two year period.

Early studies by Fieser et al. (1955) and Bischoff (1963, 1969) implicated several cholesterol oxidation products as carcinogens but these studies involved administration in oily vehicles which are now believed to have cocarcinogenic activity. Only cholestan-5α, 6α-epoxy-3β-ol was found to induce local fibrosarcomas in rats and mice when injected in an aqueous medium (Bischoff, 1963). The β-epimer, which is invariably present with the α-epoxide, may also act as a cocarcinogen (Bischoff, 1969). Results of screening other steroids for carcinogenicity revealed that a common structure was an oxygen linkage at the C_6 position for those compounds capable of inducing tumors.

Black and Douglas (1972, 1973) observed the formation of 5-cholestan-5,6α,-epoxy-3β-ol from cholesterol in the skin of human and hairless mice after exposure to ultraviolet radiation. The formation of this compound has been correlated to tumorogenesis. Chan and Black (1974,

1976) investigated the action of cholesterol-5, 6-epoxide hydrase on 5-cholestan-5,6α-epoxy-3β-ol, based on the premise that the carcinogenicity of this compound arises through the formation of an arene oxide intermediate. The hydrase enzyme converted the epoxide to a more polar species allowing rapid excretion. Hydrase activity was stimulated by factors which led to the formation of 5-cholestan-5, 6α-epoxy-3β-ol or its β-epimer, and was found to function by converting these compounds to their respective diols.

The feeding of cholesterol oxidation products to experimental animals has also been reported to promote cancer. Leduc (1980) found an increase in the incidence of liver tumors in mice after a brief feeding of cholestan-5, 6α-epoxy-3β-ol, but not after similar feeding of cholestan-3β, 5α, 6β-triol. Dehydrated egg and milk powders, in which cholesterol may be susceptible to oxidation, also were found to induce liver tumors in mice.

Cholesterol and its oxidation products have also been examined for their mutagenic effects on *Salmonella typhimurium* strains (Smith et al., 1979). Purified cholesterol was non-mutagenic in all strains tested, but gave mutagenic responses after being heated or γ-irradiated in air. Naturally air-aged commercial preparations of USP or reagent grade cholesterol also exhibited mutagenic responses, although some differences were noted between apparently similar samples to two bacterial strains. This was attributed to fine distinctions in sample compositions not evident by chromatographic analysis. In an effort to identify one or more specific compounds present in oxidized cholesterol samples, 5-cholesten-3β-ol-7-one, 3,5-cholestadiene-7-one, 5-cholesten-3-one, cholestan-3β, 5α, 6β-triol, cholestan-3β-5α-diol-6-one, cholestan-5, 6α-epoxy-3β-ol and cholestan-5, 6β-epoxy-3-β-ol were found to be nonmutagenic at levels as high as 6 mg/plate. 5-Cholesten-3β,25-diol exhibited toxicity in the assays, but conclusions as to its mutagenicity were not drawn.

Cholesterol Oxidation Products in Foods

Increased understanding of both the autoxidation reactions of cholesterol and the adverse biological effects of the oxidation products has generated concern over the possible presence of these compounds in foodstuffs containing cholesterol. Sheppard and Shin (1980) have even suggested the possible hazards to human health in the consumption of foods containing cholesterol oxidation products. Wilson (1976) stated that potential risks of atherosclerosis may be associated with the consumption of old powdered whole egg or whole milk. Kummerow (1979) considers the consumption of large doses of vitamin D during infancy hazardous in that it may initiate arterial smooth muscle cell death. This angiotoxicosis was proposed to be aggravated and accelerated in later years by the consumption of foods containing cholesterol oxidation products. Leduc (1980) found that the ingestion of dehydrated milk and egg powders increased the incidence of tumors in mice. Taylor et al. (1977) isolated necrogenic compounds from these foods.

Several researchers have speculated that certain food processing procedures may promote the formation of cholesterol oxidation products. Kummerow (1979a) stated that potatoes fried in animal fats, as well as chicken or fish, probably contain cholesterol oxidation products, since cholesterol is absorbed by fried product from the oil medium during frying. Taylor et al. (1979) implicated dried powdered foods stored in air at room temperatures as potentially major sources of angiotoxic sterol oxidation products in the diet. These foods include powdered eggs and milk as well as products containing dried whey or eggs. Also suspect were smoked meats, fish, and air-aged cheeses.

Smith (1980) emphasized a need to evaluate cholesterol-containing foods for cholesterol oxidation products via systematic analytical studies. Processing of foods, such as spray-drying, retorting, curing, frying and bleaching subject cholesterol in the food to conditions which may be conducive to oxidation. To date, relatively little information exists regarding levels of cholesterol oxidation in foods. A comprehensive list of oxidized cholesterol species isolated from foods is given in Table 2.

Table 2. Cholesterol oxidation products in foodstuffs[a].

Cholesterol oxide	Foodstuff	Reference
5α-cholesten-3β-ol-7α-hydroperoxide	Egg dough[b]	Acker and Greve, 1963
5α-cholesten-3β-ol-7β-hydroperoxide	Egg dough[b]	Acker and Greve, 1963
5-cholesten-3β,7α-diol	Egg dough[b]	Acker and Greve, 1963
	Spray dried egg yolk[b]	Chicoye et al., 1968a
	Freeze dried egg yolk[c]	Naber and Biggert, 1980, 1981, 1982
	Refined edible tallow[c]	Ryan et al., 1981
	Tallow-fried french fried potatoes[c]	Ryan, 1982; Tsai and Addis, 1984
	Butter[b]	Luby, 1982
	Bleached grated cheese	Finocchiaro et al., 1984
5-cholesten-3β,7β-diol	Egg dough[b]	Acker and Greve, 1963
	Spray dried egg yolk[b]	Chicoye et al., 1968a
	Freeze dried egg yolk[c]	Naber and Biggert, 1980, 1981, 1982
	Refined edible tallow[c]	Ryan et al., 1981
	Tallow-fried french fried potatoes[c]	Ryan, 1982; Tsai and Addis, 1984
	Butter[b]	Luby, 1982
	Bleached grated cheese	Finocchiaro et al., 1984

Table 2 (cont'd.).

Cholesterol oxide	Foodstuff	Reference
5-cholesten-3β-ol-7-one	Pork Fat	Williams and Pearson, 1965
	Spray dried egg yolk^{b}	Chicoye et al., 1968a
	Dried egg mix	Merrit, 1977
	Freeze dried egg yolk^{c}	Naber and Biggert, 1980, 1981, 1982
	Dried egg custard mix	Shoptaugh, 1982
	Dried whole milk	Shoptaugh, 1982
	Dried cheese product	Shoptaugh, 1982
	Salami	Shoptaugh, 1982
	Shrimp	Shoptaugh, 1982
	Bleached grated cheese	Finocchiaro et al., 1984
3,5-cholestadiene-7-one	Fresh egg yolk	Pennock et al., 1962
	Anhydrous milk fat	Flanagan et al., 1975
	Non-fat dry milk	Flanagan et al., 1975
	Beef^{b}	Vajdi and Nawar, 1979
	Tallow	Ryan et al., 1981
	Refined edible beef tallow^{c}	Ryan et al., 1981
	Tallow-fried french fried potatoes^{c}	Ryan, 1982; Tsai and Addis, 1984
	Bleached grated cheese	Finocchiaro et al., 1984

Table 2 (Cont'd.).

Table 2 (cont'd.).

Cholesterol oxide	Foodstuff	Reference
5-cholesten-3β,25-diol	Freeze dried egg yolk^c	Naber and Biggert, 1980, 1981, 1982
	Dried egg custard mix	Shoptaugh, 1982
	Dried whole milk powder	Shoptaugh, 1982
	Dried cheese product	Shoptaugh, 1982
	Salami	Shoptaugh, 1982
	Shrimp	Shoptaugh, 1982
	Dried beef brain	Tsai and Addis, 1984
	Dried beef liver	Tsai and Addis, 1984
	Deep-fat fried chicken^c	Tsai and Addis 1984
	French fried potatoes^c	Tsai and Addis,1984
Cholestan-5,6α-epoxy-3β-ol	Dried egg mix	Merrit, 1977
	Dried egg yolk	Tsai et al., 1979
	Bleached grated cheese	Finocchiaro et al., 1984
	Dried beef brain	Tsai and Addis, 1984
	Dried beef liver	Tsai and Addis, 1984
	Deep-fat fried chicken^c	Tsai and Addis, 1984
	French fried potatoes^c	Tsai and Addis 1984
Cholestan-5,6β-epoxy-3β-ol	Spray dried egg yolk^b	Chicoye et al., 1968a
	Dried egg mix	Merrit, 1977
	Dried egg yolk	Tsai et al., 1979
	Bleached grated cheese	Finocchiaro et al., 1984

Table 2 (cont'd.).

Table 2 (cont'd.).

Cholesterol oxide	Foodstuff	Reference
Cholestan-3β,5α,6β-triol	Dried egg yolk	Feiser and Bhattacharyya, 1953
	Spray dried egg yolk[b]	Chicoye et al., 1968a
	Dried egg mix	Merrit, 1977
	Tallow-fried french fried potatoes[c]	Ryan, 1982
	Dried beef brain	Tsai and Addis, 1984
	Dried beef liver	Tsai and Addis, 1984

[a] Adapted from Smith, (1981).
[b] Foodstuffs variously irradiated.
[c] Foodstuffs variously heated.

These data cannot be regarded as definitive since only the most recent works were conducted with adequate analytical methods (Smith, 1981).

Acker and Greve (1963) isolated cholesterol hydroperoxide and "7-hydroxy-cholesterine" from egg-containing dough mixes which were subjected to light under extreme conditions. Finely ground samples exposed to sunlight showed high levels of conversion to these products at the expense of cholesterol, while only limited formation was noted in similarly tested unground samples. Light barrier packaging of the dough mix effectively protected cholesterol from oxidative changes over a two year period. Chicoye et al. (1968) showed that induced photo-oxidation of spray-dried eggs caused the formation of 5-cholestan-5, 6α-epoxy-3β-ol and cholestan-3β, 5α, 6β-triol. Fresh egg yolk and unirraadiated spray-dried egg powder contained insignificant levels of these compounds, even when stored for one year at 25 C. Tsai et al. (1979) isolated cholestan-5, 6α-epoxy-3β-ol and its β-epimer from dry egg products by high performance liquid chromatography.

Dairy products have also been investigated to a limited degree for the presence of cholesterol oxidation products. Flanagan and Ferretti (1974) and Flanagan et al. (1975) isolated 4-cholesten-3-one and 3,5-cholestadiene-7-one from stored samples of non-fat dry milk and anhydrous milk fat. Luby (1982) investigated the photo-oxidation of cholesterol

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in butter. Results indicated that 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol and 6-cholesten-3β, 5α-diol formed after illumination of butter for extended periods of time. Finocchiaro et al. (1984) isolated 5-cholesten-3β, 7α-diol, 5-cholesten-3β,7β-diol, cholestan-5,6α-epoxy-3β-ol and 5-cholestan-5,6β-epoxy-3β-ol from stored bleached butteroils and grated cheeses. Shoptaugh (1982) analyzed a series of dairy products and tentatively identified 5-cholesten-3β, 25-diol and 5-cholesten-3β-ol-7-one from dried whole milk and dried cheese products.

Meats and meat by-products have been scrutinized to a limited extent for the presence of cholesterol oxidation products. Tu et al. (1967) analyzed freshly cooked meat for 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol and 5-cholesten-3β-ol-7-one but found no measurable amounts in lipid extracts. Vajdi and Nawar (1979) isolated 3,5-cholestadiene-7-one from beef after radiolysis. Shoptaugh (1982) tentatively identified 5-cholesten-3β, 25-diol and 5-cholesten-3β-ol-7-one in salami and shrimp. Dried liver and brain products from health food stores were found to contain significant quantities of cholestan-5,6α-epoxy-3β-ol, 5-cholesten-3β, 25-diol and 5-cholestan-3β, 5α, 6β-triol (Tsai and Addis, 1984; Park and Addis, 1984).

Ryan et al. (1981) and Ryan (1982) studied the formation of cholesterol oxidation products formed in tallow utilized as a deep fat frying medium. Results indicated that cholesterol in tallow exposed to frying temperatures oxidized to form 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol and 3,5-cholestadiene-7-one. Subsequent studies in the french fried potatoes processed in tallow for the presence of cholesterol oxidation products indicated that cholesterol and its oxidation products were preferentially absorbed by fried potatoes. The cholesterol oxides isolated from french fried potatoes processed in tallow were approximately four times more concentrated than cholesterol oxides in the frying medium. Oxidation products of cholesterol identified included 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol, 3,5-cholestadiene-7-one and cholestan-3β, 5α, 6β-triol. Commercially processed french fried potatoes fried in tallow or tallow blends were also analyzed and found to contain 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7α-diol, and 3,5-cholestadiene-7-one as well as unoxidized cholesterol.

Tsai and Addis (1984) and Park and Addis (1984) utilized high-performance liquid and capillary gas chromatography, respectively, to analyze commercially deep fat fried food products for cholesterol oxidation products. Fast food restaurant products including french fried potatoes, onion rings and fried chicken were found to contain appreciable levels of 5-cholesten-3β-ol-7-one, r

b

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sys

con

Conc

decr

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5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol, cholestan-5,6α-epoxy-3β-ol and 5-cholesten-3β, 25-diol.

Thus, as suspected by Kummerow (1979a), frying oils containing cholesterol may act as vehicles for the distribution of cholesterol oxidation products into foods of non-animal origin. Punwar and Derse (1978) evaluated various fats and oils for cholesterol content (Table 3). Frying fats from animal origins were found to contain appreciable amounts of cholesterol. Kummerow (1979a) has stated that 600 million pounds of fat are used for frying each year. Also, oils used to fry chicken and fish retain cholesterol from the product which may later be absorbed by other products fried in the same medium. Therefore, since the use of frying fats of animal origin appears to be a common practice, emphasis on stabilizing the non-saponifiable fraction, as well as triglycerides, should be implemented.

Measurement of Lipid Oxidation

The derivation of oils via fractionation processes can effect the oxidative stability of the newly produced oil system. Oils derived by fractionation have increasing concentrations of unsaturated fatty acyl moieties. Concommitant with this increase in unsaturation is a decrease in the stability of lipid systems to oxidative attack. This phenomenon, specifically in regard to beef tallow, merits concern due to the ubiquitous nature of

Table 3. Cholesterol content of various fats and oils[a].

Fat and/or oil	Cholesterol content (mg/100g)
Corn oil	ND[b]
Coconut oil	ND
Cottonseed oil	ND
Clarified shortening	77.8
Edible rendered bacon fat	108
Edible tallow	141
Lard	75
Leaf lard	101
Palm oil	1.97
Palm kernel oil	1.34
Peanut oil	ND
Pure vegetable shortening	ND
Soybean oil	ND
White clover lard	97.7
Salmon oil (human food grade)	485
Menhadin oil (human food grade)	521
Herring oil	766

[a] From Punwar and Derse, (1978).

[b] ND, not detected.

cholesterol in animal fats. As previously discussed, cholesterol present in oxidizing lipid systems is also prone to oxidation, the products of which may cause adverse physiological effects. Therefore, methods of assessing lipid oxidation in tallow or tallow-derived systems is important not only in regard to the aethestic quality of tallow-containing foods, but also the inherent toxicological threat posed by the oxidation of cholesterol present therein.

Perkins (1967) classified autoxidation processes as those occurring in fats and oils exposed to oxygen at temperatures below $100^{\circ}C$. Autoxidation occurs at sites of unsaturation in fatty acyl groups which compose the triglyceride moiety. The attack by oxygen and subsequent decomposition at these sites involves a three-step free radical chain mechanism as proposed by Farmer (1942).

Initiation
$RH + O_2$ -------- $R. + .OOH$ (3)

Propagation
$R. + O_2$ -------- $ROO.$ (4)
$RH + ROO.$ -------- $ROOH + R.$ (5)
$ROOH$ -------- $RO. + .OH$ (6)

Termination
$R. + R.$ -------- RR (7)
$R. + ROO.$ -------- $ROOR$ (8)
$ROO. + ROO.$ -------- $ROOR + O_2$ (9)

RH refers to any unsaturated fatty acid in which the H is labile by reason of being on a carbon atom adjacent to a double bond. R. refers to a free radical formed by removal of a labile hydrogen. The onset of this reaction mechanism is characterized by absorption of oxygen by the fat. During this induction period, antioxidant species in the lipid systems are consumed while free radicals or their precursors accumulate. Rapid oxygen uptake proceeds at elevated radical concentrations.

Artman (1969) comprehensively reviewed the autoxidation process. Radical abstraction of an allylic hydrogen from an unsaturated fatty acid forms a fatty acyl radical. Direct addition of atmospheric oxygen, or addition after double bond rearrangement, yields a peroxy radical which abstracts a new allylic hydrogen from an unoxidized unsaturated fatty acid. The resulting hydroperoxide may then undergo homolytic cleavage, forming two new radicals which induce an autocatalytic propagation of the autoxidation mechanism. Termination reactions may occur only when the concentration of free radicals are sufficiently great to allow kinetically preferential radical-radical interactions.

Products of autoxidation, although dependent on the fatty acid constituents and their concentrations in the lipid system, generally include short chain aldehydes, ketones, acids and alcohols. Trace amounts of esters, hydrocarbons, aromatics, cyclohexanes and lactones have also been isolated from autoxidized samples (Artman, 1969).

Therefore, the oxidative deterioration of food lipids primarily involves autoxidative reactions accompanied by secondary oxidative and non-oxidative reactions (Gray, 1978). Hydroperoxides are the major initial reaction products of unsaturated fatty acyl moieties with oxygen, particularly oleate, linoleate, and linolenate in foods.

Peroxide Value

Peroxides (hydroperoxides) represent the primary products of lipid oxidation, and as such, their concentration in oil and fat systems can be used to measure the extent of oxidation. These species however decompose when exposed to heat, light or high radical concentrations to form secondary products of oxidation such as ketones or aldehydes. Thus, the level of peroxides in fat or oil systems may not accurately reflect the actual state of oxidative rancidity (Sherwin, 1968). Despite the transitory nature of hydroperoxides, the peroxide value is a common measurement of lipid oxidation, and if used properly under these constraints, is particularly useful in evaluating fats and oils in the initial stages of oxidation.

Gray (1978) reviewed numerous analytical procedures for the measurement of peroxide values for fats and oils. The majority of determinations are iodemetric in nature, yet alternative methods employ stannous chloride and ferrous salts as well as other oxidation-reduction indicators (Lea, 1962). Iodometric techniques are based on the measurement of iodine liberated from potassium iodide by peroxides present in a fat or oil. Although this method is widely used, Melenbacher (1960) identified two primary sources of error associated with iodometric techniques. Iodine, liberated from potassium iodide in the presence of peroxides, may absorb to unsaturated fatty acyl groups. Also, atmospheric oxygen may itself liberate iodine from potassium iodide during analysis, referred to as oxygen error, which causes high peroxide values (Gray, 1978). Other sources of error commonly associated with this technique include variations in sample weight, inconsistencies in solvent grades, variations in time and temperature during analysis, and the constitution and reactivity of the peroxides being titrated.

The official AOCS iodometric method Cd - 8 - 53 (AOCS, 1971) for peroxide value determinations is commonly used for all normal fats and oils; however, Gray (1978) stated that this method is highly empirical and that any procedural variation is reflected by variation in results. This method also is limited at low peroxide values due to difficulties in the determination of titration endpoints. Fiedler (1974) proposed replacing titration with an electrochemical technqiue in which liberated iodine is reduced at a platinum electrode maintained at a constant potential. Utilizing this technique, the sensitivity was improved for peroxide values ranging from 0.06 to 20 meq/kg lipid material.

Thiobarbituric Acid Test

The thiobarbituric acid (TBA) test is a commonly employed method for assessing the oxidative stability of fats, oils, and fatty foods. In this method, colorimetric evaluation of oxidized lipid systems are used to assess the extent of oxidation (Gray, 1978). Sinnhuber et al. (1938) proposed the mechanism for pigment formation as occurring via the condensation of two moles of TBA with one mole of malonaldehyde (Figure 5). However, malonaldehyde was not known to be common to all oxidized lipid systems. Dahle et al. (1962) postulated that malonaldehyde was derived from peroxides having unsaturated linkages β and γ to the peroxide group, which eventually underwent cyclization to form malonaldehyde. Under these constraints, only fatty acids possessing three or more double bonds were capable of producing malonaldehyde. Pryor et al. (1976) later elucidated the mechanism of formation of TBA-reactive substances from oxidized fatty acids. TBA-reactive substances, such as prostiglandin-type endoperoxides decompose under the TBA test conditions to produce malonaldehyde which eventually reacts with TBA.

The TBA test has been utilized in various ways. TBA reagent can be added directly to foods and the mixture heated until maximal color development has occurred. This

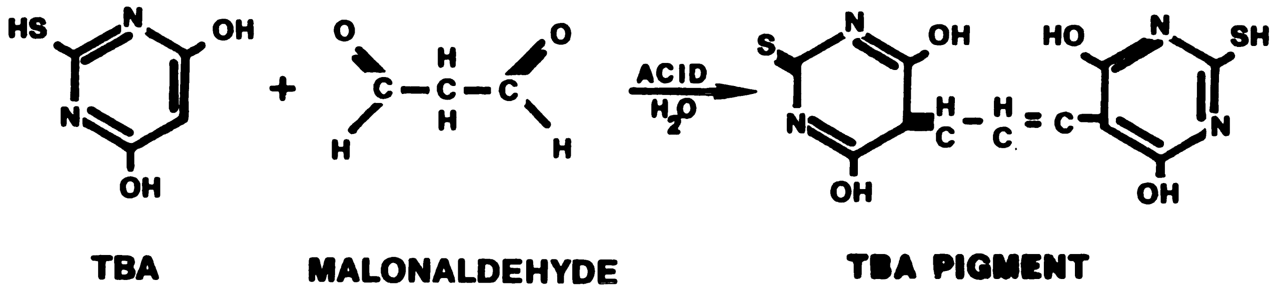


Figure 5. Proposed mechanism for the formation of red pigment from malonaldehyde and thiobarbituric acid in the TBA test (Gray, 1978).

is followed by extraction of the colored complex and quantitation by spectrophotometric means. In an alternate technique, foods are steam distilled from an acid solution. The distillate is then reacted with TBA and heated, after which the pigment is quantitated spectrophotometrically (Gray, 1978). Both methods require acidic conditions (pH 0.9-1.5) to liberate malonaldehyde from peroxides as well as catalyze the condensation of malonaldehyde with the TBA reagent.

Despite its widespread use, the TBA test has been criticized for a variety of reasons. Gray (1978) reviewed the results of many studies and reported a major problem with the TBA test is the formation of pigments with absorption maxima at wavelengths other than 532 nm. Therefore, individual lipid systems may react with TBA in ways different to that given in Figure 3, mandating a priori investigation of specific oxidized lipid systems for compatibility with the TBA method.

The TBA test is most frequently employed to assess the oxidative stability of meat lipids (Bailey et al., 1980). The use of this method in meat systems is due to a number of studies which indicate that increases in TBA values parallel flavor deteriorations in meat samples (Watts, 1961; Zipser et al., 1964). These studies indicate high correlation between rancid odor and TBA numbers in cured and uncured pork samples. However, the TBA test has never been standardized as a measure of meat acceptability and it is not always reliable as a measure of undesirable meat flavor (Bailey et al., 1980). This has been attributed to the possible interaction of malonaldehyde and closely related compounds with other food components such as amino acids, proteins and glycogen (Kwon et al., 1965). It is particularly unreliable in frozen foods and for certain cured meat products (Bailey et al., 1980).

Gas Chromatographic Evaluation of Lipid Oxidation

In recent years, the use of gas chromatography has been more extensively used in the separation and identification of the products of lipid oxidation in model systems (Gray, 1978). Although these methods were developed to elucidate mechanisms of oxidation, several researchers have developed procedures for measuring oxidation of unsaturated fatty acyl moieties in food systems, including vegetable oils, ground beef and cheese (Nawar and Fagerson, 1962; Jarvi et al., 1971; Fioriti et al., 1973; Dupuy et al., 1973; Dupuy et al., 1976; Melton, 1983). These methods include analysis of headspace and direct analysis by gas chromatography both with and without pre-enrichment of the volatile fractions.

Studies conducted on oxidized methyl linoleate (Horvat et al., 1964) and soybean oil (Selke et al., 1970) indicated that saturated hydrocarbons arise early during the oxidation of lipids when aldehydes are either absent or present in non-detectable levels. Evans et al. (1967) reported that pentane is liberated as the predominant short chain hydrocarbon in thermally decomposed oil systems. Pentane formation and flavor score correlations have been used to determine rancidity of oils via direct application of oils onto the gas chromatographic column (Jarvi et al., 1971; Evans et al., 1967). Warner et al. (1974) reported significant correlations between the amount of pentane developed and the number of rancid descriptions of aged vegetable oils and potato chips. Samples in this study required only 0.08 parts per million in the headspace to be perceived as rancid by 90% of the flavor panel. Both oil and potato chips in this study were more stable to the development of pentane as the linoleate content and iodine values decreased. Min and Schweizer (1983) reported correlations between the oxidation of potato chips processed in soybean oil and the evolution of pentane. This study indicated that the concentration of pentane in the headspace of potato chips increased as the storage time increased and flavor scores decreased. Thus, pentane concentration was suggested as a flavor quality indicator in the storage of potato chips.

Aldehydes, arising from the decomposition of hydroperoxides have been studied extensively in the flavors of oxidized oils by gas chromatography. Swoboda and Lea (1965) first studied the volatile compounds in oxidized and heated sunflower oil. The vacuum distillate from the treated oils was subjected to gas chromatographic analysis. Although over 40 compounds were indicated, hexanal, 2-heptenal and the isomeric 2,4-decadienals proved to be the major volatile components in the oxidized lipid system. Other compounds isolated and identified in this study were the aldehydes pentanal, 2-hexenal, heptanal, octanal, 2.4-heptadienal, 2-octenal, nonanal, 2-nonenal, the isomeric 2,4 nonadienals, and 2-undecenal. Also isolated via gas chromatographic evaluation were ketones, hydrocarbons and alcohols including: heptane, octane, 1-octen-3-one, 1-octen-3-ol and 2-heptenone.

The volatile profile method of assessing the extent of lipid oxidation was investigated further by other researchers. Dupuy (1976) obtained high correlations between oil flavor and peak areas of pentenal, hexenal, 2,5-heptadienal and total volatile peaks. Williams and Applewhite (1977) studied the correlation of specific volatile profile peaks to flavor scores of vegetable oils. Results indicated that many peaks normally present in the volatile profiling of an oxidized oil can be adequately used to assess oil flavors. However, this study did not publish the identities of the volatile flavor compounds yielding high correlations. Warner et al. (1978) examined commercially processed soybean, cottonseed and peanut oils oxidized under controlled conditions. They indicated that flavor scores for all three oils correlated well to the concentration of hexanal and pentanal in the oils.

Current studies in the area of GC profiling and correlation to flavor scores for oxidized lipid systems include determinations of the flavor intensities associated with the various carbonyl compounds isolated in oxidized lipid systems. Dixon and Hammond (1984) have devised methodology to assess the threshhold concentrations of volatile lipid oxidation products in aqueous emulsions. Compounds including 2-ketones, n-aldehydes, 2-enals and 2,4-dienals were measured for perceived flavor intensity using 2-heptanone serial standards for comparison.

Gwo et al. (1984) have also employed GC profiling of volatile flavor components to assess changes and evaluate shelf-life of fats used for deep-fat frying. These data, coupled with other indices of fat deterioration, have also been reported to be highly useful in evaluating the efficacy of antioxidants used in fats specified for frying.

Potato Chip Processing and Stability

The application of selected tallow fractions to new food uses is exemplified by the potential use of such a fraction in the processing of potato chips. Approximately 3.4 million pounds of potatoes are processed into chips yearly, representing nearly 11% of the entire potato harvest (PCIB, 1978). Clearly, the use of an oil derived from tallow may adequately be used to manufacture potato chips.

Therefore, as the attributes of the derived tallow fraction will be assessed in the manufacture of chips, a review of potato chipping operations, raw materials, and stability is pertinent to this study.

Potato Variety

Potatoes grown specifically for processing into chips must possess characteristics for ensured quality of the finished product (Potato Chip/Snack Food Association, 1976a). Included in these characteristics are high specific gravities (>1.070), low reducing sugar content (<0.2-0.3%), good size and shape, absence of defects, and suitability to storage and reconditioning. A comprehensive list of potato cultivars having differing chipping characteristics are given in Table 4.

The size of potatoes utilized by chippers is dependent on the package size specified by distributors. Small tubers are traditionally used for concession or small bag operations while larger tubers are mainly utilized for greater bulk density in larger size packages. Tuber shape is significant in that non-uniformity increases peel loss and decreases yield recovery ratios (Potato Chip/Snack Food Association, 1976a). Likewise, the absence of defects such as diseased potatoes with rot, sprouts and green areas, is important in terms of trimming losses and also in regard to finished product quality in the absence of trimming operations.

Table 4. Potato cultivar evaluation for chip manufacturing following storage (45°F) (Potato Chip/Snack Food Association, 1976a)

Excellent:	Good:	Fair:	Unsatisfactory:
Kennebec	Superior	Alamo	Triumph
Shurchip	Abnaki	Cobbler	Warba
Norchip	Chippewa	Cherokee	Pontiac
Monona	Russet Rural	Onaway	N.Y. 41
Merrimack	RussetBurbank	Pungo	Lasoda
New Haig	Peconic	Plymouth	Green Mountain
		Sebago	Houma

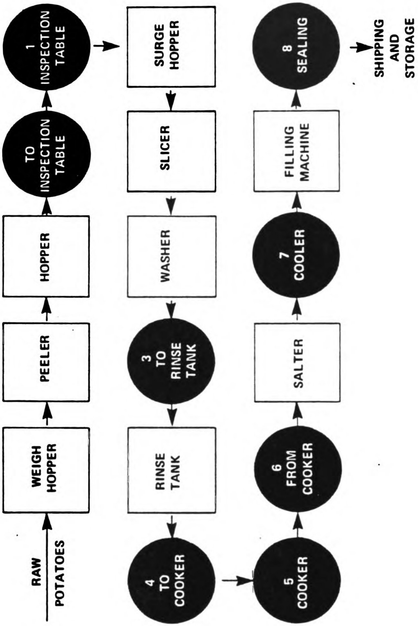
Unit Operations

The unit operations pertinent to the processing of potato chips have been outlined comprehensively by the Potato Chip/Snack Food Association (1976a). The processes include: cleaning and peeling, slicing and washing, color treatment, frying, chip through-put, salting, filling, package closure, package equipment maintenance and package coding. A flow diagram of this operation is found in Figure 6.

Studies on the Oxidative Stability of Potato Chips

Potato chips manufactured under controlled process operations contain between 30-46% lipid by weight. Therefore, the stability of potato chips during storage is dependent on the oxidative stability of the oil absorbed by

Figure 6. Typical unit operations in the processing of potato chips (Potato Chip/Snack Food Association, 1976a).


RAW POTATOES
WEIGH HOPPER
PEELER
HOPPER
TO INSPECTION TABLE
1 INSPECTION TABLE
SURGE HOPPER
SLICER
WASHER
3 TO RINSE TANK
RINSE TANK
4 TO COOKER
5 COOKER
6 FROM COOKER
SALTER
7 COOLER
FILLING MACHINE
8 SEALING
SHIPPING AND STORAGE

the potato matrix during the frying process (Min and Schweizer, 1983). Studies conducted over the past two decades have utilized the understanding of lipid oxidation to assess changes in the quality of stored potato chips.

Dornseifer and Powers (1963) investigated the volatile carbonyls produced by potato chips during storage. Steam distillates of fresh and aged potato chip samples were treated with 2,4-dinitrophenylhydrazine reagents to form the 2,4-dinitrophenylhydrazone derivatives of the volatile carbonyls present in stored chips. The carbonyls were subsequently regenerated from the phenylhydrazones and analyzed by gas chromatography. Results indicated that ethanal, propenal, 2-propanone, n-butanal, 2-pentenal, 2,3-butadione, 2-hexenal, n-heptenal and 2-heptenal were present in both fresh and aged chips. Further, these researchers noted that ethanal and 2,3-butadione which have desirable flavors decreased with storage while 2-propenal, which manifests a sharp pungent aroma, increased greatly with extended time of storage. Thus, these compounds were believed to play an important role in the flavor of potato chips. No indication of the oil system used to process the potato chips was given.

In a subsequent study, Dornseifer and Powers (1965) studied potato chip volatile constituents from potato chips processed in cottonseed oil stored at -35°C in the dark, 60°C in the dark, and room temperature in sunlight. The volatile constituents derived under these conditions were fractionated according to functional groups. Results indicated that isovaleric acid was present in all three chip distillates while butyric acid concentrations increased greatly under ambient temperature-sunlight storage. Analysis of the carbonyls derived from the stored chips indicated the presence of acetaldehyde, propionaldehyde, acrolein, 2-butanone, butyraldehyde, valeraldehyde, diacetyl, crotonaldehyde, 2-hexanone, 2-hexanal, hexanal, 2-heptanone, and 2-heptenal in all chips regardless of storage conditions. Heptanal, octanal, nonanal, 2-octanone, 2-nonanone and the isomeric 2,4 decadienals were also isolated from the steam distillates of potato chips stored at 60°C in the dark and ambient temperature-sunlight conditions. The concentrations of all carbonyls differed among storage conditions with the only identified trend being the disappearance of diacetyl and the increase in 2-hexenal in all samples with extended storage.

Mookerjee et al. (1965) studied monocarbonyl compounds in fresh and stale potato chips processed in an oil blend of equal parts corn and cottonseed oil. This investigation indicated a relationship between the types and concentrations of monocarbonyl compounds and the changes in flavor of potato chips over storage time. Results showed that as potato chips become "stale" over periods of storage, the levels of saturated aldehydes, saturated ketones and

2-enals increased while levels of 2,4-dienals decreased. The development of stale flavors in the chips was thus proposed to be due to the formation of undesirable carbonyls with a concommitant loss of 2,4-dienals, which contribute to fresh potato chip flavor. In total, nineteen monocarbonyl compounds were identified including five saturated aldehydes (C_5-C_9), seven saturated 2-ketones (C_3-C_9), six 2-enals (C_6-C_{11}) and 2,4-decadienal. With extended storage, the largest increases in the following were noted; saturated aldehydes, (hexanal and pentanal), saturated ketones, 2-pentanone and 2-propanone, and 2-enals, 2-heptenal and 2-octenal. The levels of 2,4-decadienals constituted 55% of the monocarbonyl compounds in fresh potato chips while only being 7% of the total monocarbonyl in stale chips. Since 2,4-decadienals originate from the decomposition of linoleic acid under deep fat frying conditions, these researchers maintained that oils used for potato chip frying should contain certain amounts of linoleic acid to insure the formation of fresh potato chip flavor.

Deck et al. (1973) isolated and identified volatile compounds isolated from fresh potato chips. Carbonyl compounds isolated included saturated aldehydes (C_2, C_4-C_7), 2-enals (C_7, C_8), 2,4-dienals (C_7, C_{10}) as well as benzaldehyde, 3-cis-hexenal, furfuraldehyde, phenacetaldehyde, cyclopentanone and furyl methyl ketone. Of these compounds, 2,4-decadienal, phenacetaldehyde and furfuraldehyde were identified as having great importance in the development of desirable flavors in potato chips.

Warner et al. (1974) reported that the oxidative stability of potato chips processed in corn oil, hydrogenated vegetable oil and a 7:3 blend of cottonseed oil:corn oil could be assessed by monitoring the evolution of pentane in the headspace of stored chips. Flavor panel scores steadily decreased as the levels of pentane in the headspace of stored chips increased. Results from this study also indicated that pentane development in stored potato chips is directly dependent on the concentration of linoleic acid in the frying oil moiety.

Min and Schweizer (1983) investigated the oxidation of potato chips processed in soybean oil via monitoring the evolution of volatile compounds, the disappearance of oxygen and the efficacy of antioxidants in stored potato chips. Volatile compounds including pentane, 2-heptenal, 2,4-heptadienal and the isomeric 2,4-decadienals were detected in fresh and stored potato chips as well as in unused and expressed oils, with greater concentrations noted for the aged and expressed samples. Headspace analysis of stored chips showed decreasing oxygen concentrations with concommitant increases in the concentration of pentane. The changes correlated well to perceived changes in potato chip sensory quality. Thus, the authors recommended that evaluating pentane development could be used to assess the oxidative stability of stored potato chips. This study also indicated that pentane development decreased with increasing concentrations of the antioxidant TBHQ added to the frying oil.

EXPERIMENTAL

MATERIALS

Beef Tallow

Eleven 20 pound beef tallow samples rendered under various conditions were obtained from Interstate Foods Corporation (Chicago, Illinois). Subsequently a 100 gallon drum of tallow was obtained from this source and used for the duration of this study. All tallow samples were stored at 4°C to reduce oxidative changes.

Vegetable Oils

Ten gallons each of refined cottonseed oil and partially hydrogenated cottonseed oil (antioxidant-free) were supplied by Humko Inc. (Memphis, Tenn.). Palm oil was purchased from Palmco, Inc. (Portland, Oregon). These oils were stored at 4°C until use.

Standard Cholesterol Oxidation Products

Standard 5-cholesten-3β-ol, 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol, 3,5-cholestadiene-7-one, 5-cholesten-3β-ol-7-one, 5-cholestan-5,6α-epoxy-3β-ol and cholestan-3β, 5α, 6β-triol were purchased from Steraloids Inc. (Wilton, NH). 5α-Cholestane was purchased from Sigma Chemical Company (St. Louis, MO).

Potatoes

Approximately 100 pounds of potatoes (Atlantic) were provided by Dr. Jerry Cash, Michigan State University, Department of Food Science and Human Nutrition, for use in the manufacture of potato chips. These potatoes were stored for 19 days at room temperature until processing into chips, to avoid the need for reconditioning.

Reagents and Solvents

All reagents and solvents utilized in this study were reagent grade and/or HPLC grade.

METHODS

Phase I - Characterization of Tallows

The initial phase of this study was conducted to screen tallows rendered by different industrial processes for suitability for aqueous detergent fractionation. The eleven tallow samples provided by Interstate Foods Corporation represented tallows rendered via wet and dry rendering processes at different temperatures. The tallows were characterized by the following chemical and physical analyses, as well as by olein yields at 40°C under the fractionation scheme specified by Bussey et al. (1981).

Iodine Values

Iodine values for all tallow samples were determined using the official AOCS method (Cd 1-25).

Free Fatty Acid Values

Free fatty acid values for all tallow samples were determined using the official AOCS method (Ca 5a-40).

Phospholipid Content

Phospholipid analyses for all tallow samples were conducted using the official AOCS method (Ca 12-55).

Peroxide Value

Peroxide values for all tallow fractions were obtained using the official AOCS method (Cd 8-53).

Fatty Acid Analysis

All tallow samples were methylated by the boron triflouride- methanol method of Morrison and Smith (1964) utilizing the preparative conditions for triglycerides. Analysis of fatty acid methyl esters was carried out with a 5840A Hewlett Packard gas chromatograph using a glass column (2m x 4mm, i.d.) packed with 15% diethylene glycol succinate on Chromosorb W, 80/100 mesh (Supelco Inc., Bellefonte, PA). The analyses were carried out isothermally at 190°C, with an injection port temperature of 210 C, and a flame ionization detector temperature of 300°C. Nitrogen carrier gas flow rate at the detector was 30 ml/min.

Color Analysis

A model D-25 Hunter Color Difference Meter with an inverted head was used to assess the color of all tallow samples. A standardized yellow tile (L=78.4, a_L=-1.9,

b_L=25.0) was utilized as a color reference. Approximately 70 g of each tallow sample was placed in an optical glass cylinder cup (7.4 cm x 1.9 cm). The average of four individual color measurements was recorded for each sample, aided by an inverted white-lined cannister for standard optical background.

Olein Yield via Fractionation

The aqueous detergent fractionation procedure for tallow as defined by Bussey et al. (1981) was employed to assess the eleven tallow samples with regard to fractionation characteristics. One kg of each tallow sample was allowed to equilibrate to 40°C. Six grams of SDS were intimately mixed with each softened tallow sample. After 17 hours of crystallization, 600 ml of a 5% sodium sulfate solution were added. This mixture was allowed to sit at 40°C for 1 hour followed by centrifugation for 15 min at 2700 rpm (1240 x g) at room temperature. The resulting olein and stearin fractions were separated and weighed. The olein yield (g) was determined for each tallow samples by weight of the derived olein fractions.

Phase II - Modification of the Fractionation Process

Results obtained in the initial phase of this study identified the optimal pre-fractionation rendering process required for successful fractionation of tallow. A 100 gallon drum of rendered edible tallow was obtained which had been rendered under optimal conditions. This tallow was used to conduct studies to optimize the fractionation process modified by Bussey et al. (1981).

Fractionation reagent concentration

The following systems were prepared to assess the role of reagents used in the aqueous detergent fractionation process:

Tallow	Na_2SO_4	SDS	H_2O
1000 g	30g	6 g	600 ml
1000 g	---	6 g	600 ml
1000 g	---	6 g	---
1000 g	---	---	---

These systems were then subjected to the fractionation scheme outlined in Phase I. Olein yields were determined after centrifugation and recorded. In addition, tallow samples were completely melted and allowed to equilibrate to 40°C for 17 hours, with and without the addition of SDS, followed by centrifugation and determination of olein yields.

Electrolyte type and concentration

A series of mono- and divalent anions were investigated for substitution for sodium sulfate in the fractionation process. Solutions (5%) of sodium tartrate, sodium citrate, dibasic sodium phosphate, sodium chloride, sodium potassium tartrate and potassium carbonate were used in place of sodium sulfate in the fractionation scheme by Bussey et al. (1981). The effectiveness of these electrolyte solutions in the fractionation process was assessed on the basis of olein yield at 40°C.

Further investigations were conducted using 1, 2, 3, 4, 5, 6, 8 and 10 % solutions of sodium citrate and sodium sulfate. Substitution of these solutions for 5% sodium sulfate in the fractionation process previously described was carried out. Olein yields at 40°C were recorded using these electrolyte concentrations.

Phospholipid concentration

Vegetable lecithin at a level of 0.5% (w/w) was added to tallow to assess the effect of phospholipids on olein yields in the fractionation process. After addition of the lecithin, the tallow was subjected to fractionation as previously described. Olein yields at 40°C were obtained and compared to tallow fractionated without added phospholipid.

Temperatures for subsequent fractionation

The 40°C olein fraction derived via the fractionation process previously described was exposed to temperatures of 37, 35 and 33 C. Equal parts of 40°C olein were allowed to equilibrate to these temperatures for 48 hours. Crystallization did not occur at 37 and 35°C. Olein derived at 40°C was found to partially crystallize at 33°C. Similar crystallizations were conducted on subsequent olein phases at 27, 22, and 18°C without addition of SDS, water or electrolyte.

Phase III - The Characterization of Tallow Fractions

Modified fractionation process

The results from the previous phases of this study were utilized to develop the following modified fractionation process. Tallow (1 kg) was allowed to soften for 24 hours at 40°C. SDS was subsequently added as a powder to the softened tallow at a level of 0.6% based on tallow weight. This mixture was allowed to crystallize for 18 hours at 40°C followed by the addition of 600 ml of 5% sodium citrate solution warmed to 40°C. After one hour at 40°C, the mixture was centrifuged at 1240 X g for 15 min at room temperature. The resulting stearin (solid) fraction was washed repeatedly with hot H_2O to remove the SDS while the clear olein phase was further fractionated at 33, 27, 22 and 18°C as shown in Figure 7. Additional detergent was not introduced after the initial fractionation at 40°C because the combination of decreasing temperature and centrifugation provided satisfactory separation.

The derived tallow fractions were then subjected to the following analyses for characterization.

Iodine values

Iodine values were determined for all tallow fractions as previously described in Phase I.

Fatty Acid analysis

Fatty acid analysis for all tallow fractions were performed as previously described in Phase I.

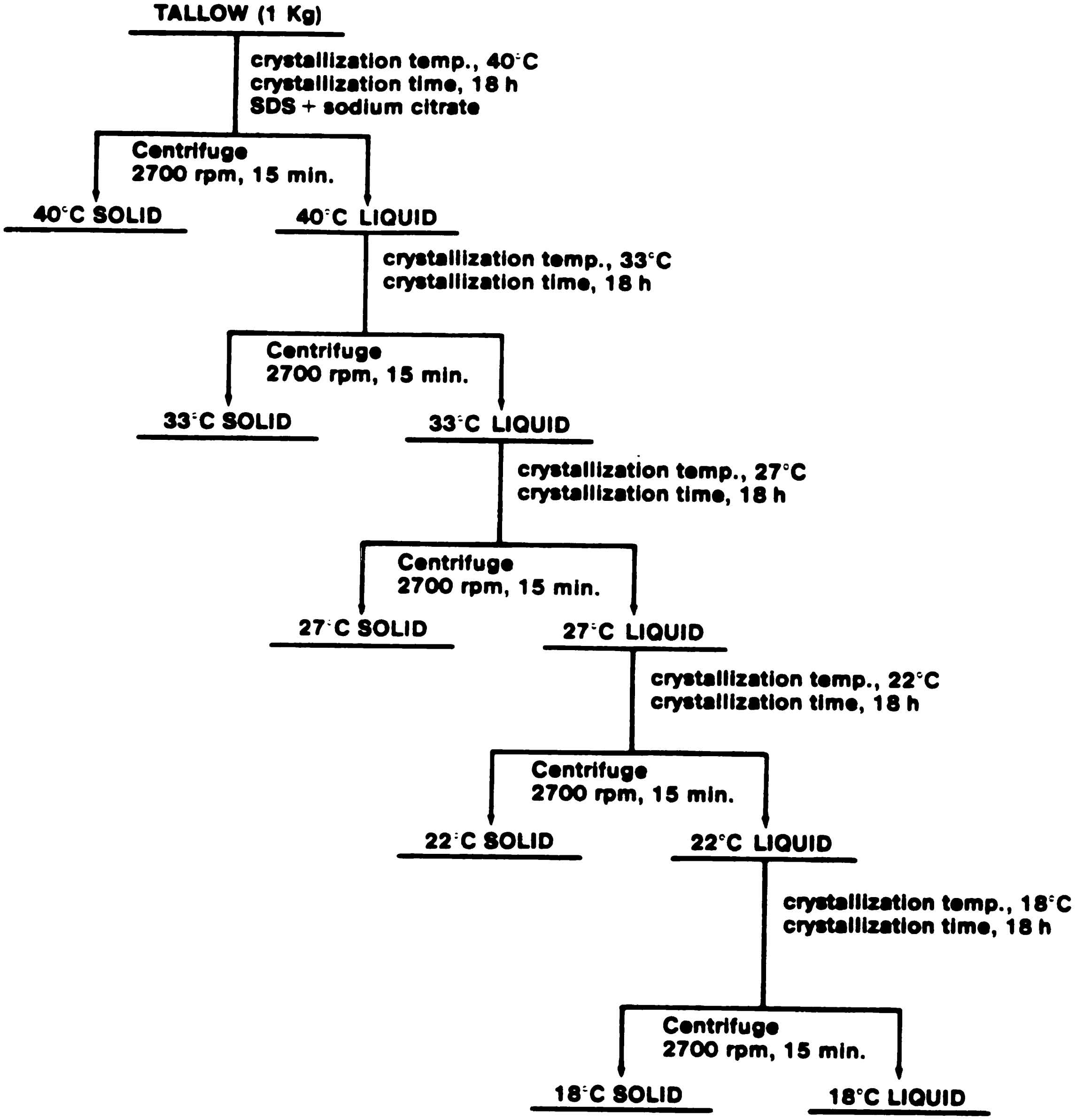


Figure 7. The modified aqueous detergent fractionation process utilized in the derivation of frying oils from refined edible beef tallow.

Analysis of Cholesterol in tallow fractions

1. Extraction of Cholesterol from tallow fractions

Original tallow and fractionated tallow samples (in duplicate) were subjected to saponification and extraction of cholesterol by the method described by Itoh et al. (1973). Ten grams of each tallow sample were refluxed for 1 h with 100 ml of 1.0 N alcoholic KOH. The saponified samples were cooled and 100 ml of distilled H_2O were added to each sample. The non-saponifiable matter was extracted from the samples using 5 x 100 ml aliquots of isopropyl ether in 1L separatory funnels. The ether extracts for each sample were combined and washed with 5 x 100 ml aliquots of distilled H_2O and then mixed with anhydrous sodium sulfate for 2 h at 4°C to remove residual water. The ether extracts were filtered, and evaporated to dryness in a Buchi Rotovapor R rotary evaporator (Buchi Inc., Switzerland). The residue was redissolved in ethyl acetate and quantitatively transferred to a 25 ml volumetric flask containing 1.0 ml of a 5 mg/ml stock solution of 5α-cholestane in ethyl acetate used as an internal standard for gas chromatographic (GC) analysis. The flasks were filled to the mark with ethyl actate, flushed with nitrogen, and stored at 4°C until analysis.

2. Standard cholesterol solutions

A stock solution of cholesterol was prepared by dissolving 250 mg cholesterol in 50 ml of ethyl acetate, yielding a working concentration of 5 mg/ml stock solution.

A series of standard cholesterol solutions was made by pipetting 0,1,2,3,4,5,6 and 7 ml of the stock solution into 25 ml volumetric flasks containing 1.0 ml of the 5.0 mg/ml solution of 5α-cholestane (internal standard), yielding standard solutions of 0,5,10,15,20,25,30 and 35 mg/25 ml.

3. Quantitation of cholesterol

Cholesterol quantitation was carried out using a 5840A Hewlett Packard gas chromatograph equipped with a glass column (2 m x 4 mm i.d.) packed with 3% SP 2100 on 100-120 mesh Supelcoport (Supelco, Bellafonte, PA). An isothermal program was utilized with column temperature, 265°C; injector temperature, 280°C; flame ionization detector temperature, 350°C; nitrogen (carrier) flow rate at the detector, 35 ml/min. The retention times of 5α-cholestane (internal standard) and cholesterol were 4.5 and 7.6 min respectively. Injection volume was 2.5 μl for all standard cholesterol solutions as well as the samples derived from fractionated and original tallow samples.

Phase IV - Cholesterol Oxidation in Lipid Model Systems

The oxidation of cholesterol in model systems consisting of triglycerides with differing levels of unsaturation was investigated. The model systems were prepared using 200 g each of triolein and tristearin, and a 1:1 mixture of triolein to tristearin. These systems were prepared in duplicate, and to each system 0.15% cholesterol (w/w) was added. These solutions were immersed in heated mineral oil held at 180°C in order to simulate frying temperatures. One set of samples were heated without aeration, while the other set was aerated with the rate of air bubbled through each system regulated at 30 ml/min. All samples were heated for 96 hours with samples removed for analysis at 24 hour intervals.

Analysis of Cholesterol

Cholesterol oxidation was assessed in the heated triglyceride systems by monitoring the loss of cholesterol over time with heating. The extraction and quantitation of cholesterol was conducted in duplicate as previously described in Phase III.

Qualitation of cholesterol oxidation products

Thin-layer chromatography was employed to tentatively identify the types of cholesterol oxidation products found in the heated model systems. Pre-coated analytical plates (0.25 mm) coated with silica gel GF (Fischer, Inc., Pittsburgh, PA) were activated at 110°C for 1 hour. One-dimensional thin-layer chromatograms were prepared by spotting 10 ul of the concentrated cholesterol extracts on the plates. Three plates were prepared representing all the cholesterol extracts used in this study. The plates were developed three times in a heptane:ethyl acetate (1:1, v/v) solvent system, with air drying between irrigations. The plates were then sprayed with 50% H_2SO_4 and heated at 110°C for 10 min to insure color development. Rf values and spot colors were recorded and compared to thin-layer chromatograms of standard cholesterol oxidation products prepared in a similar manner.

Phase V- Oxidative Stability of Tallow-Derived Frying Oils

Preparation of Oil Systems

The 22°C olein fraction from tallow was derived via the modified fractionation process previously described in Phase III. This olein phase was investigated with regard to its oxidative stability, as were unfractionated tallow, palm oil, and hydrogenated cottonseed oil. The influence of added quantities of vegetable oils on the oxidative stability of the 22°C olein fraction was studied by the preparation of the following oil blends:

95% 22°C olein + 5% refined cottonseed oil
90% 22°C olein + 10% refined cottonseed oil
95% 22°C olein + 5% hydrogenated cottonseed oil
90% 22°C olein + 10% hydrogenated cottonseed oil
85% 22°C olein + 15% hydrogenated cottonseed oil
95% 22°C olein + 5% palm oil
90% 22°C olein + 10% palm oil
85% 22°C olein + 15% palm oil

These blended oils along with the 22°C olein, unfractionated tallow, hydrogenated cottonseed oil and palm oil were prepared in triplicate and divided into three groups which were treated in the following manner:

Group I - Control (no antioxidant)
Group II - TBHQ (0.01% based on lipid weight)
Group III - Ascorbyl palmitate-mixed tocopherol
(500 ppm - 100 ppm)

These 36 samples were stored in glass screw-cap bottles and held at 65°C. Peroxide values (AOCS method Cd 8-53) were taken at intervals over a 60 day period. Samples were removed to assess the oxidative stability of cholesterol when peroxide values reached 35 and 70 meq/kg. Samples not reaching peroxide values of 35 meq/kg after 60 days at 65°C were analyzed for cholesterol stability at the end of the 60 day period. Concommitant with the analysis for cholesterol stability, fatty acid analysis were also performed.

Analysis of Cholesterol Oxidation Products

All samples were analyzed in duplicate for cholesterol content and type of cholesterol oxidation products formed utilizing the extraction, quantitation and qualitation methods specified in Phases III and IV of this study.

Fatty acid analysis

All samples were analyzed for fatty acid content in duplicate by the method described previously in Phase I.

Phase VI - Potato Chip Production and Stability Study

Preparation of Frying Oils

Oils used to process potato chips were 22°C olein derived as previously described in Phase III, unfractionated tallow blended 1:1 with hydrogenated cottonseed oil and hydrogenated cottonseed oil. Approximately 10 kg of each of the above oils were prepared for this study.

Preparation of Potato Slices

Potatoes were washed and sliced without peeling using a hand operated slicer calibrated to give slices of thickness of 0.06 inches as measured by a manual micrometer. The potato slices were soaked in water prior to frying to remove residual starch from the slice surfaces.

Frying Process

Approximately 7 kg of each oil system were placed in General Electric Hotpoint Deep-Fat Fryers (Model No. HK3) and heated to 180 ± 2°C. Washed and rinsed potato slices were placed in frying baskets and fried in the hot oil for 105 seconds. The chips were then allowed to drain and cool on racks until they reached room temperature. The chips were then lightly salted and sealed in one gallon glass jars. The jars were stored at room temperature in the dark for the duration of the stability study.

The oils in the fryers were allowed to equilibrate to 180 + 2°C between batches of chips. Approximately five pounds of chips were processed in each oil system. Fresh oil was added back to the fryers when needed.

Analysis of Potato Chips

Color

The potato chips fried in the three oil systems were visually evaluated immediately after frying and graded using the fry color standards for potato chips from the manual of the Potato Chip/Snack Food Association (1976b).

Fat Content

Potato chip samples fried in each oil system were analyzed for fat content initially and every week over the 6 week stability study. Exactly 35 g of each chip sample were ground in a mortar and quantitatively transferred to a 250 ml Virtis flask. One hundred ml of hexanes:diethyl ether (9:1, v/v) were added to the flask and the ground chip samples were allowed to soak overnight in the dark. The solvent was then decanted and the residual chip homogenate was re-extracted two times with 100 ml of the same solvent in a Virtis homogenizer. The extracts were combined, filtered and the solvent was removed in a Buchi rotary evaporator. The lipid material was weighed, flushed with nitrogen and stored at -20°C until further analysis.

Fatty Acid Analysis

Fatty acid analyses were performed weekly on the lipid extracts from the chips processed in the three oil systems as previously described in Phase I of this study.

Cholesterol Analysis

The cholesterol content of chips processed in 22°C olein and tallow:hydrogenated cottonseed oil (1:1) was determined weekly over the six week stability study. The extraction, quantitation and qualitation of cholesterol and oxidative derivatives were performed in duplicate on the chip lipid extracts as described previously in Phases III and IV of this study.

Stability Testing of Potato Chips

Sensory analysis

Weekly taste panels were conducted using a 10 member panel for the duration of the 6 week stability study. Three chips fried in each oil system were presented to taste panelists in individual booths lit with flourescent lights to simulate daylight. Characteristics evaluated were color, greasiness, flavor and overall acceptability using a linear 100 mm scale (see Appendices for scorecard).

Statistics

Numerical scores for potato chip attributes obtained from random sensory panelists were statistically analyzed utilizing a Completely Randomized Design (CRD) due to the nature of the panel members. An analysis of variance package (ANOVA) was used in conjunction with a microcomputer to process the numerical scores according to the method described by Gill (1978a). The data (scores) were analyzed for changes in specific attributes with time in each chip system, as well as for differences among chip sample attributes at given time intervals in the storage period. The data generated in this study were tested for homogenous variance and treatment effects and the calculated F statistics were used to define probability statements as specified by Gill (1978b).

Volatile Profiling of Stored Potato Chips

In order to evaluate the oxidation of potato chips processed in 22°C olein chemically, the following procedure was utilized. Thirty grams of potato chips (22°C olein processed) were placed in a 2 l boiling flask. 500 ml of water was subsequently added and the mixture was placed in a Likens-Nickerson extraction apparatus. Pentane (30 ml) was used as the extracting solvent. This system was allowed to reflux for 4 hours followed by concentration of the pentane fraction, which contained the volatile flavor components. A stream of nitrogen was used to concentrate to concentrate this extract from 30 ml to 0.1 ml. Sodium sulfate was used to dry the volatile flavor extracts. The concentrated volatile extract was transferred to a screw-top graduated vial and stored at -20°C until analysis. This profiling was performed on new chips and chips stored for 5 weeks which had been described as oxidized by trained panelists.

Capillary Gas Chromatography of Volatile Flavor Extracts

The analysis of the volatile flavor extracts from fresh and oxidized 22 olein processed potato chips were carried on with a 5840A Hewlett Packard gas chromatograph using a 20 M Carbowax capillary column. The analyses were carried out using a temperature program at an initial temperature of 40°C which was maintained for 10 min. The rate of temperature increase was 2 C/min to a final temperature of

190°C. The run was maintained at this temperature for 15 min, yielding a total run time of 100 min. The operating parameters for the analysis included injecting port temperatures of 275°C, and flame ionization detector temperatures of 300°C. Helium carrier gas was maintained throughout this system at a back pressure of 2.0 kg/cm^2.

Packed column chromatographic analyses of potato chip volatiles.

Potato chip flavor extracts were analyzed using a 3 m x 2 mm (i.d.) glass column packed with 10% Carbowax 20 M TPA on Chromosorb WHP (80/100 mesh). A HP 5840A Gas chromatograph was used. The GC was operated with a temperature program having an initial temperature of 55°C held for 10 min, followed by a temperature increase of 2°C/min up to 190°C and held at this final temperature for 60 minutes. Flame ionization temperature was 300°C, injection port temperature 275°C and the helium carrier gas flow rate was 30 ml/min at the detector. Injection volume was 0.5 .l.

Standard aldehydes and ketones in pentane were also injected at 0.5 µl volumes and retention times were recorded and compared to those of compounds present in the potato chip extracts.

GC - MS analysis of potato chip flavor extracts

GC-MS analysis was performed on the flavor extract from a 5 week old potato chip sample. Five µl of sample were injected into a Hewlett packard 5840A gas chromatograph equipped with a 3 M x 2 mm (i.d.) glass column packed with 10% Carbowax 20 M TPA on Chromosorb WHP (80/100 mesh). The GC was operated with a temperature program with an initial temperature of 55^{o}C which was held for 10 min. The rate of temperature increase was 2^{o}C/min up to 190^{o}C, followed by a 60 min hold at this temperature. Helium carrier gas was regulated at 30ml/min. The effluent passed into a Hewlett Packard 5985A mass spectrometer (quadrapole) unit having the following parameters; electron impact voltage, 70 e V; election multiplier voltage, 2410 e V; threshold 0.6; source temperature, 200^{o}C; analog/digital measurements, 3/sec; and ion detection in the positive mode.

RESULTS AND DISCUSSION

Phase I. Characterization of Refined Edible Tallows

As stated in the review of literature, the chemical composition of triglycerides found in tallow is dependent on climate, carcass location, rendering procedures and to a lesser degree diet (Swern et al. 1979). Of these, rendering procedures are the most easily controlled. Klein and Craver (1974) have reported that the best conditions for highest yield and optimal quality involves preheating crude tallow to 180-200^{O}F followed by alkali refining to remove free fatty acids. Therefore, the initial impetus of this study was to characterize tallows rendered from several processing plants using both wet and dry rendering techniques at a series of different temperatures. These studies were performed in order to evaluate different rendering processes for the production of tallows suitable for fractionation, as well as to identify possible chemical differences among tallow samples which may influence fractionation.

Eleven tallow samples were obtained from Interstate Foods Corporation representing tallows rendered at three temperatures under both wet and dry rendering conditions. The chemical and physical characteristics of these tallows are found in Table 5 and Table 6. The information given in

Table 5. Chemical and physical characteristics of tallow samples received from a commercial supplier.

Sample	Iodine value[a]	Peroxide value[a] (meq/kg)	Free fatty acid[a] (%, as oleic)	Phospholipid[a] (%, as lecithin)	Color		
					L	a_L	b_L
120°F, dry rendered	48.7	0.0	0.21	N.D.[b]	+41.4	-5.3	+17.9
120°F, wet rendered (1)	48.0	0.0	0.23	0.02	+41.3	-5.3	+17.1
120°F, wet rendered (2)	42.6	0.1	0.45	0.04	+42.5	-5.7	+17.6
120°F, wet rendered (3)	43.3	0.1	0.67	0.09	+40.6	-6.8	+24.6
120°F, wet rendered (4)	46.3	0.3	0.60	0.03	+42.4	-4.6	+14.6
145°F, wet rendered (1)	48.4	0.2	0.26	0.03	+42.0	-4.0	+11.5
145°F, wet rendered (2)	47.3	0.1	0.25	0.16	+42.4	-4.1	+12.3
180°F, dry rendered (1)	45.9	0.0	0.65	0.03	+35.5	-1.0	+16.6

Table 5 (cont'd.).

Sample	Iodine Value[a]	Peroxide Value[a] (meq/kg)	Free fatty acid[a] (%, as oleic)	Phospholipid[a] (%, as lecithin)	Color		
					L	a_L	b_L
180°F, dry rendered (2)	47.5	0.2	0.61	N.D.[b]	+34.5	+0.1	+15.5
180°F, dry rendered (3)	46.2	0.0	0.06	0.23	+42.6	-4.8	+14.1
180°F, wet rendered	45.3	0.1	0.12	0.138	+41.9	-2.3	+16.0

[a] Values represent averages of analysis performed in duplicate.
[b] N.D., not detected.

Table 6. Fatty acid composition of tallow samples obtained from a commercial supplier.

Sample	Fatty Acid	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
120°F, Dry		3.4	0.7	25.7	3.7	19.0	43.9	2.4	1.0	0.2
120°F, Wet (1)		3.7	0.7	26.9	3.9	17.9	42.9	1.6	0.7	1.6
120°F, Wet (2)		3.6	0.9	27.2	4.0	20.2	40.8	2.2	0.8	0.4
120°F, Wet (3)		3.6	0.9	28.3	4.2	21.4	38.6	1.8	1.0	0.3
120°F, Wet (4)		3.8	1.0	27.0	4.1	17.9	42.7	2.4	0.9	0.3
145°F, Wet (1)		3.5	0.9	26.5	4.1	18.8	42.6	2.5	0.9	0.2
145°F, Wet (2)		3.6	0.8	27.1	4.1	19.2	41.6	2.4	0.7	0.4
180°F, Dry (1)		3.8	0.8	27.1	4.1	17.9	42.8	2.4	0.9	0.4

Table 6 (cont'd.).

Sample	Fatty Acid	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
180°F, Dry (2)		4.0	0.7	28.4	3.7	18.3	39.3	1.3	0.6	3.7
180°F, Dry (3)		3.7	0.9	25.4	3.5	19.3	43.3	2.7	1.0	0.2
180°F, Wet		4.2	0.8	27.1	3.5	20.5	40.2	2.7	0.9	0.3

these tables indicate that the tallows varied in many physical and chemical characteristics. The tallows ranged in iodine values from 42.6 to 48.7, in free fatty acid content from 0.06 to 0.67% and in phospholipid content from no detectable amount to 0.23%.

Results of fatty acid analyses indicated that all tallow samples had fatty acid profiles within the ranges reported by Swern et al. (1976). Color evaluations of the tallow samples are shown in Table 5 and ranged visually from very clear to deep yellow in color. Thus, from a chemical and physical perspective, tallow samples rendered at different temperatures under wet and dry conditions had different characteristics.

Fractionation of these tallow samples under the conditions specified by Bussey et al. (1981) indicated sizeable differences in olein yields at 40°C which were dependent on pre-fractionation rendering processes. The results of olein yield determinations for the tallows are found in Table 7 and Figure 8 and appear to indicate that the rendering process is directly related to the suitability of tallows for fractionation. Those tallows rendered at low temperatures (120°F) had substantially higher olein yields at 40°C than did those tallows rendered at intermediate temperatures (145°F), while those rendered at high temperatures (180°F) were observed to have very poor olein yields. Tallows rendered at 120°F produced fractions of

Table 7. Olein yields of tallow samples obtained from a commercial supplier fractionated at 40°C utilizing the method of Bussey et al. (1981).

Sample	Olein yield (%)[a]
120°F, dry rendered	62.6
120°F, wet rendered (1)	69.4
120°F, wet rendered (2)	65.7
120°F, wet rendered (3)	68.2
120°F, wet rendered (4)	70.0
145°F, wet rendered (1)	53.4
145°F, wet rendered (2)	51.5
180°F, dry rendered (1)	37.7
180°F, dry rendered (2)	38.4
180°F, dry rendered (3)	3.9
180°F, wet rendered	19.8
120°F, wet rendered (4) +0.5% vegetable lecithin	67.5

[a] Values represent average values of duplicate fractionations.

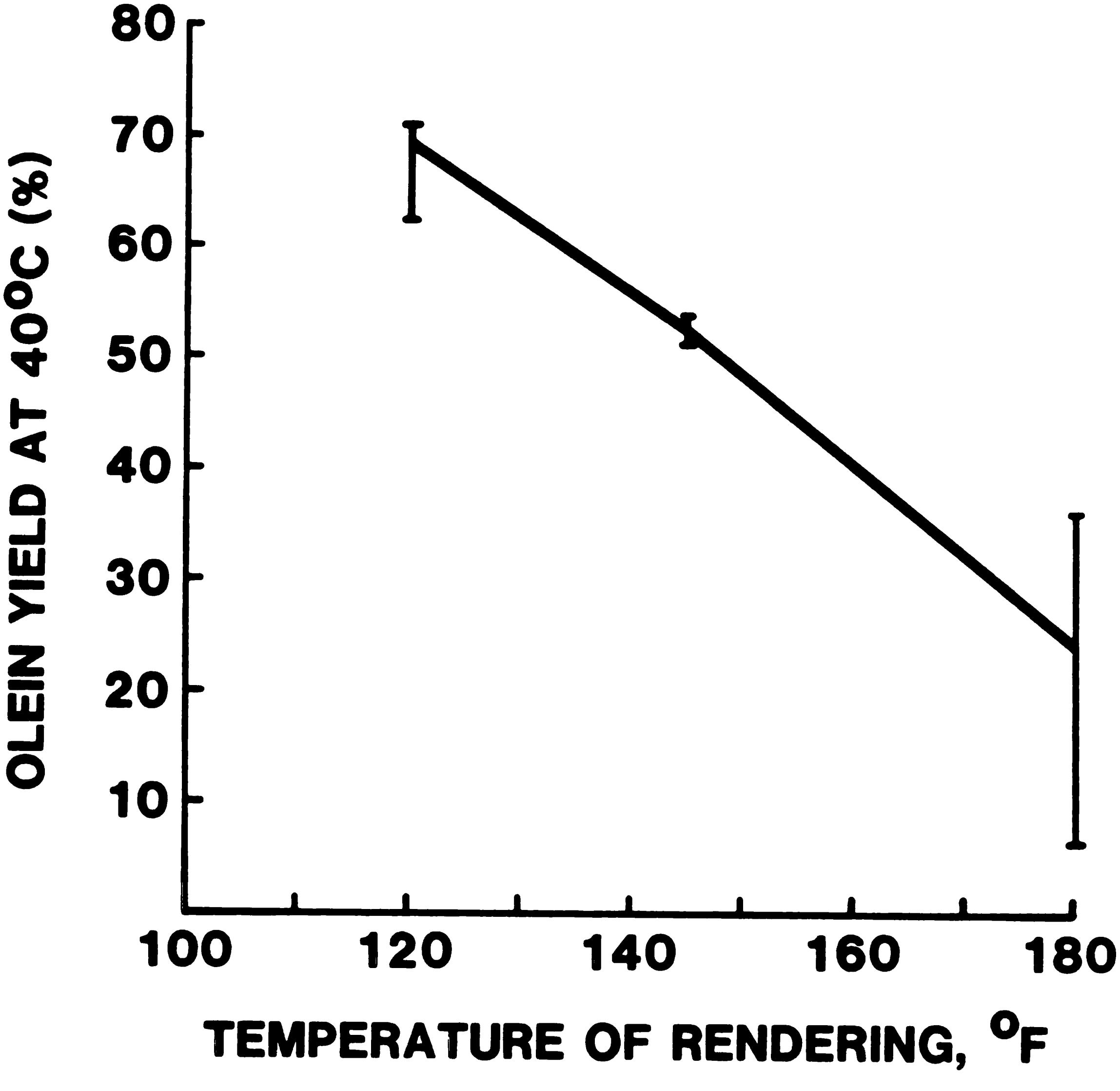


Figure 8. Olein yields of tallows fractionated at 40°C versus temperature of rendering, °F.

62.6-70.0%, while those rendered at 145°F yielded olein fractions of 51.5-53.4%. Those tallows rendered at 180°F ranged in olein yield from 3.9-38.4%. Due to a lack of an adequate number of samples, coupled with the variability of the chemical constituents, it was not possible to ascertain the effect of wet versus dry rendering on fraction yield at specific rendering temperatures. However, at a rendering temperature of 120°F, wet procedures consistently produced tallows which gave higher yields of olein when fractionated at 40°C.

It appears from these fractionation data that the rendering processes used in tallow production are important to the optimization of olein yields in subsequent fractionation procedures. Rendering of tallow at 180°F will completely melt GS_3 and GS_2U triglyceride types in the fatty tissues trimmed from the carcass. As these triglyceride types melt, they eventually may be dispersed in the tallow system, yielding rendered tallow with higher GS_3 and GS_2U concentrations than those tallows produced by rendering at lower temperatures. Rendering tallow at 120°F may prevent GS_3 and GS_2U triglyceride types from completely melting, and these may be partially removed as the rendering process proceeds through the filtration or centrifugation processes.

Speculation regarding higher olein yields for tallows rendered at lower temperatures may also involve viscosity effects. At lower temperatures of rendering, fats remain more viscous, thus preventing the release of the higher melting glycerides into the liquid fat phase. Low temperatures of rendering may also fail to totally disrupt adipocytes in pre-render tallow, resulting in only partial removal of triglycerides. These factors, combined with failure to reach the melting point of the tallow moiety may be responsible for the higher olein yields of tallows rendered at lower temperatures. These speculations could be reinforced by mass balance data of the actual rendering processes, as well as by proximate analysis of the residues of rendering.

Also noted in this phase of the study was the presence of an emulsion between the crystalline stearin and liquid olein phases in many of the fractionated tallow samples. It was observed that this emulsion formed at the expense of olein, thus affecting subsequent olein yields. Glassner et al. (1984) studied the formation of these emulsions and found that emulsion formation was dependent on surfactant concentration used in the process, as well as the shear forces applied to the system during mixing procedures.

The concentration of phospholipids present in the tallow samples was also investigated in regard to emulsion formation in the fractionation process due to the emulsification properties of these compounds (Bennion, 1980). The effects of phospholipids in tallow fractionation and emulsion formation was asessed by the addition of 0.5% vegetable lecithin to a tallow sample with known olein yield of 70.0% at 40°C. At the 0.5% level, an olein yield of 67.5% was observed (Table 7). Thus, it appears that phospholipid concentration has little effect on the efficiency of fractionation.

Phase II. Modification of the Fractionation Process

Results from the initial phase of this study indicated that tallows rendered at low temperatures were better suited for fractionation than those rendered at higher temperatures. Having identified tallow samples suitable for fractionation purposes, the next phase of this study was directed toward the optimization of the fractionation process, including optimization of reagent concentrations (water, electrolyte and surfactant), as well as alternative electrolyte studies for substitution for sodium sulfate. In addition, the development of a fractionation scheme yielding tallow fractions having characteristics which permitted their use as pourable shortenings and deep fat frying media was addressed.

Effects of reagent concentration on olein yield at 40°C.

Tallow rendered at 120°F was utilized to assess the role of selected reagents in the fractionation scheme proposed by Bussey et al. (1981). The results of fractionations, expressed as olein yield at 40°C, using this method while varying the reagents are found in Table 8. These data suggest that the combined application of electrolyte, water and surfactant are essential in the aqueous detergent fractionation of tallow. In the absence of any one or more of the reagents specified, fractionation yields were found to be considerably reduced, or no phase separation was observed to occur. In addition, tallow completely melted by heating to approximately 65°C followed by equilibration to 40°C was observed to fractionate without the addition of any reagent. However, the olein derived in this manner partially crystallized after three hours when held at 40°C. The addition of 0.6% SDS (w/w) to completely melted tallow, followed by equilibration to 40°C, resulted in olein formation, but at substantially lower yields than tallows fractionated according to Bussey et al. (1981).

It appears that an aqueous solution of electrolyte and a suitable surfactant are essential for the separation of olein from stearin at 40°C. A proposed mechanism by which these reagents aid the fractionation process of tallow has been given by Glassner (1983). When tallow is equilibrated from lower temperatures to 40°C, the resultant mixture is composed of liquid olein and stearin crystals. The addition of a surfactant (SDS) may function by adsorbing to the stearin crystal surfaces, or by being incorporated in some way in the crystal matrices or agglomerates, yielding crystal formations with relatively greater hydrophilicity

Table 8. The effect of reagents and their concentration on olein yield produced by fractionation of tallow (120°F rendered) at 40°C.

Tallow (g)	Water (g)	Electrolyte (Na_2SO_4) (g)	Surfactant (SDS) (g)	Olein yield (%)
<u>Aqueous</u>				
1000	600.0	30.0	6.0	70.0
1000	600.0	0.0	6.0	9.3
1000	600.0	30.0	0.0	N.S.[a]
<u>Dry</u>				
1000	0.0	30.0	6.0	29.6
1000	0.0	0.0	6.0	N.S.[a]
1000 (melted)	0.0	0.0	0.0	85.4[b]
1000 (melted)	0.0	0.0	6.0	35.3

[a] N.S., no separation

[b] Olein subsequently crystallized after 3 hours at 40°C.

because of the sulfate moiety of the SDS molecule. The subsequent addition of aqueous electrolyte solution in turn is suscepted to neutralize ionic charges across the stearin crystal surfaces or by orienting the SDS in a more uniform manner. The results of these actions cause greater attraction and agglomeration characteristics among the stearin crystals. When this system is subjected to centrifugation, packing of the stabilized, hydrophilic crystals occurs, resulting in efficient separation of the olein and stearin phases. This being the case, it becomes apparent that the reagents specified in the fractionation process are inherent to successful fractionation.

Effects of electrolyte type and concentration on olein yield.

Having established the essential nature of the reagents used in the fractionation scheme proposed by Bussey et al. (1981), studies were conducted to investigate alternative electrolytes that could be used in the fractionation process and to optimize their concentrations in the aqueous solution. This was due to concern expressed by an edible tallow supplier (Regutti, personal communication) over potential residual sodium sulfate in the tallow fractions, since sodium sulfate is only approved for specific food uses. Thus. electrolytes normally utilized in food applications were investigated as possible substitutes for sodium sulfate in the fractionation process (Table 9). Five

Table 9. Olein yields obtained by using electrolyte solutions in the aqueous fractionation (40°C of tallow rendered at 120°F.

Electrolyte	Concentration in aqueous phase (%)	Olein yield (%)
Sodium sulfate	5.0	70.0
Sodium citrate	5.0	75.5
Sodium tartrate	5.0	52.6
Sodium chloride	5.0	19.3
Sodium potassium tartrate	5.0	51.7
Sodium phosphate (dibasic)	5.0	24.7
Potassium carbonate	5.0	22.6

percent solutions of these electrolytes were prepared and used in the fractionation process. Olein yields at 40°C for tallows fractionated using these solutions are indicated in Table 9. These data suggest that substitution of sodium sulfate with sodium citrate was effective in maintaining or increasing the olein yield at 40°C. The inability of the other electrolytes investigated to maintain olein yields at approximately 70% precluded their further use in this study.

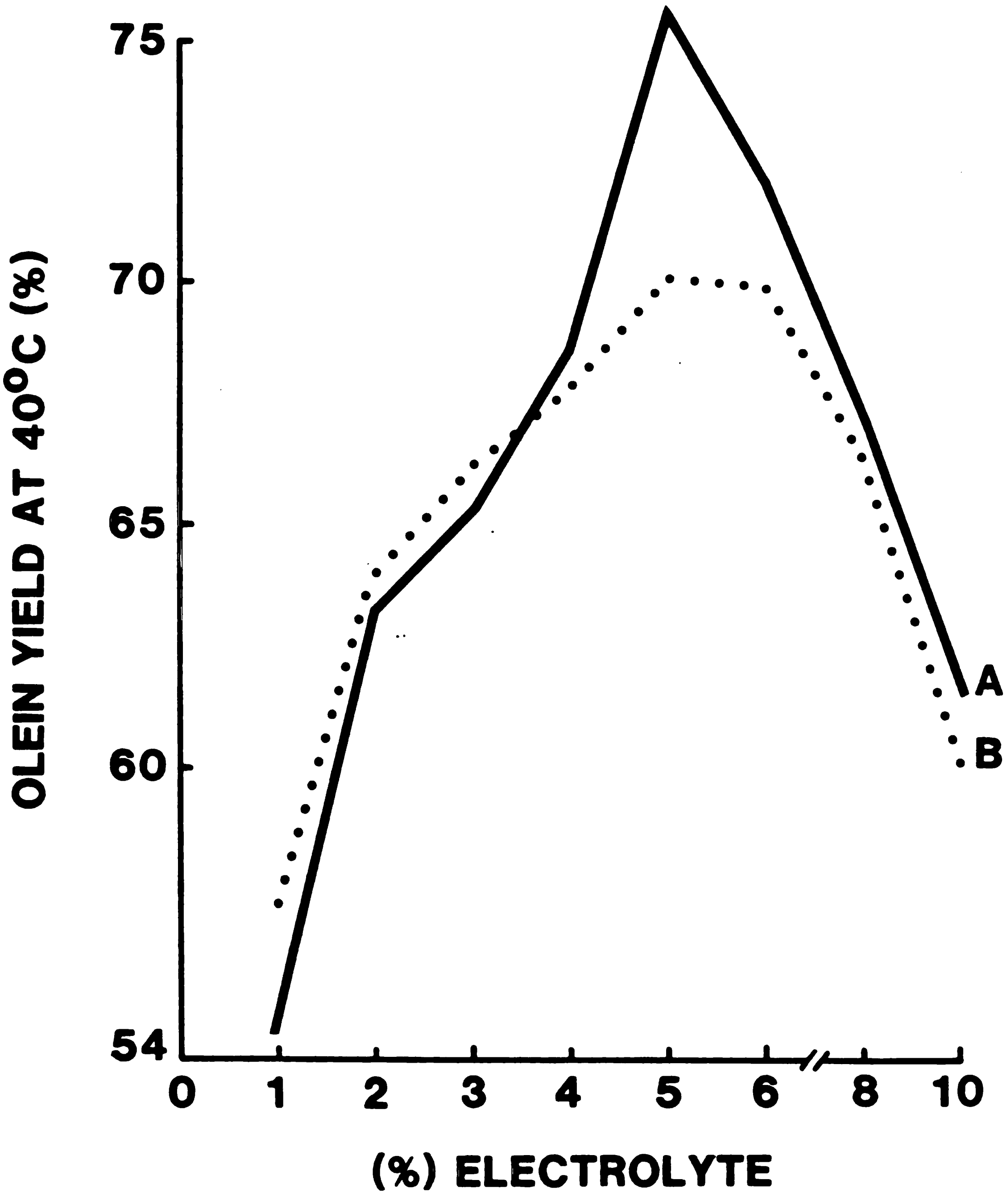
The optimum concentrations of sodium citrate and sodium sulfate were investigated with respect to olein yield at 40°C. The results of this study are found in Table 10 and Figure 9. Five percent solutions of both sodium citrate and sodium sulfate proved optimal for maximum olein yield. Also noted was the greater efficacy of sodium citrate to produce more olein per unit weight of tallow. At the 5.0% level, the use of sodium citrate resulted in olein yields of 75.5% while sodium sulfate only yielded 70.0%, representing an increase in olein yield at 40°C of approximately 8.0%. Glassner (1983) utilized sodium citrate to fractionate tallow and also found the optimal electrolyte concentration to be 5.0%. Thus, on the basis of these findings, sodium citrate was found to be a suitable replacement for sodium sulfate in the fractionation process. In addition, residual levels of sodium citrate may also enhance the oxidative stability of the derived olein fractions due to the ability

Table 10. The effect of electrolyte concentration on olein yield of tallow fractionated at 40°C.

Electrolyte concentration (%)	Olein yield (%)[a]	
	Sodium citrate	Sodium sulfate
1.0	54.6	57.1
2.0	63.2	64.0
3.0	65.3	66.2
4.0	68.5	67.8
5.0	75.5	70.0
6.0	72.1	69.8
8.0	67.2	66.3
10.0	61.6	60.0

[a] Olein yields represent average of duplicate fractionations.

Figure 9. The effect of electrolyte concentration on olein yield obtained by aqueous fractionation of tallow at 40°C where A represents sodium citrate, B sodium sulfate.


OLEIN YIELD AT 40°C (%)
75
70
65
60
54
0
1
2
3
4
5
6
8
10
(%) ELECTROLYTE
A
B

of citric acid to chelate pro-oxidant metal ions (Dugan, 1976).

Investigations of subsequent fractionation temperatures.

Results from this phase of the study indicated that for optimal fractionation of tallow at 40°C, reagent levels of 600 ml of 5% sodium citrate and 0.6% SDS per kilogram of tallow were required. Previous studies by Bussey et al. (1981) indicated that although the use of aqueous detergent fractionation at 40°C was essential in removing higher melting glyceride types from tallow, only a decrease in temperature was required for subsequent fractionations. No reagents were added in subsequent fractionation steps to obtain lower melting tallow fractions. Thus, the development of a subsequent fractionation scheme to derive fractions from 40°C olein having characteristics suitable for use as a pourable deep-fat frying medium for foods consumed at ambient temperatures was investigated.

In this study, three 1 kg samples of the 40°C olein fraction in glass beakers were placed in cubicles with temperatures of 37, 35 and 33°C. The olein samples were allowed to equilibrate at these temperatures for 24 hours. After equilibration, only the 40°C olein sample held at 33°C underwent partial crystallization, while those held at 35 and 37°C showed little to no crystal growth. Previous studies by Bussey et al. (1981) indicated that sequential fractionation is required to optimize the yield of olein at lower temperatures. This was due to the large amounts of entrapped olein which could not be removed by centrifugation when the ratio of liquid to solid phase was low.

Therefore, the fractionation scheme for tallow yielding the pourable frying medium was designed to eliminate sequentially the higher melting glycerides in each olein phase by subsequent decreases in temperature of equilibration of 4-7°C. The next phase of this study will define this process and also characterize the significant components of each fraction.

Phase III. Characterization of Tallow Fractions

Utilizing the modifications specified in the previous phase of this study, a fractionation scheme for tallow to derive a deep-fat frying oil with pourable characteristics was developed and is presented schematically in Figure 10. This phase of the study addressed the chemical characterization of the tallow fractions derived in this process. Parameters investigated included the quantitation of fraction yields, cholesterol contents, fatty acid composition, iodine values and saturated to unsaturated fatty acid ratios (Table 11).

Unfractionated tallow was found to contain 0.14% cholesterol by weight via GLC analysis, while subsequent tallow fractions contained 0.10-0.15% cholesterol. These findings are in agreement with reported cholesterol levels

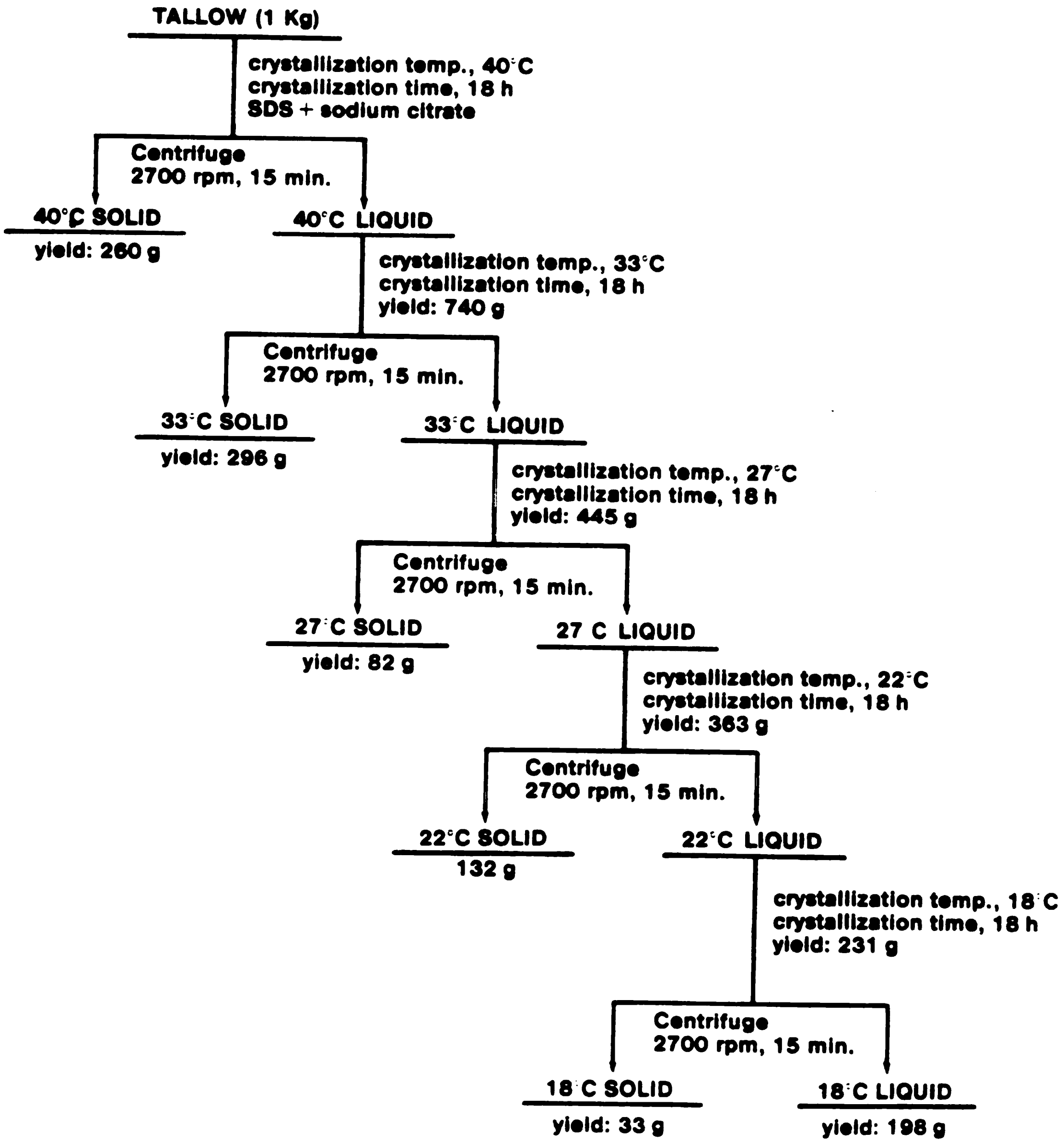


Figure 10. The fractionation scheme of edible tallow using sodium dodecyl sulfate and sodium citrate.

Table 11. Fraction weight, cholesterol content, and fatty acid composition of edible tallow and tallow fractions obtained by aqueous detergent fractionation.

	Original tallow	40°C		33°C		27°C		22°C		18°C	
		Solid	Oil	Solid	Oil	Solid	Oil	Solid	Oil	Solid	Oil
Fraction weight (g)	1,000	247[a]	740[b]	296[b]	445	82[b]	363	132[b]	231	33[b]	198
Total cholesterol (mg)	1,355	259	970	360	611	93	475	152	332	37	290
%Cholesterol	0.14	0.10	0.13	0.12	0.14	0.11	0.13	0.12	0.14	0.11	0.15
Iodine Value	46.5	36.5	47.1	41.5	48.7	41.8	49.9	44.6	52.4	46.2	55.0
Fatty Acid Composition (%)											
C14:0	3.2	3.0	2.9	3.0	2.8	3.2	2.6	2.7	2.7	2.9	2.6
C16:0	24.6	25.7	23.6	24.5	22.6	24.6	21.4	22.1	21.8	23.2	22.0
C18:0	16.4	20.5	16.3	17.6	15.5	17.0	14.0	14.3	13.4	15.1	13.3
C20:0	0.2	0.1	0.1	0.1	0.1	--	0.2	--	0.1	--	--
C14:1	1.4	1.1	1.3	1.1	1.3	1.3	1.3	1.3	1.3	1.4	1.4
C16:1	4.2	3.8	4.2	4.1	4.3	4.1	4.6	4.5	4.8	4.6	4.9
C18:1	40.5	37.1	42.2	41.2	44.3	40.6	47.6	46.5	47.2	46.0	48.7
C18:2	4.0	3.4	3.8	3.5	3.9	3.7	3.8	3.8	3.8	1.7	2.0
C18:3	2.0	1.8	2.0	1.5	2.0	1.6	1.6	1.6	1.6	1.8	2.0
% Saturated	44.4	49.3	42.9	45.2	41.0	44.8	38.2	39.1	38.0	41.2	37.0
%Unsaturated	52.1	47.2	53.7	51.4	55.8	51.5	58.9	57.7	58.8	55.5	59.0
Ratio S/U	0.85	1.04	0.80	0.88	0.73	0.87	0.65	0.68	0.65	0.74	0.64

[a] Actual amount recovered after hot water extraction to remove SDS.

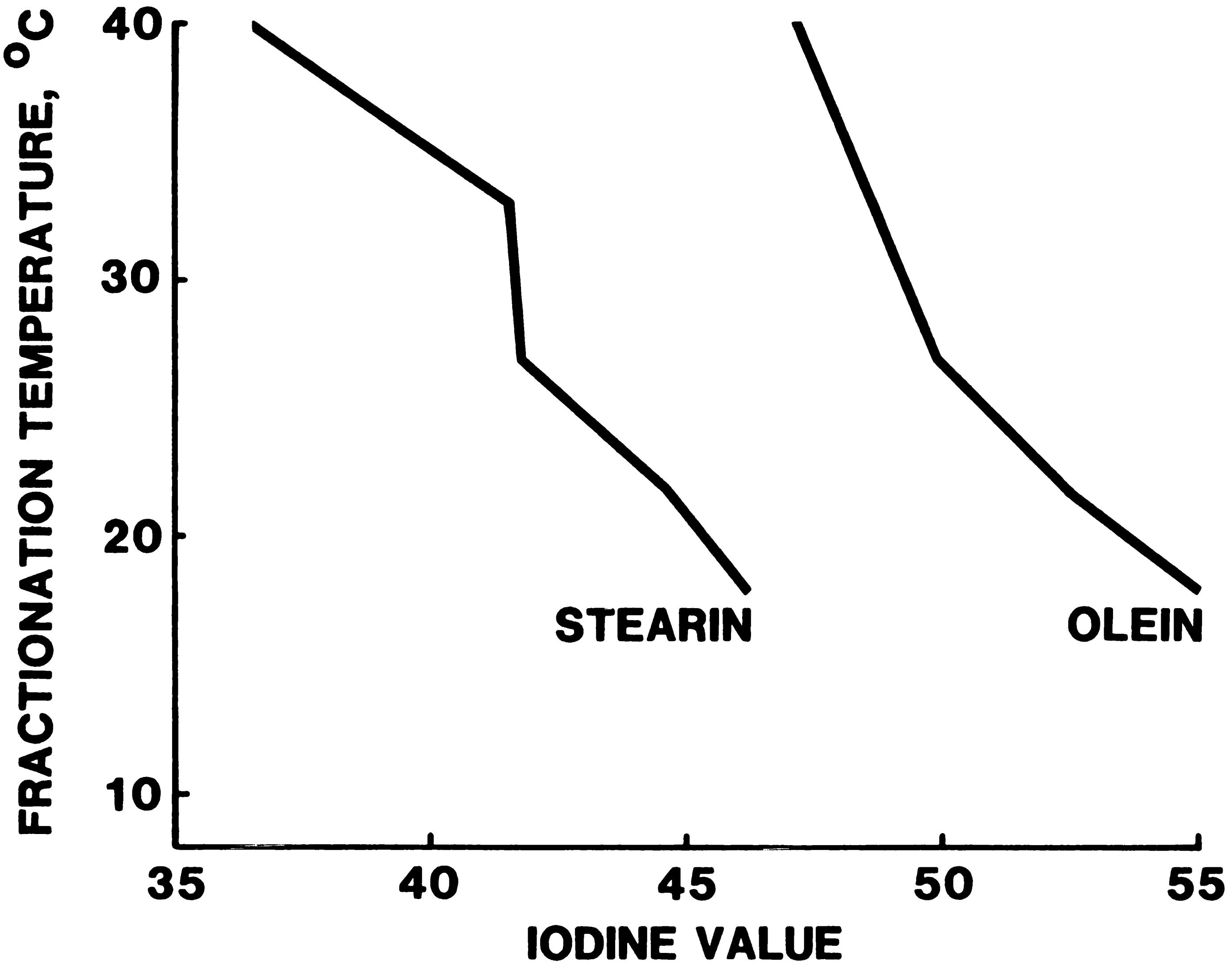
[b] Solid weight determined by difference.

in tallow of 0.15-0.20% by Punwar and Derse (1978). Observed was the fact that the liquid fraction derived at each temperature contained slightly higher levels of cholesterol than the corresponding solid fraction (Table 11).

Cholesterol recoveries ranged from 91-102% based on the total cholesterol content (mg) in the liquid phase compared to the sum of cholesterol (mg) in the subsequent solid and liquid fractions. The low cholesterol content (0.10%) observed in the 40°C solid fraction may be attributed to the use of SDS and electrolyte solution in the primary fractionation step. The use of these reagents may cause partial emulsification of cholesterol or cholesteryl esters which are subsequently lost in hot water washing techniques needed to remove residual SDS. This phenomenon is reinforced by the observation that approximately 12 g of 40°C stearin, representing 5% of this fraction, were also lost in the hot water washing procedure probably due to emulsification by SDS with the aqueous electrolyte solution.

Fatty acid analyses and iodine value determinations for all derived tallow fractions are given in Table 11. These data indicate that the levels of unsaturated fatty acids increased, as did the iodine values, for olein fractions as temperatures of fractionation decreased (Figure 11). Likewise, as the fractionation process proceeds, the level of unsaturation and iodine values for stearin fractions also increased. These phenomena were also observed in fractions

Figure 11. Changes in the iodine values of olein and stearin phases isolated in the fractionation process for tallows at different temperatures of fractionation.


FRACTIONATION TEMPERATURE, °C
40
30
20
10
35
40
45
50
55
IODINE VALUE
STEARIN
OLEIN

derived at different temperatures by Bussey et al. (1981). Therefore, it was generally observed that the overall unsaturated fatty acid concentration increased in all fractions as the temperature of fractionation decreased.

This phase of the study also indicated that at temperatures of fractionation of 27°C or less, the saturated/unsaturated fatty acid ratios remained relatively constant for olein fractions at approximately 0.65, despite obvious differences in clarity and the extent of solidification when these samples were held at room temperature. This fact may indicate that the mode of separation of glyceride types in these low temperature fractionations may be due to the arrangement of fatty acid types on the glycerol moiety besides the net level of unsaturation of the entire triglyceride system.

Results of these characterizations, coupled with the description of desirable attributes prescribed by a commercial tallow supplier (Regutti, personal communication) indicated that the most desirable fraction derived from this process for use as a pourable deep-fat frying medium was the olein phase derived at 22 C. This fraction had an iodine value of 52.4, a saturated/unsaturated fatty acid ratio of 0.65 and contained 0.14% cholesterol by weight. This olein was observed to exist as a free pouring clear yellow liquid at 22°C, having characteristic tallow odor and flavor. This fraction represented 23.1-25.0% of the parent tallow from which it was derived. The 22°C olein was noted to remain free-flowing, although cloudy, at temperatures as low as 16°C. These characteristics are consistent with those prescribed by a commercial tallow supplier desiring a pourable tallow fraction to be used to fry food traditionally consumed at ambient temperatures (Regutti, personal communication).

Likewise, these characterizations also indicated that this fractionation process yields other fractions ranging in iodine value from 36.5 to 55.0 which may also have possible applications in food systems as hardening agents, confectionary coatings, shortenings, cooking fats and frying media (Morris et al., 1956; Luddy et al., 1973; Taylor et al., 1976; Holsinger et al., 1978; De Fouw et al., 1981; Bussey et al., 1981; Bundy et al., 1981; Dillery, 1982).

The major hindrance in the development and marketing of this fraction for use as a deep-fat frying medium is the presence of cholesterol at 0.14% levels. Previous studies (Ryan et al., 1981; Ryan, 1982) indicated that cholesterol present in tallow systems exposed to conditions representative of deep-fat frying undergoes oxidation. Unfortunately, some oxidative derivatives of cholesterol have been implicated as being angiotoxic (Imai et al., 1976; Peng et al., 1978) and/or carcinogenic (Bischoff, 1969; Smith and Kulig, 1975). However, preliminary studies of heated triolein and tristearin systems have indicated that under static conditions of heating, unsaturated fatty acyl moieties have a sparing effect on the oxidation of cholesterol (Luby, unpublished data). In these systems, unsaturated fatty acids were preferentially oxidized over cholesterol when the systems were held for two weeks at 180°C. Thus, the oxidative stability of the cholesterol present in the 22°C olein fraction may be enhanced by the greater concentration of unsaturated fatty acids in this fraction. Therefore, prior to assessing the oxidative stability of the 22°C olein fraction from tallow, the oxidation of cholesterol in model systems of triglycerides with differing levels of unsaturation was first assessed.

Phase IV. Cholesterol Oxidation in Model Systems.

The use of olein fractions derived by aqueous detergent fractionation of tallow as deep-fat frying media requires an understanding of the stability of cholesterol present in such fractions. Previous studies of heated tallow systems indicate that exposure of tallow to frying temperatures for extended periods of time causes a time-dependent oxidation of the inherent cholesterol (Ryan et al., 1981; Ryan, 1982). Thus, tallow fractions when used as frying media may be sources of oxidized sterols in the diet (Ryan, 1982; Tsai and Addis, 1984). Concern over the presence of oxidized sterols in the diet is based on reports that several of these compounds are angiotoxic (Imai et al., 1976; Peng et al., 1978) and/or carcinogenic (Bischoff, 1969; Smith and

Kulig, 1975). Previous results of this study indicated that cholesterol is distributed somewhat evenly throughout the derived tallow fractions. Therefore, it was deemed important to develop an understanding of the effects of fatty acid unsaturation and exposure to air on the oxidative stability of cholesterol. To investigate these factors, triglyceride model systems of different levels of unsaturation and containing 0.15% cholesterol (w/w), were exposed to a high temperature similar to those encountered in deep-fat frying operations. Studies with each model system were performed under aerated and non-aerated (static) conditions.

The model systems consisted of solutions of 0.15% cholesterol (w/w) in triolein, tristearin and a 1:1 mixture of triolein and tristearin. These solutions were heated for 96 hours at 180°C under aerated (30 ml/min) and non-aerated conditions. The cholesterol content for each system was determined every 24 hours by quantitative GLC analysis. The changes in cholesterol concentration, expressed as percent original cholesterol remaining are presented in Table 12 and Figure 12. These results indicate that unsaturated bond concentration and aeration have a pronounced effect on the oxidative stability of cholesterol present in these systems. These data also confirm earlier studies by Ryan (1982) in which tallow heated at 180°C for 24 hours resulted in cholesterol oxidation. Thus, the primary finding of this

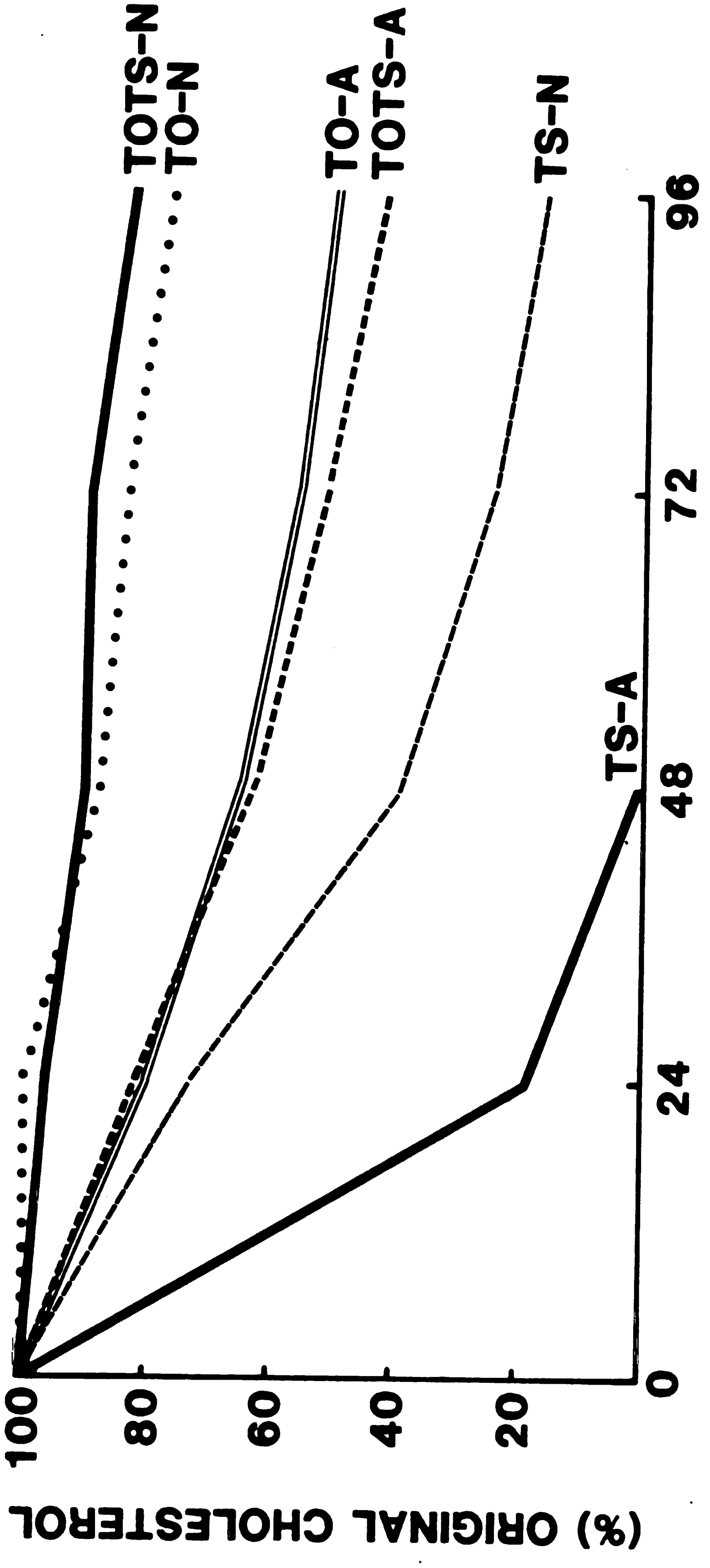
Table 12. Loss of cholesterol in lipid model systems at 180°C.

	Original Cholesterol remaining (%)				
Time of heating (hr)	0	24	48	72	96
Model System					
Triolein, aerated	100(7.46)[a]	80.2(5.98)	64.8(4.83)	55.6(4.15)	49.7(3.71)
Triolein, non-aerated	100(7.51)	99.5(7.47)	87.8(6.59)	83.4(6.26)	76.6(5.75)
Tristearin, aerated	100(7.43)	18.5(1.37)	N.D.[b](0.0)	N.D.[b](0.0)	N.D.[b](0.0)
Tristearin, non-aerated	100(7.48)	72.7(5.44)	38.6(2.89)	24.1(1.80)	16.3(1.22)
Triolein:Tristearin (1:1, w/w), aerated	100(7.40)	81.9(6.06)	63.1(4.67)	51.9(3.84)	41.5(3.07)
Triolein:Tristearin (1:1, w/w), non-aerated	100(7.45)	97.1(7.23)	90.3(6.73)	89.8(6.69)	82.8(6.17)

[a] Numbers in parentheses refer to mg cholesterol quantitated in a 5.0 g sample of the model system triglyceride solution.

[b] N.D., not detected.

Figure 12. Loss of cholesterol in model systems at 180°C. TO-A = aerated triolein, TO-N = non-aerated triolein, TS-A = aerated tristearin, TS-N = non-aerated tristearin, TOTS-A = aerated triolein and tristearin (1:1, w/w) and TOTS-N = non-aerated triolein and tristearin (1:1, w/w).


(%) ORIGINAL CHOLESTEROL
100
80
60
40
20
0
24
48
72
96
TIME, HOURS AT 180°C
TOTS-N
TO-N
TO-A
TOTS-A
TS-N
TS-A

study was that cholesterol present in triglyceride systems undergoes oxidative deterioration at temperatures representative of deep-fat frying (Figure 12 and Table 12).

The degree of unsaturation in the triglycerides was observed to have an effect on the rate of cholesterol oxidation in the model systems. Data presented in Table 12 and Figure 12 clearly show that cholesterol present in the systems containing saturated fatty acyl groups, i.e., the tristearin systems, is more prone to oxidation than cholesterol present in more highly unsaturated systems, i.e. the triolein systems. Statistical evaluation of the data presented in Table 12 indicates that when linear regression models were used to evaluate the loss of cholesterol with time at 180°C, the slopes of the linear equations approximate the rate of cholesterol degradation. Thus, in the non-aerated systems, cholesterol degraded at a rate of 0.48 mg/day in the triolein solution, while a degradative rate of 1.68 mg/day was observed for cholesterol in the tristearin solution. Cholesterol present in the triolein:tristearin (1:1, w/w) system degraded at a rate of 0.31 mg/day. In the aerated systems, the rates of cholesterol degradation were 0.94 mg/day and 1.08 mg/day for the cholesterol present in the triolein and triolein:tristearin (1:1, w/w) systems, respectively. In the aerated tristearin solution, cholesterol was observed to undergo complete degradation after only 48 hours at 180°C.

Results of this study also indicate that aeration at 180°C has a damaging effect on cholesterol in these model systems. Cholesterol was observed to deteriorate much more rapidly in the model systems aerated at a rate of 30 ml/min when compared to the non-aerated systems. In the aerated triolein systems, cholesterol decomposed at a rate of 0.94 mg/day compared to 0.48 mg/day in the non-aerated solution. Rates of 1.08 mg/day and 0.31 mg/day were observed for cholesterol degradation in the aerated and non-aerated triolein:tristearin (1:1, w/w) systems, respectively. The tristearin systems (Figure 12), which were noted to have the greatest extent of cholesterol degradation, were greatly affected by aeration at 180°C. Cholesterol present in the aerated tristearin system had totally degraded after 48 hours of heating at 180°C, while in the non-aerated system, cholesterol degraded at a rate of 1.68 mg/day. Thus, in all three triglyceride systems, aeration was observed to increase the rate of cholesterol degradation at 180°C.

Thin-layer chromatography (TLC) analysis was performed on the sterol extracts isolated from these model systems in order to identify the types of oxidative compounds formed as cholesterol deteriorated. These analyses indicated that cholesterol oxides were present in all the model systems; however, the concentration of these oxidation products paralleled the extent of cholesterol deterioration. The identity and Rf values of several cholesterol oxides isolated from the model systems as well as standard cholesterol oxidation products are listed in Table 13. TLC chromatograms indicated that 3,5-cholestadiene-7-one, 5-cholesten-3β-ol-7-one, 5-cholesten-3β,7α-diol, 5-cholesten-3β,7β-diol and cholestan-3β,5α,6β-triol were formed at the expense of cholesterol in the model systems. These compounds have been isolated from intermittently and continuously heated tallow (Ryan et al., 1981), as well as potato products fried in tallow (Ryan, 1982; Tsai and Addis, 1984). Also noted was the isolation and tentative identification of 5-cholesten-3β-ol-7-one in the heated model systems. This compound has been reported to be destroyed by saponification steps in the analysis of cholesterol oxidation products (Chicoye et al., 1968; Finocchiaro et al., 1984), yet was isolated from these systems using the saponification and extraction methods specified by Itoh et al. (1973). The unidentified compounds isolated are assumed to be other oxidation products or those derived from thermal decomposition of cholesterol.

These results suggest that the oxidation of cholesterol is influenced by the chemical composition of the model systems and by the thermal and oxidative stress to which these systems are subjected. Farmer et al. (1942) identified autoxidative processes as free radical chain mechanisms and it is in this context that the oxidation of cholesterol in these model systems can be best explained.

Table 13. TLC analysis of cholesterol oxides isolated from heated model systems and standard cholesterol oxidation products.

Compound	Rf value[a]	Color[b]
Heated Model Systems		
Solvent front	1.00	---
3,5-cholestadiene-7-one	0.90	brown
unindentified	0.84	yellow
unindentified	0.81	brown
unindentified	0.78	brown
unindentified	0.74	brown
5-cholesten-3β-ol	0.72	magenta
unindentified	0.68	brown
unindentified	0.58	brown
unindentified	0.53	peach
5-cholesten-3β-ol-7-one	0.47	yellow-brown
unindentified	0.34	yellow
5-cholesten-3β,7β-diol	0.30	blue
5-cholesten-3β,7α-diol	0.25	blue
unindentified	0.18	yellow
cholestan-3β,5α,6β-triol	0.12	brown
Standards		
Solvent front	1.00	---
3,5-cholestadiene-7-one	0.90	brown
5-cholesten-3β-ol	0.73	magenta
cholesterol-α-oxide	0.55	yellow
5-cholesten-3β-ol-7-one	0.47	yellow-brown
5-cholesten-3β,7β-diol	0.31	blue
5-cholesten-3β,7α-diol	0.25	blue
cholestan-3β,5α,6β-triol	0.12	brown

[a] Solvent system, ethyl acetate-heptane (1:1, v/v).
[b] After 50% H_2SO_4 spray, 10 min heat treatment at 110°C.

Cholesterol, being an unsaturated lipid, is prone to oxidative deterioration and the mechanism by which cholesterol in bulk undergoes oxidation has been reviewed extensively by Smith (1981). Fatty acids with unsaturated bonds also undergo oxidative deterioration by free radical chain mechanisms as described in the review of literature (Equations 3-9). The disparity in the rate of cholesterol oxidation noted between systems having different levels of unsaturation can be attributed to the point at which cholesterol becomes involved in the autoxidative process.

In the tristearin systems, cholesterol represents the only source of unsaturation and thus will undergo oxidation very early on in the heating process. This autoxidation begins with the abstration of the C_7 hydrogen of cholesterol, followed by the addition of atmospheric oxygen to form a cholesterol peroxy radical. This radical in turn will abstract another labile hydrogen, which in this case would likely be another C_7 hydrogen from an unoxidized cholesterol molecule. The result of this reaction is the formation of a new cholesterol peroxy radical and a 7α or β-hydroperoxycholesterol which decompose rapidly at elevated temperatures to liberate more radical species into the oxidizing system (Smith, 1981). Thus, cholesterol is involved in both the initiation and propagation stages of the autoxidative process. It should be noted that in this system, the radical species liberated during propagation reactions most likely attack unoxidized cholesterol molecules, since they represent the most labile species in these lipid systems.

In those systems containing unsaturated fatty acyl species, cholesterol may become involved in the autoxidative process through different mechanisms. In the triolein and triolein:tristearin (1:1, w/w) systems, the initiation reactions occurring at the onset of autoxidation most likely take place at sites of unsaturation on the fatty acyl moieties. This is logical since there are a greater number of fatty acyl sites of unsaturation in these systems, and these sites are more susceptible to the loss of labile hydrogens. Thus, the result of initiation reactions in these systems is the formation of fatty acyl hydroperoxides which decompose spontaneously at elevated temperatures to liberate more free radicals into the lipid system (Artman, 1969). These free radicals not only will be involved in propagative reactions involving fatty acyl groups, but also will be involved in the abstraction of the C_7 hydrogens of cholesterol, i.e. initiation of cholesterol oxidation.

The role of heat (180°C) and oxygen concentration in the autoxidative process has been extensively reviewed by Artman (1969). Heat serves to decompose hydroperoxides, thus increasing the rate of formation and concentration of free radicals in the lipid system. Oxygen concentration is also related to autoxidation, with the rate of oxidation increasing as the partial pressure of oxygen increases in lipid systems (Artman, 1969). The effects of both heat and oxygen (aeration) were noted to enhance the oxidation rate of cholesterol in all the model systems studied.

From this perspective, the data obtained in this phase of the study indicate that in the model systems studied, the presence of unsaturated fatty acyl groups exerts a protective or sparing effect on cholesterol. Clearly, the cholesterol present in the tristearin system oxidized at a greater rate than did cholesterol in the triolein system, when these systems were aerated and non-aerated. The implications of these results are important in the development of a pourable deep-fat frying medium derived from tallow. On the premise that increasing the level of unsaturation in triglyceride systems containing cholesterol enhances cholesterol stability, the removal of higher melting glyceride types from tallow by fractionation may influence the stability of cholesterol in the more unsaturated tallow fractions. The results of this study indicate that although the 22°C olein fraction singled out for use as a deep-fat frying medium contains cholesterol, the oxidative stability of this cholesterol may be greater than that of cholesterol present in original tallow by merit of the higher level of unsaturation in the 22°C olein fraction triglycerides.

The next phase of this study will investigate the oxidative stability of this fraction and also serve to identify factors which influence the extent of oxidation of both the triglycerides and cholesterol present in the 22°C olein fraction.

Phase V. The Oxidative Stability of Tallow-Derived Frying Oils.

Previous phases of this study indicated that the 22° olein fraction derived by the aqueous detergent fractionation of tallow possessed characteristics desirable for use as a deep-fat frying medium for foods. This fraction maintained clarity at room temperature and had a characteristic tallow flavor. This phase of the study was conducted to assess (i) the oxidative stability of this fraction; (ii) to study the effects of blending small amounts (5-15%) of vegetable oils with this fraction on the overall oxidative stability (iii) to evaluation the use of selected antioxidant systems in preventing oxidation in these lipid systems, and (iv) to assess these factors from the perspective of cholesterol stability in the oil systems.

To obtain pertinent oxidative stability data, the oil systems were evaluated by monitoring peroxide development under Schaal Oven Test conditions. This methodology was chosen since it yields results relevant to oxidative stability and because this method can be used to assess the effectiveness of various antioxidants (Dugan and Kraybill, 1956). Testing of synthetic antioxidants in oil systems at elevated temperatures, such as those used for deep-fat frying, results in a rapid loss of these antioxidants from the oils (Artman, 1969). For these reasons, stability assessment was not conducted at temperatures representative of frying.

The stability of the 22° olein tallow fraction.

Imperative to the application of novel tallow fractions to food systems is their ability to maintain flavor quality for reasonable periods of time. Results from this phase of the study indicated that the olein derived from tallow at 22°C oxidized more rapidly than did original tallow. Peroxide development occurred more rapidly in the 22°C olein when compared to unfractionated tallow (Table 14). These data indicated that at 65°C, peroxide values in tallow reached 36.3 and 75.2 meq/kg after 14 and 34 days, respectively. The 22° olein fraction developed peroxide values of 43.2 and 92.0 meq/kg after 14 and 22 days, respectively, when held at 65°C. Fatty acid analysis of the respective oils indicate that unfractionated tallow had a saturated/unsaturated fatty acid ratio of 0.65 (Table 15). Therefore, the difference in stability between these two oil systems is explainable on the basis of differences in their fatty acid composition. Fractionation sequentially removes saturated glyceride types from the total triglyceride pool,

Table 14. Peroxide value data for oil systems held at 65°C in a 60 day stability study.

	Peroxide values, meq/kg[f]										
Time, days at 65°C	0	3	7	11	14	22	27	34	40	51	60
Oil System											
Tallow	0.1	7.5	18.1	28.2	36.3	61.0	63.7	75.2			
Tallow + TBHQ[a]	0.1	2.7	3.3	4.0	4.0	3.0	3.8	2.7	5.0	4.5	7.8
Tallow + AP-MT[b]	0.1	2.7	3.2	3.9	4.3	7.3	8.9	8.2	14.7	22.4	30.6
22°C Olein	0.2	9.3	21.7	28.9	43.2	92.1					
22°C Olein + TBHQ[a]	0.2	7.2	16.6	24.9	41.2	80.7					
22°C Olein + AP-MT[b]	0.2	3.5	4.6	6.7	7.6	13.2	19.1	26.0	68.0	90.6	
5% CSO[c]	0.1	5.5	15.2	24.8	38.5	88.3					
5% CSO + TBHQ[a]	0.1	3.3	5.7	8.1	7.3	11.7	32.4	42.6	93.9		
5% CSO + AP-MT[b]	0.1	4.5	7.4	9.9	14.8	35.6	58.9	48.1	60.0	47.9	65.5
10% CSO[c]	0.1	7.9	17.7	27.9	33.1	70.5					
10% CSO + TBHQ[a]	0.1	5.1	7.8	12.2	15.8	46.9	84.0				
10% CSO + AP-MT[b]	0.1	5.6	9.5	13.7	23.8	50.7	63.0	77.3			
5% HCSO[d]	0.1	5.2	14.4	27.2	35.0	74.2					
5% HCSO + TBHQ[a]	0.1	5.2	11.5	16.1	23.1	38.2	56.2	74.0			
5% HCSO + AP-MT[b]	0.1	3.6	4.8	6.8	8.0	13.3	21.0	39.2	45.8	116.7	
10% HCSO[d]	0.0	5.5	9.9	15.6	22.6	43.4	74.9				
10% HCSO + TBHQ[a]	0.0	4.5	6.0	7.6	7.3	9.0	12.1	14.6	26.9	32.3	40.3
10% HCSO + AP-MT[b]	0.0	3.6	8.5	9.4	18.3	24.9	36.4	55.6	98.3		
15% HCSO[d]	0.0	6.3	10.3	12.6	19.3	63.6	100.2				
15% HCSO + TBHQ[a]	0.0	6.0	9.2	13.5	13.7	42.1	71.9	88.0			
15% HCSO + AP-MT[b]	0.0	3.6	5.1	9.7	11.7	32.4	36.6	51.9	67.1	90.2	
5% PO[e]	0.0	4.4	13.7	25.3	40.6	90.9					
5% PO + TBHQ[a]	0.0	2.6	3.3	4.6	4.2	4.7	4.2	5.3	5.8	10.0	20.4
55 PO + AP-MT[b]	0.0	3.0	5.1	6.4	9.1	17.9	30.7	63.3	64.1	138.0	
10% PO[e]	0.0	3.7	6.7	9.7	17.5	53.8	67.0	62.3	72.9		
10% PO + TBHQ[a]	0.0	2.6	3.3	4.2	4.2	4.3	4.4	4.2	5.8	7.2	6.4
10% PO + AP-MT[b]	0.0	3.4	4.9	6.8	8.2	18.9	20.1	57.6	58.0	90.0	
15% PO[e]	0.0	3.8	6.4	7.8	9.1	24.2	41.1	44.4	73.5		
15% PO + TBHQ[a]	0.0	2.2	3.9	4.6	4.6	5.0	5.5	5.6	5.8	8.8	13.5
15% PO + AP-MT[b]	0.0	2.1	4.7	6.6	7.0	12.3	13.6	21.1	32.7	45.0	57.1
HCSO[d]	0.0	13.5	28.7	44.2	49.1	95.9					
HCSO + TBHQ[a]	0.0	6.0	23.2	45.6	49.7	96.5					
HCSO + AP-MT[b]	0.0	2.6	14.5	33.2	53.2	85.6					
PO[e]	0.0	1.3	7.5	15.0	19.5	30.8	50.3	77.2			
PO + TBHQ[a]	0.0	0.8	2.5	3.7	4.3	6.7	16.1	24.4	38.8	45.8	61.3
PO + AP-MT[b]	0.0	1.3	2.9	9.6	18.6	34.2	47.8	68.0	90.0		

---

[a] TBHQ, tertiary butyl hydroxyquinone, added at 0.01% (w/w).

[b] AP-MT, ascorbyl palmitate-mixed tocopherols, added at 500 ppm:100 ppm levels.

[c] CSO, refined cottonseed oil. 5% and 10% CSO refer to 22°C olein fraction blended with CSO at 5 and 10% levels.

[d] HCSO, partially hydrogenated cottonseed oil. 5% HCSO, 10% HCSO and 15% HCSO refer to 22°C olein fraction blended with HCSO at 5, 10 and 15% levels.

[e] PO, palm oil. 5% PO, 10% PO and 15% PO refer to 22°C olein fraction blended with PO at 5, 10 and 15% levels.

[f] PV given represent average values of duplicate analyses.

Table 15. Fatty acid composition of oil systems used in a 60 day stability study[d].

Sample	Tallow	22°C olein	5%CSO[a]	10%CSO[a]	5%HCSO[b]	10%HCSO[b]	15%HCSO[b]	5%PO[c]	10%PO[c]	15%PO[c]	CSO[a]	HCSO[b]	PO[c]
Fatty Acid													
C14:0	3.0	2.8	2.9	2.7	2.6	2.7	2.7	3.2	2.6	2.9	0.7	0.9	1.3
C16:0	23.6	22.1	22.6	22.3	21.6	22.5	22.5	25.4	24.8	26.6	21.9	21.1	46.6
C18:0	17.1	14.2	13.0	12.5	13.7	13.2	12.7	12.8	13.1	12.5	2.7	4.7	4.7
C20:0	1.1	1.1	1.0	1.1	1.2	1.1	1.1	1.1	--	--	0.5	0.6	--
C14:1	0.9	1.0	1.1	1.0	0.9	1.0	0.9	1.1	0.9	1.7	--	--	--
C16:1	4.3	4.7	4.8	4.7	4.6	4.5	4.4	5.0	4.3	3.4	0.6	0.4	--
C18:1	43.6	48.4	46.3	44.7	48.5	48.4	48.8	45.6	47.5	45.8	17.8	52.9	38.1
C18:2	2.7	2.1	4.9	7.5	3.5	3.3	3.6	2.4	3.6	3.8	55.2	19.5	9.6
C18:3	0.2	0.2	0.2	0.4	0.2	0.3	0.3	0.2	0.2	0.4	0.3	--	--
S/U Ratio	0.87	0.69	0.69	0.66	0.68	0.69	0.67	0.78	0.72	0.76	0.35	0.38	1.10

[a] CSO, refined cottonseed oil. 5% CSO and 10% CSO refer to 22° olein fraction blended with CSO at 5 and 10% levels.

[b] HCSO, partially hydrogenated cottonseed oil. 5% HSCO, 10% HCSO and 15% HCSO refer to 22° olein fraction blended with HCSO at 5, 10 and 15% levels.

[c] PO, palm oil. 5% PO, 10% PO and 15% PO refer to 22°C olein fraction blended with PO at 5, 10 and 15% levels.

[d] Fatty acid levels represent average values of duplicate analyses.

thus increasing the concentration of the unsaturated triglycerides. These observations confirm results of previous studies reviewed by Artman (1969) noting the increased susceptibility of unsaturated lipid systems to autoxidation.

The effect of blended vegetable oils on the stability of the 22°C olein fraction.

Model system studies indicated that increasing the total unsaturation of oil systems can have a sparing effect on the oxidation of cholesterol contained therein. Therefore, small amounts (5-15%) of cottonseed oil (CSO), hydrogenated cottonseed oil (HCSO) and palm oil (PO) were blended with the 22°C olein fraction to assess the effect of these oils on the oxidative stability of the resultant blended oils as well as their effect on the cholesterol contained therein, which will be discussed later in this section.

The addition of CSO to the 22°C olein fraction at 5 and 10% levels had little effect on the stability of these systems (Table 14). Those systems containing 5 and 10% CSO developed peroxide values of 88.3 and 70.5 meq/kg, respectively, after 22 days at 65°C, while 22° olein alone was found to have a peroxide value of 92.1 meq/kg after the same period of time at 65°C. Hydrogenated cottonseed oil, on the other hand, was observed to have a stabilizing effect on the 22° olein fractions with which it was blended. Although the addition of 5% HCSO had no demonstrable effect on oxidative stability, 10-15% added HCSO extended the time required for these oil systems to exceed 70 meq/kg by 5 days when compared to unblended 22°C olein (Table 14).

These effects may be due to a combination of various factors. The vegetable oils used, CSO and HCSO, were found to have saturated/unsaturated ratios of 0.35 and 0.38 respectively (Table 15). However, CSO contained 55.2% linoleic acid while HCSO had a linoleic acid content of 19.5%. Thus, the oxidative stability of the 22°C olein blended with CSO is predictably lower than the oxidative stability of the fraction blended with HCSO due to its higher linoleic acid content (Artman, 1969). Although this effect was noted in this study, both CSO and HCSO have saturated/unsaturated fatty acid ratios lower than that of the 22° olein fraction. This being the case, the addition of either of these oils to the 22°C olein fraction should destabilize the resultant oil systems. The enhancement of stability noted by the addition of 10 and 15% HCSO and the lack of effect of the addition of 5 and 10% CSO and 5% HCSO to the 22° olein fractions may be due to the presence of tocopherols in these oil systems. The antioxidant effects of tocopherols is well documented in literature (Dugan and Kraybill, 1956; Cort, 1974; Klaui, 1976; Reinton and Rogstad, 1981). Thus, the presence of tocopherols in these vegetable oils may offset the loss of stability due to the increased level of unsaturation caused by their addition, and at higher levels of vegetable oil addition, may even be responsible for the enhanced oxidative stability of the blended 22°C olein fractions. Dugan and Kraybill (1956) and Cort (1974) have stated that tocopherols having antioxidant activity are especially effective in retarding autoxidative mechanisms in fats and oils from animal sources.

Palm oil was also used in this study and was found to increase the stability of the 22°C olein fraction at all levels of addition (Table 14). Palm oil subjected to fatty acid analysis was found to have a saturated/unsaturated fatty acid ratio of 1.10 (Table 15). Thus, the addition of palm oil at any level would be expected to stabilize oil systems by merit of its low unsaturated fatty acid content. This effect was noted in the 22 C olein fractions blended with palm oil at levels of 10 and 15%. In these systems, peroxide development reached 72.9 and 73.5 meq/kg after 40 days at 65°C respectively for 22° olein fractions blended with 10 and 15% palm oil. This represents an enhancement of the oxidative stability of 22°C olein of 18 days with respect to the time required for these oil systems to reach peroxide values of 70 meq/kg. This enhancement can be attributed to the ability of added palm oil to lower the concentration of unsaturated fatty acids in the resultant oil systems and may also be in part due to the presence of tocopherols in palm oil.

Overall, the results from this study indicate that at certain levels of addition, small amounts of CSO, HCSO and PO to the 22°C olein served to enhance the stability of the resultant oil systems. In other cases, the addition of these oils did not enhance or decrease oxidative stability. No 22°C olein blend was found to have decreased stability characteristics due to the addition of these oils when compared to unblended 22°C olein. Peroxide value data for HCSO and PO indicate that these oils alone had poorer oxidative stability characteristics than the resultant oil systems in which they were blended (Table 14). Therefore, there appears to be in some cases, a synergistic effect of blending with respect to oxidative stability in the blended oil systems. This effect may be due to the presence of tocopherols in the resultant oil systems, since the efficacy of certain tocopherols in oils of animal origin has been reported (Dugan and Kraybill, 1956; Cort, 1974).

The effects of select antioxidants on tallow derived oil systems.

A study was conducted to evaluate the effect of selected antioxidants on the oxidative stability of the tallow derived oil systems. TBHQ (tertiary butyl hydroquinone) is a synthetic antioxidant and is commonly used to stabilize snack foods with high fat contents (Potato Chip/Snack Food Association, 1976a; Min and Schweizer, 1983). This antioxidant was added to the 22°C olein and olein blends at the legal limit of 0.01% (w/w). Also investigated was a synergistic mixture of ascorbyl palmitate and mixed tocopherols (AP-MT) at concentrations of 500 ppm and 100 ppm, respectively, in the tallow-derived oil systems. This antioxidant system was chosen due to reports that mixtures of 500 ppm ascorbyl palmitate, 500 ppm lecithin and 100 ppm α-tocopherol had antioxidant properties equal to or better than synthetic phenolic antioxidants (Pongrancz, 1973; Klaui, 1976). However, since these oil systems were developed for deep-fat frying, lecithin was not used in the mixture, since phospholipids tend to decrease the smoke point of frying fats (Dugan, 1976).

The effects of these antioxidants were monitored by the development of peroxide values in the oil systems in which they were used (Table 14). The presence of either of these antioxidants in the oil systems increased the oxidative stability with respect to the control samples. TBHQ exhibited a greater potential than AP-MT to retard oxidation in the following systems; tallow, 22°C olein + 10% HCSO, and all 22° olein fractions blended with palm oil. The AP-MT synergistic mixture was more effective in stabilizing unblended 22°C olein, as well as those systems consisting of 22° olein blended with CSO and HCSO, with the above noted exception.

As evidenced by the peroxide value data in Table 14, TBHQ was very effective in stabilizing unfractionated beef tallow. In this system, 0.01% added TBHQ was observed to inhibit the development of rancidity over the 60 day period at 65°C. A final peroxide value in this system at day 60 was found to be only 7.8 meq/kg. Tallow treated with AP-MT reached peroxide values of 30.6 meq/kg over the same time period.

The 22°C olein fraction treated with these antioxidants had the opposite response than its parent tallow. TBHQ-treated 22°C olein reached a peroxide value of 80.7 meq/kg after 22 days at 65 C, while 22°C olein treated with AP-MT attained a peroxide value of 90.6 meq/kg after 51 days at 65°C. Thus, from these data, it appears the AP-MT (500 ppm:100 ppm) is better suited to stabilize tallow fractions than synthetic antioxidants.

The effects of both TBHQ and AP-MT (500 ppm:100ppm) in the blended oil systems were less clear. Antioxidants were found to enhance the oxidative stability of the blended oil systems relative to controls. TBHQ was especially effective in stabilizing palm oil blends. Mixtures of 22°C olein and 5, 10 and 15% palm oil generated peroxides at levels of 20.4, 6.4 and 13.5 meq/kg, respectively, after 60 days at 65°C when 0.01% TBHQ was applied.

The relative effectiveness of both TBHQ and AP-MT in these systems may be due to compositional factors in the specific oil systems. TBHQ may act synergistically in those 22° olein fractions blended with palm oil due to the presence of tocopherols. This synergistic effect has been reported by Pongrancz (1973) who found TBHQ to be a very effective antioxidant in palm oil containing 460 ppm mixed tocopherols. Likewise, the ability of the AP-MT system to stabilize the 22°C olein can be attributed to the effect of tocopherols in animal fats (Dugan and Kraybill, 1956; Cort, 1974) and the synergistic effect of ascorbyl palmetate (Pongrancz, 1973; Klaui, 1976). Thus, the data from this study confirmed the results found in previous studies.

As mentioned previously, the use of these two antioxidants is being investigated and/or used by producers of high fat snack foods. TBHQ is widely used in oils used for frying potato chips as well as being added to the fried chips themselves (Potato Chip/Snack Food Association, 1976a). The use of ascorbyl palmitate-mixed tocopherol-lecithin systems has only recently been investigated. Pongrancz (1973) and Klaui (1976) studied antioxidant mixtures of 500 ppm ascorbyl palmitate, 500 ppm lecithin and 100 ppm α-tocopherol in sunflower, soybean, peanut and palm oil as well as lard and butterfat. This mixture was compared to 100 ppm each of BHA and BHT. These researchers found that in oils such as sunflower oil, the antioxidant effect of the AP-lecithin-α-tocopherol system was 200 times greater than that of the combined phenolic antioxidants after 3 days at 100 °F. Similar effects were also noted for lard and butterfat.

Cort (1974) utilized 0.02% ascorbyl palmitate and 0.02% dl-γ-tocopherol to stabilize chicken, beef and pork fat applied to thin layer plates held at 45°C. This antioxidant system proved effective in all fats and manifested twice the protective effective of both BHA and BHT used at the 0.02% level. When used at the 0.02% level, ascorbyl palmitate was found to be more effective in stabilizing potato chips processed in cottonseed oil than were BHA and BHT used at the same level. Thus, this antioxidant system merits further investigation in regard to food applications.

The oxidation of cholesterol in tallow-derived frying oils.

The results obtained earlier in this phase of the study indicated that the addition of 5-15% of CSO, HCSO and PO and/or antioxidants to the 22°C olein fraction derived from tallow influenced the oxidative stability of the blended oils. Based on these results, studies were undertaken to assess the stability of cholesterol present in these systems, and to determine the influence of vegetable oils and antioxidants on the oxidation rate of cholesterol.

GLC and TLC analyses were employed to monitor the oxidative deterioration of cholesterol in the oil systems. These analyses were performed on all oil systems at the onset of the study (PV= 0.0 meq/kg), as well as at time intervals when peroxide values of the oil systems reached 35 and 70 meq/kg. Results indicated that as peroxide values increased over time in the oil systems, a concommitant deterioration of cholesterol was observed. Further, the extent of cholesterol oxidation in these systems appeared to be dependent on the oxidative stability of the triglycerides of the oil systems. This study also showed that factors influencing the stability of these tallow-derived oils, namely the addition of vegetable oils and/or antioxidants, influenced the oxidative stability of the inherent cholesterol in the same manner.

Peroxide values and cholesterol deterioration data are presented in Figures 13-22. These figures also depict the effects of both 0.01% TBHQ (w/w) and ascorbyl palmitate-mixed tocopherol (500 ppm-100 ppm) on the various oil systems with respect to cholesterol oxidation. The observed differences in oxidative stability among the various oil systems, as well as between similar systems containing antioxidants will be discussed in this section.

Figure 13 graphically illustrates peroxide value increases and cholesterol oxidation in tallow and tallow-derived 22°C olein. The extent of cholesterol deterioration follows peroxide value increases in both systems. Unfractionated tallow was more stable than the 22°C olein system, as indicated by the more rapid rate of peroxide development in the latter system. This difference was attributed to the higher level of saturated fatty acyl species in the unfractionated tallow. Also, the 22°C olein

Figure 13. Peroxide value development and loss of cholesterol in tallow held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

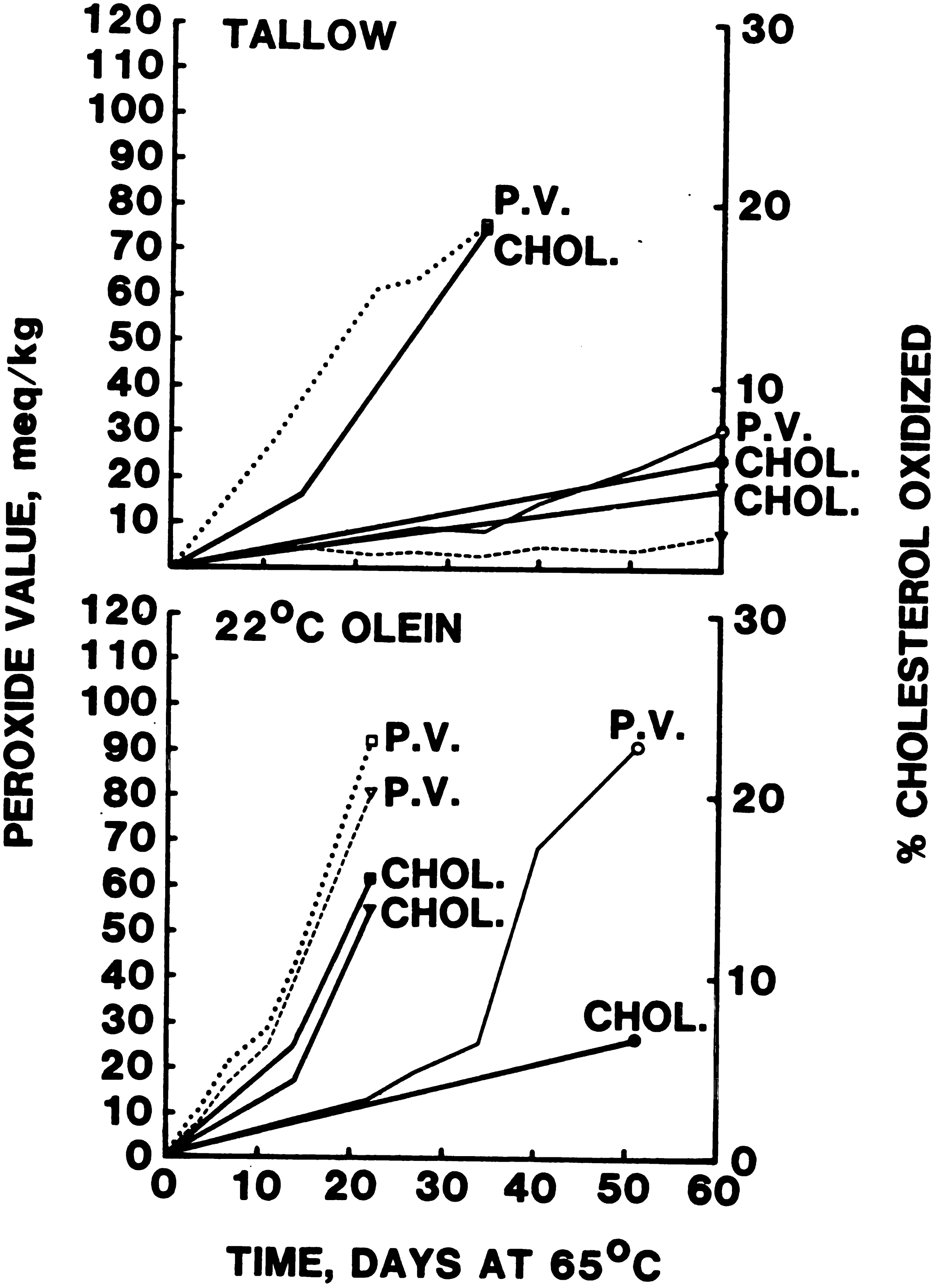
○AP-MT, PV ; ●AP-MT, cholesterol

Figure 14. Peroxide value development and loss of cholesterol in 22°C olein held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol


TALLOW
22°C OLEIN
PEROXIDE VALUE, meq/kg
% CHOLESTEROL OXIDIZED
TIME, DAYS AT 65°C
P.V.
CHOL.
P.V.
CHOL.
CHOL.
P.V.
P.V.
CHOL.
CHOL.
P.V.
CHOL.
120
110
100
90
80
70
60
50
40
30
20
10
0
30
20
10
0
0
10
20
30
40
50
60

fraction may have developed some hydroperoxides during the fractionation process. Consequently, the cholesterol present in the 22°C olein system oxidized at a more rapid rate than cholesterol in the parent tallow. The addition of both TBHQ and AP-MT to these systems enhanced triglyceride stability, and thus cholesterol stability; however the effect of these antioxidants was much more pronounced in the unfractionated tallow as was expected.

The oxidative stability of 22°C olein blended with 5 and 10% CSO at 65°C is shown in Figures 15 and 16. In these systems, the addition of CSO at both the 5 and 10% level had little effect on the stabilities of the triglycerides and cholesterol when compared to unblended 22°C olein (Figure 14). As was the case with the tallow and 22°C olein systems, the extent of cholesterol oxidation paralleled the observed increases in peroxide values for these blended systems as well. The addition of TBHQ and AP-MT to these blended oils apparently stabilized cholesterol to approximately the same extent that they stabilized the triglycerides in the oil systems. This effect was also observed in those oil systems containing 5, 10 and 15% HCSO to which TBHQ and AP-MT had been added (Figure 17-19). The addition of HCSO did not have a pronounced effect on the stability of the triglycerides to which no antioxidant had been added. However, the addition of HCSO to 22°C olein did appear to enhance the efficacy of the antioxidants in retarding oxidation in the resultant oil

Figure 15. Peroxide value development and loss of cholesterol in the 5% cottonseed oil - 95% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol

Figure 16. Peroxide value development and loss of cholesterol in the 10% cottonseed oil - 90% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol

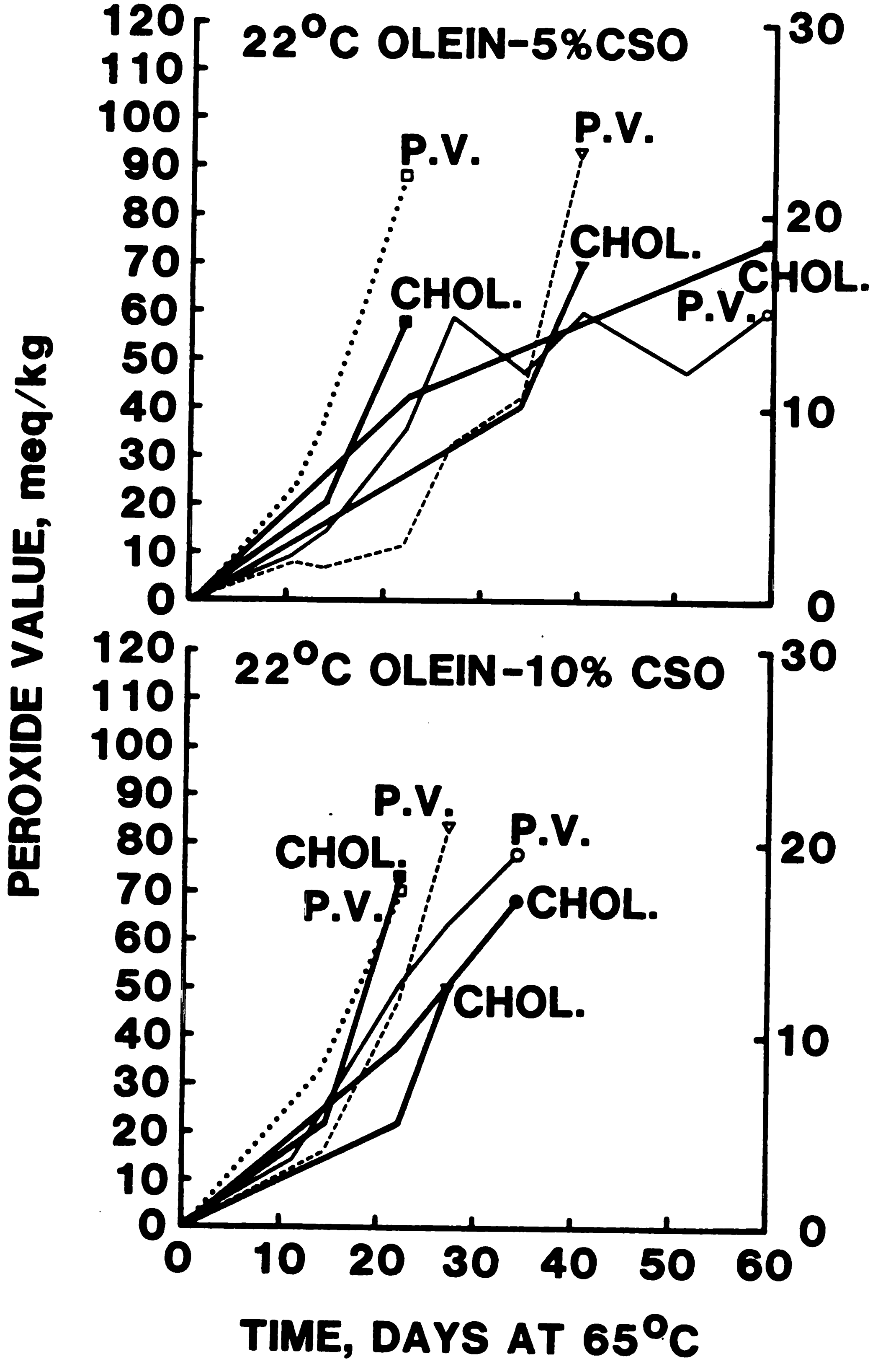

22°C OLEIN-5%CSO
22°C OLEIN-10% CSO
P.V.
CHOL.
PEROXIDE VALUE, meq/kg
% CHOLESTEROL OXIDIZED
TIME, DAYS AT 65°C
0
10
20
30
40
50
60
70
80
90
100
110
120
0
10
20
30
0
10
20
30
40
50
60

Figure 17. Peroxide value development and loss of cholesterol in the 5% hydrogenated cottonseed oil - 95% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol

Figure 18. Peroxide value development and loss of cholesterol in the 10% hydrogenated cottonseed oil - 90% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol

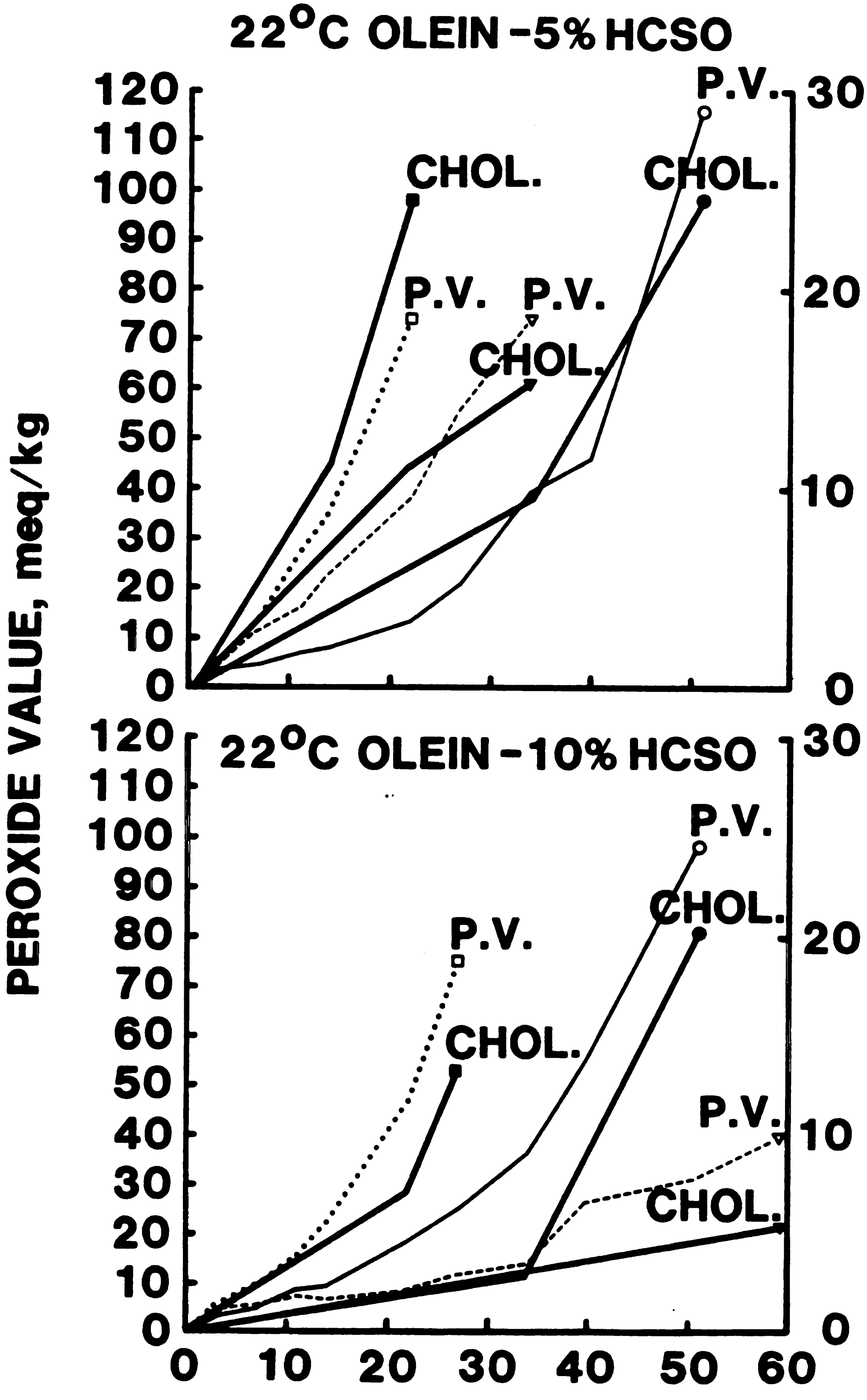

22°C OLEIN -5% HCSO
22°C OLEIN -10% HCSO
PEROXIDE VALUE, meq/kg
% CHOLESTEROL OXIDIZED
TIME, DAYS AT 65°C
CHOL.
P.V.
0
10
20
30
40
50
60
70
80
90
100
110
120
0
10
20
30
40
50
60

Figure 19. Peroxide value development and loss of cholesterol in the 15% hydrogenated cottonseed oil - 85% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

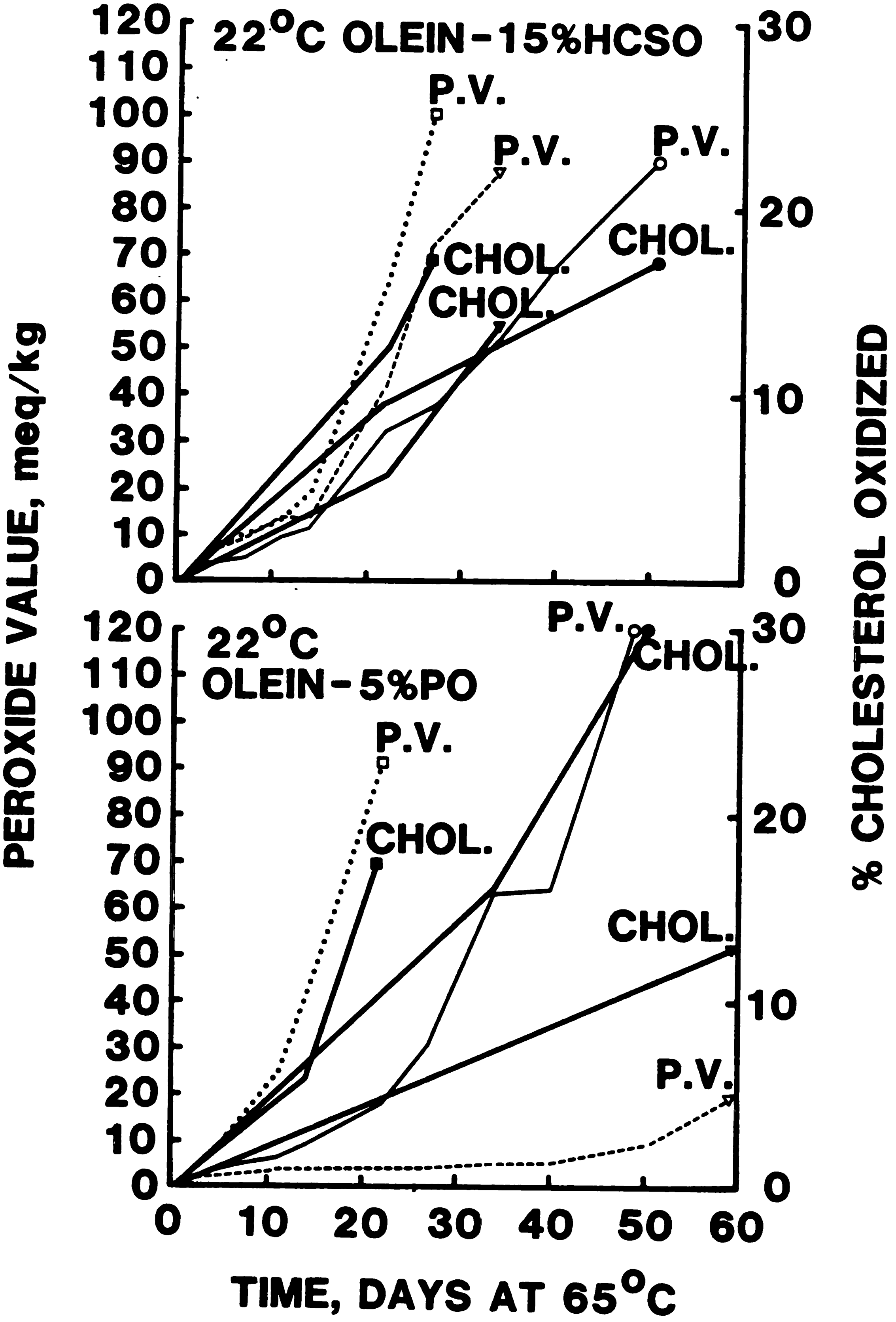
○AP-MT, PV ; ●AP-MT, cholesterol

Figure 20. Peroxide value development and loss of cholesterol in the 5% palm oil - 95% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol


22°C OLEIN-15%HCSO
P.V.
P.V.
P.V.
CHOL.
CHOL.
CHOL.
22°C
OLEIN-5%PO
P.V.
CHOL.
P.V.
CHOL.
CHOL.
P.V.
PEROXIDE VALUE, meq/kg
% CHOLESTEROL OXIDIZED
120
110
100
90
80
70
60
50
40
30
20
10
0
30
20
10
0
0
10
20
30
40
50
60
TIME, DAYS AT 65°C

systems. Thus, the addition of both HCSO and antioxidants (TBHQ or AP-MT) protected the cholesterol present in these oils by merit of their ability to stabilize the oil system triglycerides.

Palm oil was found previously to enhance the stability of oils to which it was added at 5, 10 and 15% levels (Figures 20-22). Likewise, analysis of the cholesterol found in the systems containing palm oil indicated that it too was more stable to oxidation. Since palm oil has a high saturated/unsaturated fatty acid ratio (Table 14) this effect was expected. Figures 20-22 also show that the application of antioxidants to palm oil-containing 22° olein systems further enhances stability towards peroxide development. The cholesterol present in these systems also was observed to undergo very little oxidative deterioration over the 60 day period at 65°C.

TLC analysis of the cholesterol extracts from several representative oil systems was performed when these oils developed peroxide values of 70 meq/kg or at the end of the 60 day study at 65°C. The results of these TLC analyses are found in Table 16 and indicate that the loss of cholesterol in the oil systems occurred via autoxidative mechanisms since the characteristic cholesterol oxidation products were identified. The oil systems chosen were the 22°C olein samples (control, TBHQ and AP-MT), the least oxidized system (15% palm oil - 85% 22°C olein with 0.01% TBHQ [w/w]) and

Figure 21. Peroxide value development and loss of cholesterol in the 10% palm oil - 90% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol

Figure 22. Peroxide value development and loss of cholesterol in the 15% palm oil - 85% 22°C olein system held at 65°C.

□control, PV; ■control, cholesterol

△TBHQ, PV ; ▲TBHQ, cholesterol

○AP-MT, PV ; ●AP-MT, cholesterol

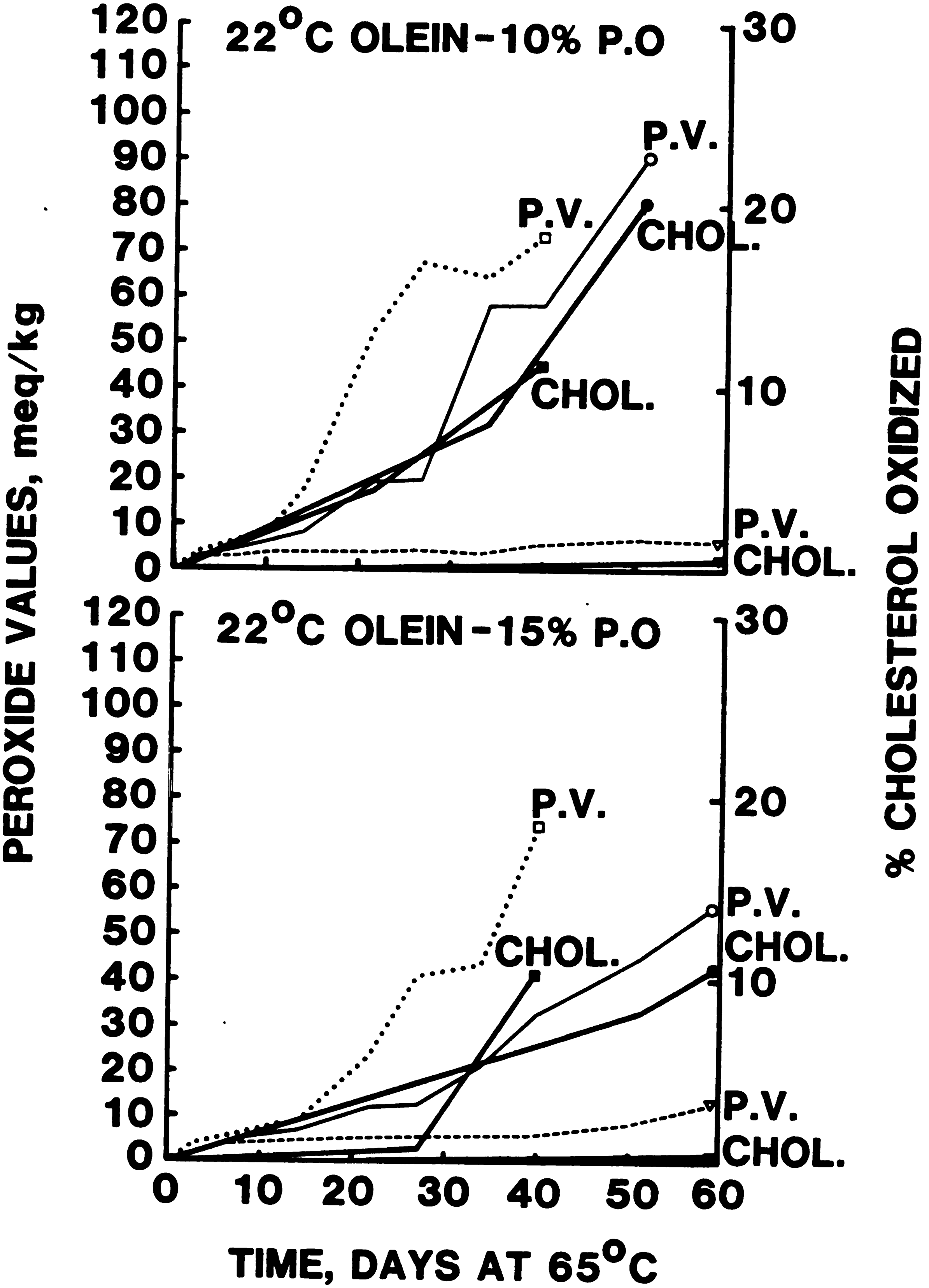

22°C OLEIN-10% P.O
22°C OLEIN-15% P.O
PEROXIDE VALUES, meq/kg
% CHOLESTEROL OXIDIZED
TIME, DAYS AT 65°C
P.V.
CHOL.
0
10
20
30
40
50
60
70
80
90
100
110
120
30
40
50
60

Table 16. TLC results from sterol extracts of selected oil systems utilized in the oxidative stability study of tallow-derived frying oils.

Oil System	Peroxide value (meq/kg)	Rf value[a]	Color[b]	Identity[c]
22°C olein	92.1	0.88	brown	3,5-cholestadiene-7-one
22°C olein + 0.02% TBHQ	80.7	0.70	magenta	5-cholesten-3β-ol
22°C olein + AP-MT	90.6	0.48	yellow	5-cholesten-3β-ol-7-one
		0.33	blue	5-cholesten-3β,7β-diol
		0.26	blue	5-cholesten-3β,7α-diol
22°C olein (85%), Palm oil (15%)	13.5	0.88	brown	3,5-cholestadiene-7-one
TBHQ (0.02%)		0.70	magenta	5-cholesten-3β-ol
22°C olein (95%), palm oil (5%)	138.0	0.88	brown	3,5-cholestadiene-7-one
AP-MT (500 ppm-100 ppm)		0.82	magenta	unindentified
		0.70	magenta	5-cholesten-3β-ol
		0.48	yellow	5-cholesten-3β-ol-7-one
		0.33	blue	5-cholesten-3β,7β-diol
		0.26	blue	5-cholesten-3β,7α-diol

[a] Solvent system, ethyl acetate:heptane (1:1, v/v).

[b] 50% H_2SO_4 spray, 10 min at 110°C.

[c] Identities determined by Rf values and color development of standard cholesterol oxidation products.

the most oxidized sample (5% palm oil - 95% 22°C olein with ascorbyl palmitate-mixed tocopherol [500 ppm-100 ppm]) encountered over the duration of this study. Cholesterol oxidation products isolated and tentatively identified from the 22° olein systems were 3,5-cholestadiene-7-one, 5-cholesten-3β-ol-7-one, 5-cholesten-3β,7β-diol and 5-cholesten-3β,7α-diol. These compounds formed at the expense of cholesterol and in total represented 15.1%, 13.2% and 6.9% of the original cholesterol present in the 22° olein systems without antioxidant, with TBHQ and with AP-MT added, respectively (Figure 14). The 15% palm oil - 85% 22°C olein with 0.01% TBHQ system was found to contain 99.5% of the original cholesterol present after 60 days at 65°C (Figure 22). In this system, only 3,5-cholestadiene-7-one was identified in addition to cholesterol. This compound has also been identified in unoxidized tallow systems (Ryan, 1982). The most oxidized system, i.e. the system reaching the highest peroxide value (138.0 meq/kg) over the stability study, was the 5% palm oil - 95% 22°C olein system with AP-MT added. Cholesterol oxidation products included 3,5-cholestadiene-7-one, 5-cholesten-3β-ol-7-one, 5-cholesten-3β,7β-diol and 5-cholesten-3β,7α-diol in this system. The formation of these compounds corresponded to a loss of cholesterol of 30.2% as determined by quantitative GLC analysis (Figure 20).

From these results it is apparent that cholesterol present in oil systems undergoes oxidation and that the extent of this oxidation is contingent on the stability of the triglycerides composing those systems. This effect was also observed in model system studies from Phase IV where cholesterol underwent oxidation in triolein and triolein: tristearin systems only when the fatty acyl groups had begun to deteriorate. Thus, it appears that cholesterol oxidizes in oil systems as a consequence of fatty acid free radical formation when unsaturated fatty acids comprise a moderate amount of the total fatty acid content. This proposed effect is reinforced by the fact that the addition of antioxidants (free radical scavengers) to the oil systems also cause a consequential increase in the oxidative stability of cholesterol. The antioxidants used in this study retard oxidation by donating hydrogen to fatty acyl radicals and also by adducting with radicals to form stable antioxidant-fatty acyl adducts. Thus, the effectiveness of these compounds in these oil systems suggests that cholesterol indeed undergoes oxidation as a consequence of fatty acid free radical propagation.

Phase VI- The Production and Stability of Potato Chips Processed in 22°C Olein Derived from Tallow.

Results of previous phases of this study indicated that the 22°C olein fraction derived from tallow by aqueous detergent fractionation had stability characteristics equivalent to commercial frying oils, and compositional characteristics suitable for use as a deep-fat frying medium. This fraction also was observed to possess a typical tallow flavor which has been reported to produce aesthetically desirable flavors in foods fried therein (Bauerlein et al., 1968; Chang, 1979; De Fouw, 1981). This flavor attribute, combined with the pourable consistency of the 22°C olein fraction at room temperature, support the contention that this oil system has excellent potential as a deep-fat frying medium for foods consumed at ambient temperature (Regutti, personal communication). This phase of the study was conducted to assess the performance of this fraction as a deep-fat frying medium in potato chip production, since potato chips represent the largest market share of the snack food industry (Potato Chip Information Booklet, 1978). This study entailed both the manufacture of chips using this frying fat, and evaluation of the stability characteristics of the potato chips thus processed.

Frying oil characteristics

The frying media used in the production of potato chips were characterized prior to their use. The 22°C olein fraction was a clear yellow liquid at room temperature, with a typical tallow odor and an iodine value of 52.3. Hydrogenated cottonseed oil (HCSO) was obtained from a commercial supplier and was similar to that specified by commercial chippers. This oil was partially crystallized at room temperature, had a very bland taste and an iodine value of 78.2. This oil was noted to have a reddish-yellow color. The tallow:HCSO blend (1:1) was completely solid at room temperature and had an iodine value of 63.7. Although this blend was observed to have a moderate tallow odor at frying temperatures, only a slight tallow odor was discernable at room temperature. This was attributed to the solid nature of this system, causing entrapment of flavor volatiles in the solid fat matrix. This blend was used in order to assess the feasibility of tallow flavor attributes in frying oils by blending rather than fractionation techniques.

Potato chip characteristics

Potato chips of excellent quality were produced in each of these frying oils and were of uniform size and shape and free of defects. These chips were visually graded for color using the Potato Chip Color Manual of the Potato Chip/Snack Food Association (1976b) immediately after frying. The chips produced in the 22°C olein and the tallow:HCSO (1:1) were graded number 1, the highest possible grade, while chips processed in HCSO were graded number 2 due to their darker color. It was observed that the chips processed in the tallow:HCSO (1:1) developed a white waxy appearance on cooling. This was attributed to the high melting characteristics of the blended tallow:HCSO system.

Potato chips produced in the 22°C olein had typical tallow odor and flavor immediately after frying, while those chips processed in HCSO had flavor and odor characteristics similar to commercially processed potato chips. The chips produced in the tallow:HCSO (1:1) blend had very bland odor characteristics, attributed to the aforementioned solid nature of the adsorbed frying medium.

The fat content of the potato chips produced in the three oil systems was determined by solvent extraction, immediately after frying and weekly during the subsequent five week stability study (Table 17). In all three chip systems, the fat contents were in the acceptable ranges specified for chips of 0.06 inches in thickness (Potato Chip/Snack Food Association, 1976a). The results of fatty acid analysis for the extracted fats from each chip system are found in Table 18.

The potato chips were also subjected to sensory analysis by a random ten member panel. Panelists evaluated the chips for color, greasiness, flavor and overall acceptability (See Appendix I for scorecard). Analysis of sensory data indicated that panelists perceived differences among the chips processed in different oils when evaluated immediately after frying. Analysis of variance for panel responses indicated that color, flavor and overall acceptability differed in the potato chips processed in the three oil systems ($P>99.9$). Although no statistical

Table 17. Fat content (%) of potato chips processed in the three tested oil systems.

Oil system used for frying	Fat Control (%), wet basis[a,b]						
	Time (weeks) of storage						
	0	1	2	3	4	5	Ave
22°C olein	41.1	40.3	47.4	40.0	46.6	47.4	43.9
HCSO	46.3	44.3	42.3	39.1	41.6	45.7	43.2
Tallow:HCSO (1:1)	38.3	41.7	43.1	43.4	38.9	43.1	41.4

[a] Based on grams of lipid recovered/35.0 g potato chips x 100.

[b] Analysis carried out immediately after frying and weekly during the storage period.

Table 18. Fatty acid composition of fat extracted from potato chips processed in the three tested oil systems[a].

Fatty Acid	C14:0	C16:0	C18:0	C20:0	C14:1	C16:1	C18:1	C18:2	C18:3	S/U
Oil System										
22°C olein	2.7	22.6	14.9	--	1.0	4.5	47.8	2.8	0.9	0.66
HCSO	0.9	22.2	5.2	0.8	0.2	0.6	51.5	17.3	0.1	0.42
Tallow:HCSO (1:1)	1.9	23.1	12.1	0.8	0.6	2.3	46.1	10.2	1.0	0.63

[a] Stated values represent averages of duplicate analyses.

preference evaluation was made due to the high level of variance in the responses for each chip tested, average responses indicated that chips produced in the 22°C olein had superior flavor and overall acceptability characteristics. Using a numerical scale where 0 represents high quality and 10 represents very poor quality, the chips fried in the 22°C olein had average flavor and overall acceptability scores of 2.0 and 1.7 (Tables 19 and 20), while the chips processed in tallow:HCSO (1:1) and HCSO had average flavor and overall acceptability scores of 3.2, 2.8 and 6.1, 5.6, respectively (Tables 19 and 20). Comments made by panelists also indicated a preference for the chips fried in the 22°C olein.

Differences in color among the chips processed in the three oil systems was also perceived by sensory panelists ($P>99.9$). The average color scores for the potato chips were 2.9, 4.5 and 7.0 for those fried in tallow:HCSO (1:1), 22°C olein, and HCSO, respectively immediately after frying (Table 21). Since the scale for color ranged from lighter (0.0 numerical score) to darker (10.0 numerical score), a score of 5.0 was assumed to be ideal. Thus, the 22°C olein processed chips were perceived to have optimal color characterisics when compared to the other chips tested. The color scores for the potato chips processed in tallow:HCSO (1:1) were found to be very low, since these chips took on a

Table 19. Means and standard deviations for flavor[a] responses by panelists for potato chips over the 5 week stability study.

Oil System	22°C Olein	Tallow:HCSO (1:1)	HCSO
Time (weeks)			
0	2.0 ± 0.8	3.2 ± 1.1	6.1 ± 1.1
1	2.1 ± 1.5	3.5 ± 1.8	4.9 ± 1.3
2	2.8 ± 1.4	3.5 ± 2.0	4.8 ± 1.9
3	3.4 ± 1.3	4.2 ± 1.2	4.6 ± 0.7
4	5.3 ± 2.3	3.0 ± 1.2	5.3 ± 1.0
5	7.2 ± 2.1	4.3 ± 2.1	5.9 ± 1.5

[a] Scale of 0 (excellent flavor) to 10 (very poor flavor).

Table 20. Means and standard deviations for overall acceptability[a] responses by panelists for potato chips over the 5 week stability study.

Oil System	22°C Olein	Tallow:HCSO (1:1)	HCSO
Time (weeks)			
0	1.7 ± 0.7	2.8 ± 1.8	5.6 ± 1.8
1	2.3 ± 1.8	3.3 ± 1.3	4.9 ± 1.5
2	2.1 ± 1.1	3.6 ± 2.0	4.6 ± 2.0
3	3.2 ± 1.6	3.9 ± 1.9	4.3 ± 1.7
4	4.8 ± 2.1	3.4 ± 0.9	5.1 ± 0.9
5	7.2 ± 2.1	4.3 ± 2.3	6.0 ± 1.7

[a] Scale of 0 (acceptable) to 10 (unacceptable).

Table 21. Means and standard deviations for color[a] responses by panelists for potato chips over the 5 week stability study.

Oil System	22°C Olein	Tallow:HCSO (1:1)	HCSO
Time (weeks)			
0	4.5 ± 0.9	2.9 ± 1.3	7.0 ± 1.1
1	5.0 ± 0.6	4.2 ± 1.4	5.2 ± 1.2
2	4.9 ± 1.0	2.8 ± 1.5	5.2 ± 0.8
3	3.9 ± 1.5	3.0 ± 1.5	5.4 ± 1.1
4	4.7 ± 1.3	3.1 ± 1.5	5.7 ± 0.8
5	5.0 ± 1.2	3.5 ± 0.9	6.4 ± 0.7

[a] Scale of 0 (lighter) to 10 (darker), where 5.0 was ideal.

Table 22. Means and standard deviations for greasiness[a] responses by panelists for potato chips over the 5 week stability study.

Oil System	22°C Olein	Tallow:HCSO (1:1)	HCSO
Time (weeks)			
0	3.9 ± 1.7	3.5 ± 1.1	4.5 ± 2.0
1	3.5 ± 1.1	4.0 ± 1.5	4.6 ± 2.1
2	3.9 ± 2.0	2.5 ± 1.2	4.2 ± 2.4
3	3.8 ± 1.6	4.1 ± 2.0	3.5 ± 1.5
4	4.6 ± 2.3	4.5 ± 1.7	4.7 ± 2.2
5	4.6 ± 2.1	5.1 ± 2.0	4.2 ± 1.5

[a] Scale of 0 (no greasiness) to 10 (very greasy).

waxy white appearance due to the solid nature of the absorbed frying medium.

No perceived differences in greasiness were observed among the three chip samples after frying (P>50). This was expected since the fat contents determined by solvent extraction indicated similar fat absorption characteristics among the chips processed in the three oil systems (Figure 22).

Stability of potato chips during storage.

Min and Schweizer (1983) stated that the average shelf-life of potato chips processed in oils without antioxidants is approximately 17-21 days. Thus, the potato chips used in this study were stored and analyzed over a five week period to assess quality changes.

The processed chips were stored in glass jars in the dark and evaluated weekly for the development of rancidity. Chemical and sensory evaluations were carried out during the five week storage period. The chips were evaluated for fat content (Table 17), changes in fatty acid composition, cholesterol content and deterioration, changes in volatile flavor profiles and sensory analysis.

The fat content of the chips remained somewhat constant throughout the stability study as was expected (Table 17). Fatty acid compositional analyses of the absorbed oil in the chips indicated that the fatty acids did not change appreciably throughout the five week period of storage (Table 23).

Cholesterol analysis of the chips fried in the 22°C olein and tallow:HCSO (1:1) indicated that the absorption of cholesterol by the fried potato chips paralleled the adsorption of the frying media by the chips (Table 24). Potato chips processed in the 22°C olein contained 53.8±4.2 mg cholesterol/100 g chips while those fried in tallow:HCSO (1:1) contained 30.0±2.3 mg cholesterol/100 g chips. The amount of cholesterol contained in these chips indicated that no preferential absorption of cholesterol occurred during frying, despite previous reports of preferential absorption of cholesterol by french fried potatoes processed in tallow (Ryan, 1982). TLC analysis of the non-saponifiable fraction of the lipids extracted from the potato chips indicated that no cholesterol oxidation had occurred over the five week stability study, despite the fact that the chips did undergo oxidative deterioration as evidenced by both sensory analysis and GC volatile profiling. Thus, cholesterol present in the frying media was absorbed by the chips, yet remained in an unoxidized form over the acceptable shelf-life of the product.

Results of sensory analyses indicated that flavor and overall acceptability scores changed over time for the potato chips processed in 22°C olein (P>99.9). This was reinforced by comments of the panelists regarding the

Table 23. The effect of storage on the fatty acid composition of fat extracted from potato chips processed in the three tested oil systems[a].

Oil System	Fatty Acid Time of Storage (weeks)	C14:0	C16:0	C18:0	C20:0	C14:1	C16:1	C18:1	C18:2	C18:3	S/U
22°C Olein	0	2.7	22.6	14.9	--	1.0	4.5	47.8	1.8	0.9	0.71
	1	2.6	21.8	14.8	--	1.4	5.0	45.4	3.8	1.7	0.68
	2	2.8	22.4	14.8	--	1.5	4.9	45.0	3.9	1.7	0.70
	3	2.7	21.3	14.7	--	1.5	5.1	44.6	4.0	2.1	0.68
	4	2.6	21.9	14.3	--	1.2	4.7	47.6	3.6	1.3	0.66
	5	2.7	21.5	14.9	--	1.4	4.9	45.2	3.9	2.0	0.68
Tallow:HCSO	0	1.9	23.1	12.1	0.8	0.6	2.3	46.1	10.2	1.0	0.63
	1	1.9	23.3	12.0	0.2	0.6	2.5	46.6	10.5	0.5	0.62
	2	2.0	22.9	11.6	0.3	0.8	2.5	45.2	11.5	1.3	0.60
	3	1.9	23.5	12.0	0.2	0.6	2.5	46.4	10.4	0.5	0.62
	4	1.9	23.7	11.9	0.3	0.8	2.9	44.8	10.4	1.3	0.63
	5	2.1	24.0	11.9	0.2	0.7	2.6	45.7	10.4	0.9	0.63
HCSO	0	0.9	22.2	5.2	0.8	0.2	0.6	51.5	17.3	0.1	0.42
	1	0.9	22.2	5.3	0.6	0.2	0.6	51.6	17.3	--	0.42
	2	0.9	21.9	5.3	0.7	0.3	0.4	51.1	17.1	1.1	0.41
	3	0.9	22.8	5.4	0.8	0.3	0.6	51.1	17.2	0.2	0.41
	4	0.9	22.2	5.4	0.8	0.3	0.5	51.8	17.3	0.1	0.42
	5	0.9	21.6	5.4	0.9	0.4	0.3	50.6	17.1	1.4	0.43

[a] Values given represent averages of duplicate analyses.

Table 24. Cholesterol content of potato chips processed in 22°C olein and tallow:HCSO (1:1).

	Time of storage (weeks)	mg cholesterol/5g lipid extract[a]	mg cholesterol 100 g chips
<u>Oil System</u>			
22°C olein			
	0	6.40	52.6
	1	6.31	50.8
	2	5.84	55.0
	3	6.03	48.0
	4	6.42	59.6
	5	6.01	56.9
Tallow:HCSO (1:1)			
	0	3.71	28.3
	1	3.81	31.7
	2	3.74	31.9
	3	4.03	34.7
	4	3.82	29.6
	5	3.44	29.3

[a] Stated levels represent averages of duplicate analyses.

development of rancid flavors in the chips stored for four and five weeks. Statistical evaluation of panel responses for the tallow:HCSO (1:1) processed chips over time indicated that the overall acceptability had changed ($P \geq 99$), while changes in flavor were perceived to be much less ($P \geq 50$). Similar results were also observed in the panel scores for the chips processed in HCSO. Therefore, these data suggest that although the chips processed in 22°C olein maintained superior flavor during storage for three weeks, oxidative deterioration had occurred in the fourth and fifth week of storage.

Gas chromatographic evaluation of rancidity in potato chips processed in 22°C olein.

In order to assess the extent of oxidation and the compounds responsible for the development of off-flavors and odors in the 22°C olein processed chips, GC profiling techniques were implemented. In this study, volatile compounds were extracted from fresh (0 week) and rancid (5 week) potato chips using a Likens-Nickerson extraction apparatus. The flavor extracts were analyzed by capillary GLC (Figure 23). As evidenced, several volatile compounds present in these extracts changed in concentration over the five week storage period. Attempts to identify selected peaks were not successful since the capillary column used was not compatible to the GC-MS system. However, these chromatograms indicate that many volatile compounds are

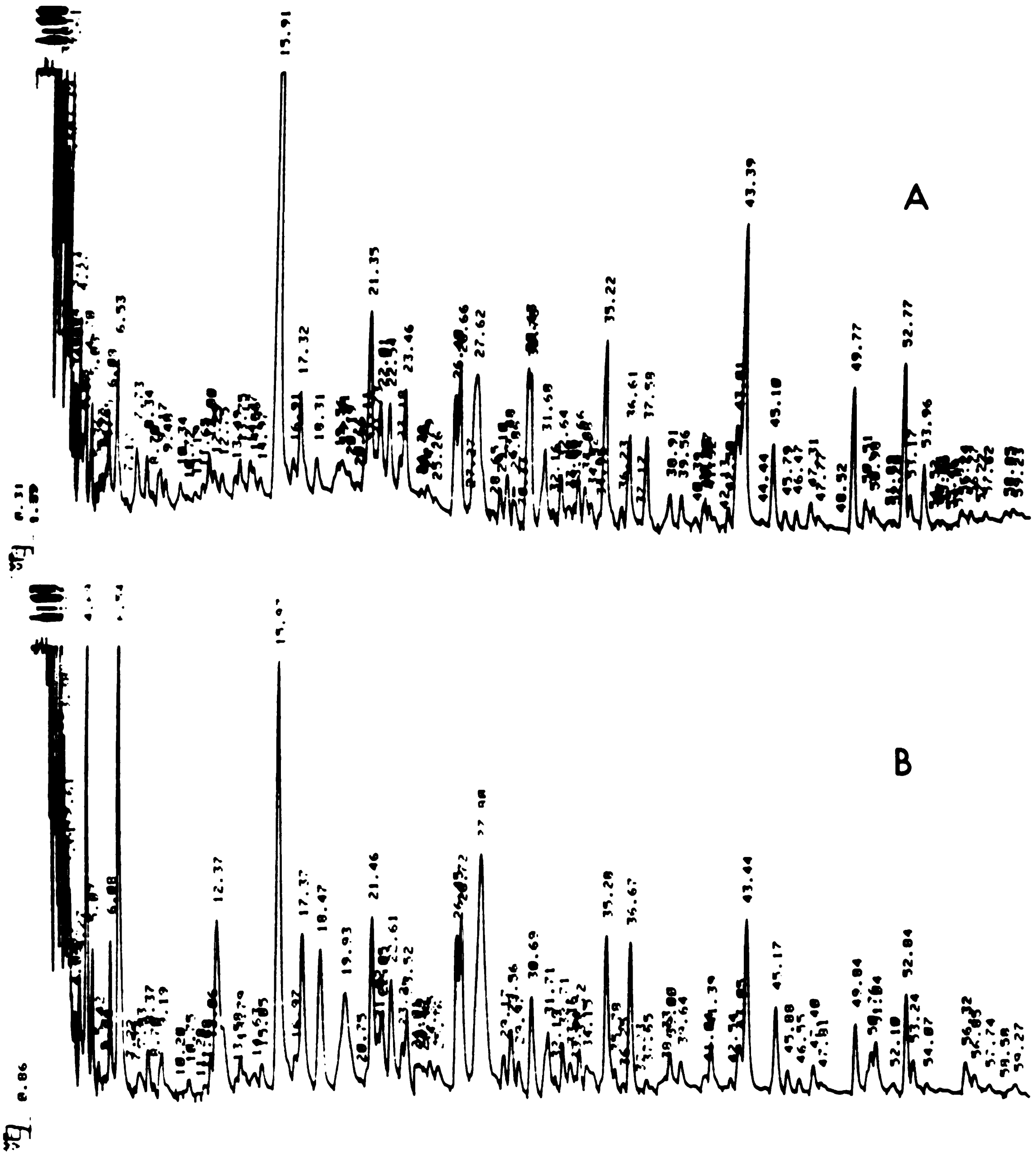


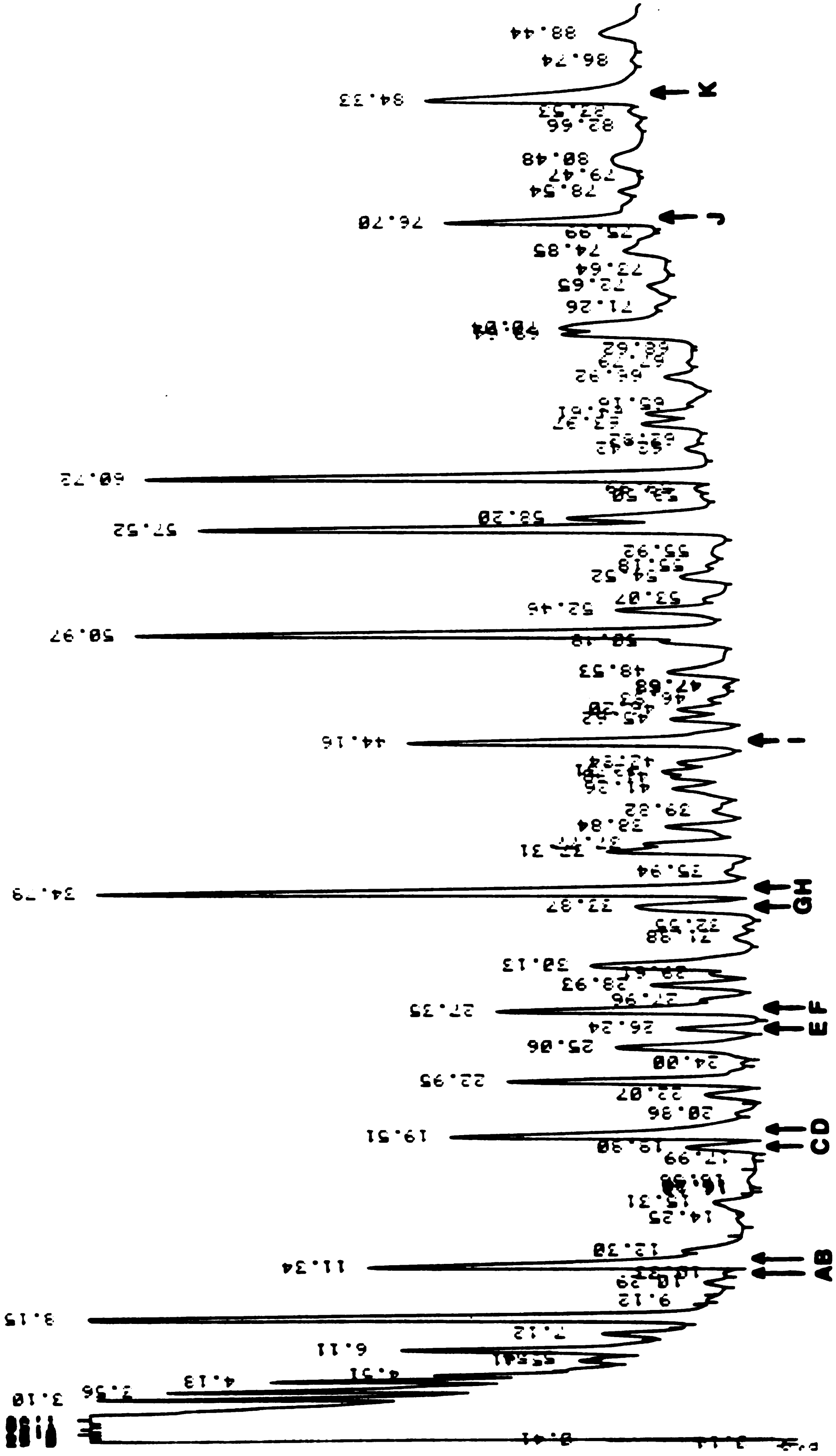
Figure 23. Capillary GC chromatograms of volatile flavor extracts of potato chips fried in 22°C olein, after storage for 0 weeks (A) and 5 weeks (B) at room temperature.

produced during the production and storage of the 22°C olein processed potato chips.

Further analyses were conducted on the volatile flavor extracts from the oxidized five week old potato chips using a packed column system. The chromatogram for this extract is shown in Figure 24. Using standard aldehydes and ketones associated with the oxidation of oleic and linoleic acids, several compounds were tentatively identified in the potato chip flavor extract. These compounds included saturated aldehydes (hexanal, heptanal, octanal, nonanal and decanal), 2-ketones (2-hexanone, 2-heptanone, 2-octanone and 2-nonanone) and 2,4 dienals (t,t, 2,4-heptadienal and t,t, 2,4-decadienol). The presence of hexanal, heptanal, octanal, nonanal, decanal, t,t, 2,4-heptadienal and t,t 2,4-decadienal were confirmed by GC-MS analysis. Mass spectra of these compounds are listed in Appendix II. Further, GC-MS analysis confirmed the presence of 2-hexenal, 2-octenal and 2-nonenal in the chip flavor concentrate.

The compounds identified in the oxidized potato chips are similar to those reported in potato chips processed in oils containing oleic and linoleic acyl species. Mookerjee et al. (1965) reported that heptanal, octanal and nonanal were present in rancid potato chip samples and that these compounds originated from the oxidation of oleic acid esters in the potato chips. Hexanal, 2-heptenal, 2-octenal, 2-nonenal and 2,4-decadienal originated from the oxidation

Figure 24. Gas chromatogram (packed column) of flavor extract from oxidized (5 week old) 22°C olein fried potato chips.
A. 2-hexanone, B. hexanal, C. 2-heptanone, D. heptanal,
E. 2-octanone, F. octanal, G. 2-nonanone, H. nonanal
I. decanal, J. t,t,2,4-heptadienal, K. t,t,2,4-decadienal.


K
J
I
GH
EF
CD
AB
88.44
86.74
84.33
82.66
80.48
79.47
78.54
76.70
74.85
73.64
72.65
71.26
70.04
68.62
65.92
65.16
63.37
62.42
60.72
58.20
57.52
55.92
54.52
53.07
52.45
50.97
48.53
47.68
44.16
41.26
39.82
38.84
37.31
35.94
34.79
33.37
31.88
30.13
28.93
27.35
26.24
25.06
24.00
22.95
22.07
20.36
19.51
19.30
17.99
15.31
14.25
12.30
11.34
10.29
9.12
8.15
7.12
6.11
4.51
4.13
3.56
3.10
0.41

of linoleic acid esters. The presence of similar compounds in the potato chips fried in the 22°C olein after five weeks of storage is not unexpected since this oil system contained 47.8 and 2.8 mole percent oleic and linoleic acid, respectively (Table 23). Further, the most prevalent peaks in the chromatogram were those for nonanal, octanal and heptanal, which again is consistent with the higher concentration of oleic acid in the 22°C olein compared to linoleic acid.

Overall, these results indicate that the 22°C olein fraction from tallow provided superior potato chips when compared to chips processed in commercial potato chipping oils. The chips were found to be stable to flavor changes over a three week storage period. Cholesterol content of these chips was found to be 53.8±4.2 mg/100 g chips and no oxidative changes in cholesterol were observed over the five week period of storage.

Packed column and capillary column GC and GC-MS analyses indicated that potato chips fried in 22°C olein produced compounds during storage which contributed to off-flavor development (as confirmed by sensory data). These compounds were identified as products of oxidation of mono- and di-unsaturated fatty acids.

SUMMARY AND CONCLUSIONS

The utilization of a liquid fraction obtained through aqueous detergent fractionation of tallow as a pourable deep-fat frying medium was investigated. The major findings of the study are as follows:

1. Low temperature rendering (120^{o}F) produces tallows better suited for fractionation than those produced by rendering at moderate (145^{o}F) or higher temperatures (180^{o}F), as determined by olein yield at 40^{o}C.
2. Modifications of the fractionation process specified by Bussey et al. (1981) in the derivation of frying oils included substitution of sodium citrate for sodium sulfate, subsequent fractionation at 33, 27 and 22^{o}C and reagent concentrations of 0.06% SDS and 5% sodium citrate aqueous solution used at 60%, based on tallow weight.
3. The addition of surfactant and electrolyte solution to tallow was necessary only at the primary fractionation step at 40^{o}C. Subsequent fractionations, achieved by holding tallow olein fractions at successive lower temperatures, were required at 33, 27 and 22^{o}C to

insure the removal of higher melting glyceride types from the olein fractions.

4. Characterization of the tallow fractions derived in this fractionation process indicated gradual increases in unsaturation as temperatures of fractionation decreased. Also, cholesterol was found to be distributed evenly throughout the derived fractions.
5. The 22°C olein fraction had characteristics suitable for use as a deep-fat frying medium. This fraction was a clear yellow liquid at 22°C, possessed typical tallow flavor, and maintained a pourable consistancy at temperatures of 16°C. This fraction was determined to have an iodine value of 52.4 and contained 0.14% cholesterol by weight. The 22°C olein fraction represented 26-30% of the parent tallow from which it was derived.
6. Model triglyceride system studies revealed that unsaturated fatty acids exert a protective effect on cholesterol contained therein and that aeration enhances the oxidation of cholesterol in all model systems studied.
7. Accelerated stability studies of the 22°C olein fraction indicated that this oil possesses stability characteristics equal to that observed for commercial hydrogenated cottonseed oil and palm oil, both of

which are currently used in the manufacture of potato chips.

8. The addition of vegetable oils (CSO, HCSO and PO) to 22°C olein enhanced the oxidative stability of this fraction. Likewise, TBHQ (0.01%) and ascorbyl palmitate-mixed tocopherol (500 ppm-100 ppm) when added to the 22°C olein and 22° C olein-vegetable oil blends effectively retarded autoxidation. The oxidation of cholesterol in the 22°C olein and 22°C olein blends paralleled the oxidation of the unsaturated fatty acyl groups present in these systems.
9. Potato chips produced in the 22°C olein fraction possessed typical tallow-fried flavor and were apparently preferred by panelists over potato chips processed in HCSO over the first three weeks of a five week study. The potato chips processed in 22°C olein were stable to flavor changes for three weeks, the normal shelf-life of commercially processed chips.
10. The loss of desirable flavor characteristics in the potato chips fried in 22°C olein was determined to be due in part to the formation of aldehydes and ketones (C_6-C_{10}) from oxidized unsaturated fatty acyl components in the absorbed frying medium.
11. Analysis of the frying oil absorbed by potato chips during processing revealed the presence of cholesterol

at levels similar to that present in the frying medium itself. This cholesterol was observed to be stable to oxidative deterioration, even after the potato chips from which it was isolated were scored by sensory panelists as rancid.

These results indicate that the 22°C olein fraction possesses characteristics which yield potato chips with desirable and unique flavor attributes. Also, the products processed in this oil were sufficiently stable, and did not require the addition of antioxidants. Thus, results of this study indicate that fractionation of tallow can produce a frying oil which has potential for the processing of foods consumed at ambient temperatures.

Proposals for Further Research

During the course of this study, several observations were noted and points raised which merit further investigation. These include;

1. The possible applications of other fractions derived in this fractionation scheme to foods as well as to non-food uses.
2. Rate kinetic studies of cholesterol oxidation in triglyceride systems with differing levels of unsaturation.
3. The stability of the 22°C olein over the course of its use in frying operations, and the stability of the cholesterol contained therein.
4. Cost analysis studies of the production of this fraction, including raw material, process, labor and set-up costs, as well as cost comparisons to other oil alternatives.
5. The use of antioxidants, especially ascorbyl palmitate-mixed tocopherol systems, in retarding oxidation reactions in both oils and products processed in oils.

6. Extensive market analysis of both the acceptability of products produced in an oil such as the 22°C olein fraction and sensory analysis of various snack foods processed in this fraction.

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Appendix I

Potato Chip Sensory Scorecard

POTATO CHIP SCORECARD

NAME ______________________________ SAMPLE NO. ________________

INSTRUCTIONS:

1. Please check to make sure that the sample number on this sheet matches that on the sample being evaluated.
2. Place an "X" along the horizontal line where your perception of each attribute is best represented.

Color	Lighter	Darker
Greasiness	No Greasiness	Very Greasy
Flavor	Excellent	Very Poor
Overall Acceptability	Acceptable	Unacceptable

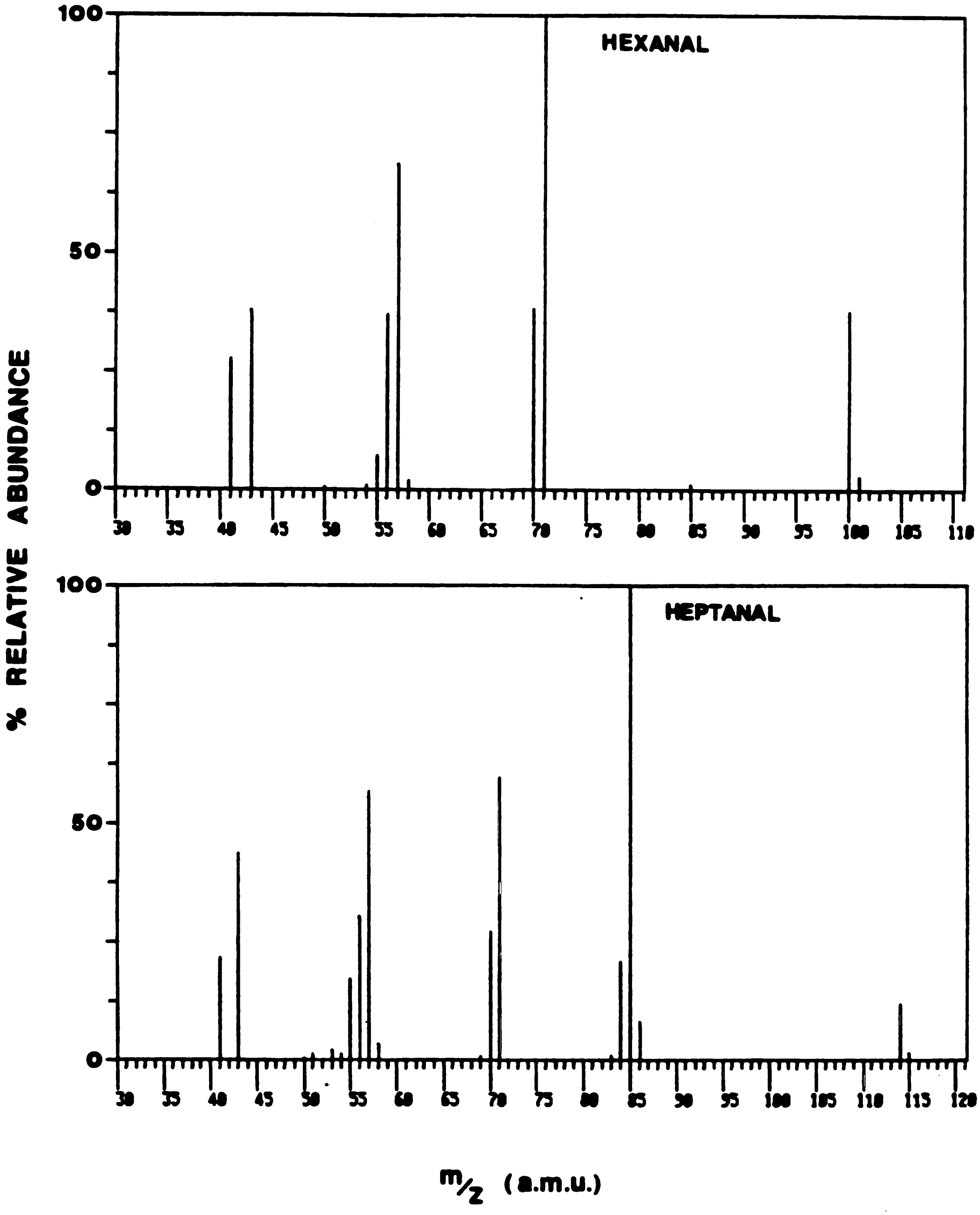
Comment: ____

____

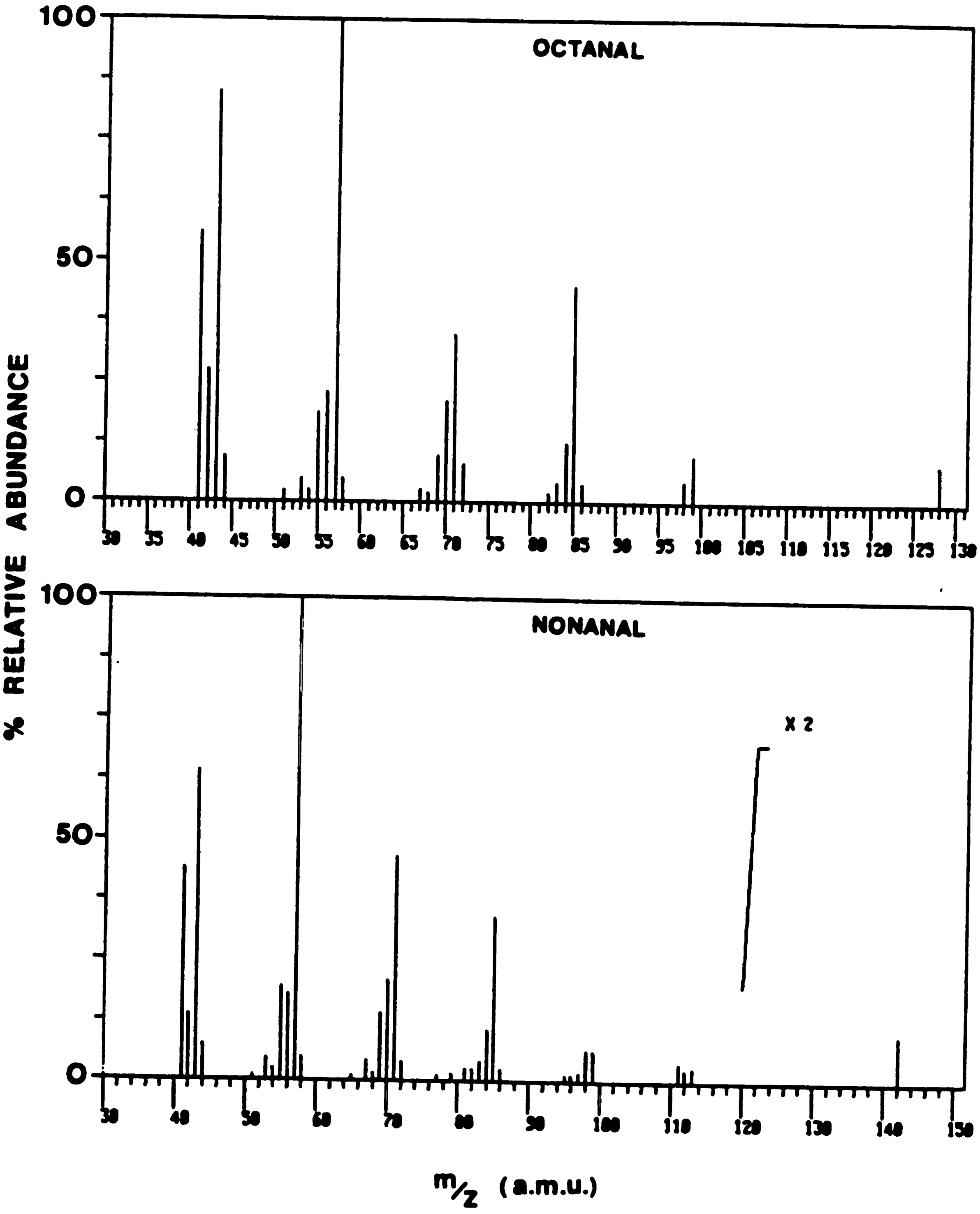
THANK YOU!

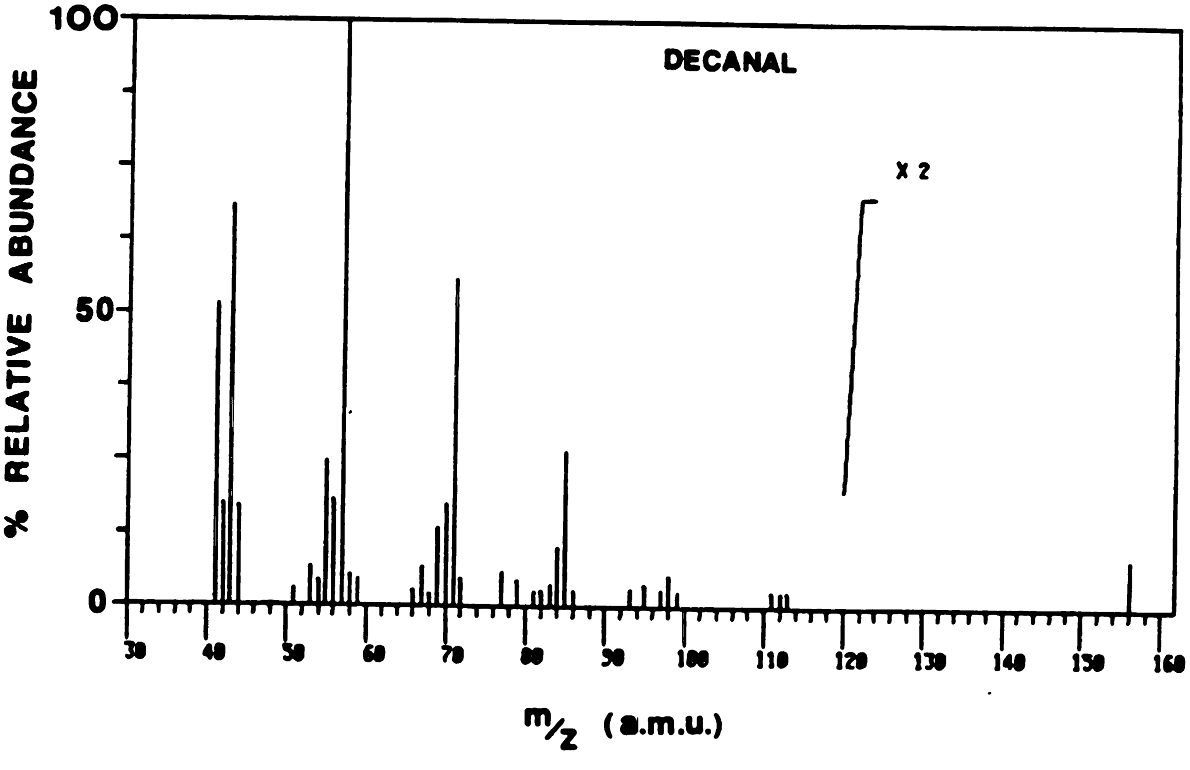
Appendix II

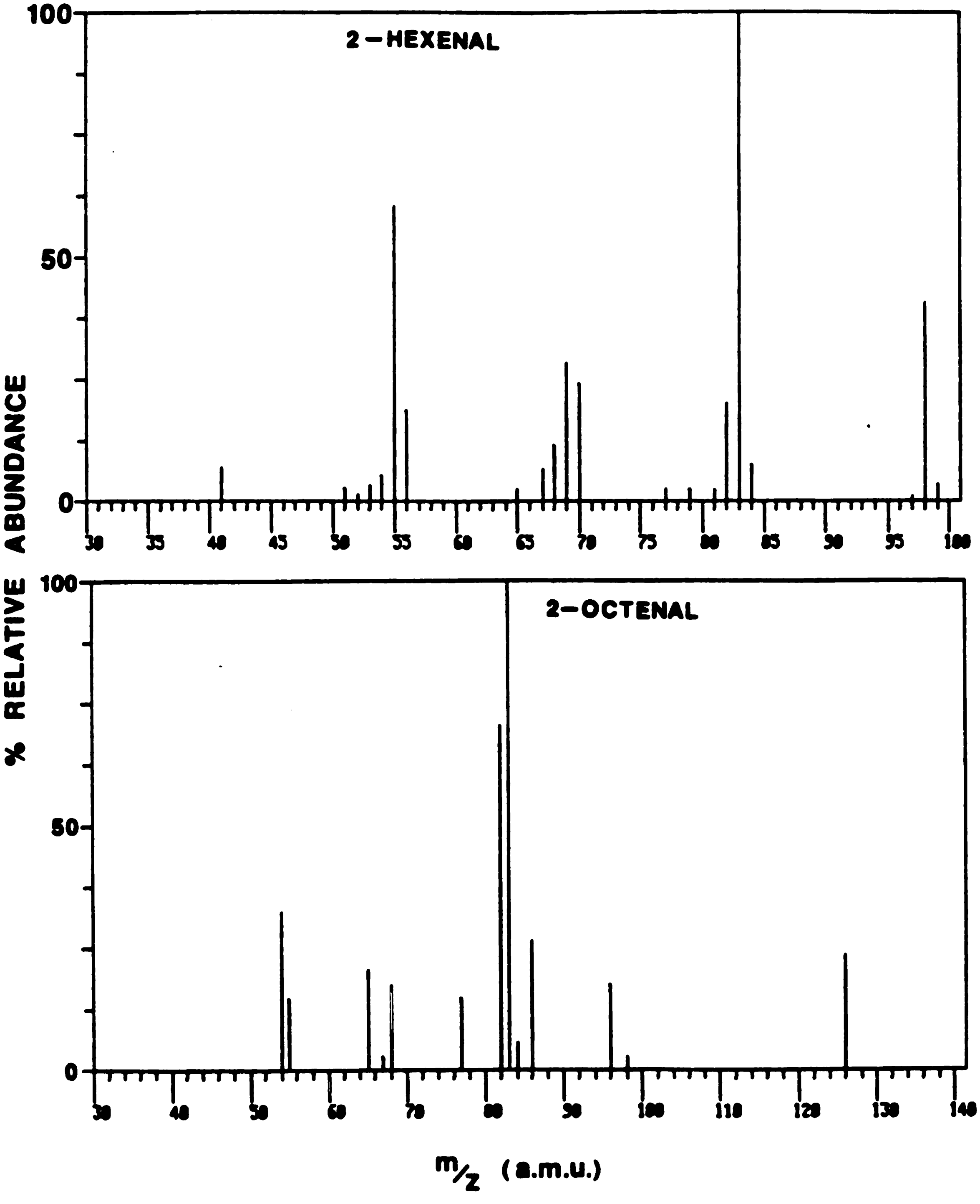
Mass Spectra for Compounds Isolated from Oxidized Potato Chips

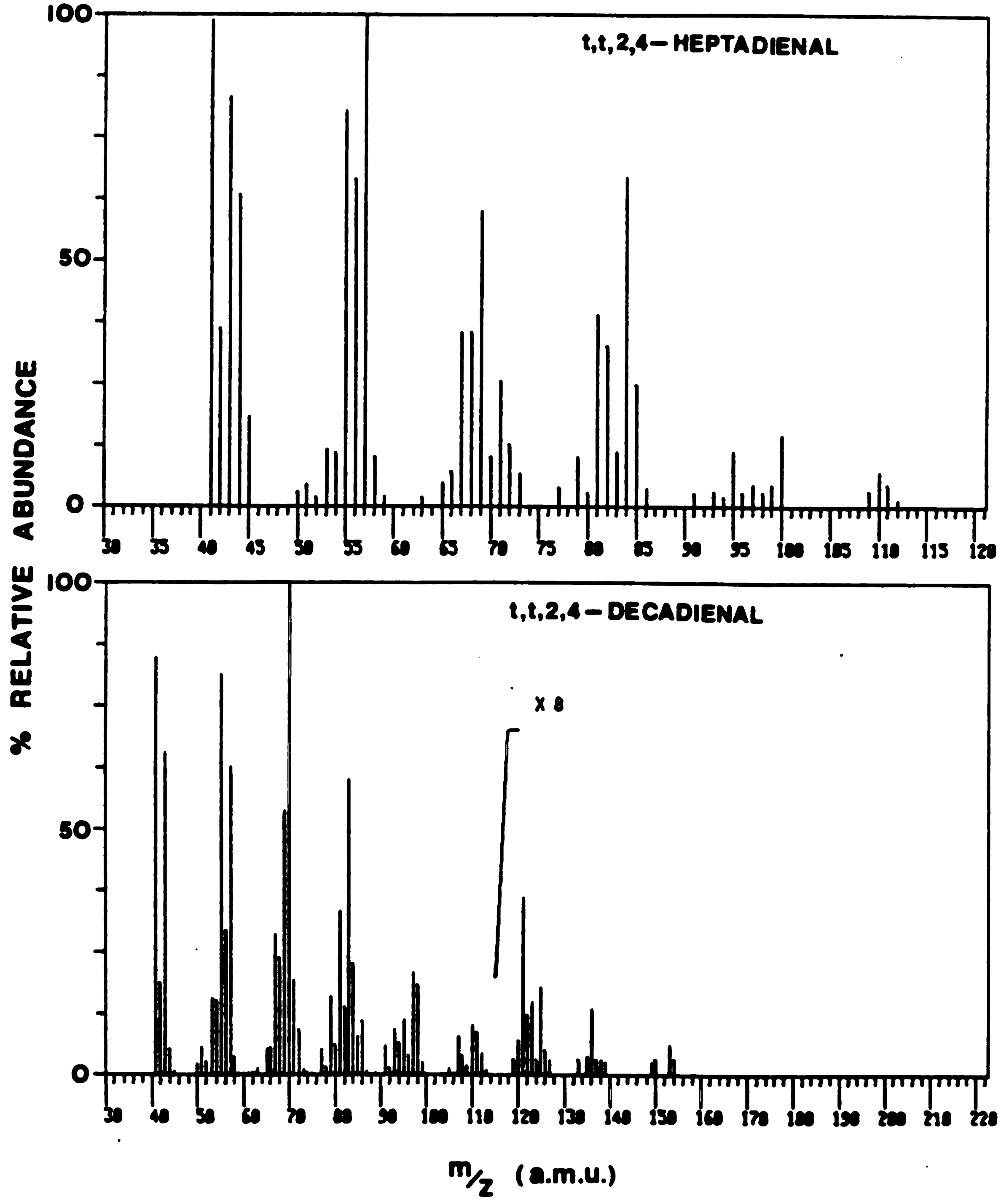


HEXANAL
HEPTANAL
% RELATIVE ABUNDANCE
100
50
0
30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
m/z (a.m.u.)


OCTANAL
NONANAL
X 2
100
50
0
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
140
150
% RELATIVE ABUNDANCE
m/z (a.m.u.)


100
50
0
% RELATIVE ABUNDANCE
DECANAL
X 2
30
40
50
60
70
80
90
100
110
120
130
140
150
160
m/z (a.m.u.)


2-HEXENAL
2-OCTENAL
% RELATIVE ABUNDANCE
100
50
0
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
110
120
130
140
m/z (a.m.u.)


t,t,2,4– HEPTADIENAL
t,t,2,4– DECADIENAL
X 8
% RELATIVE ABUNDANCE
100
50
0
30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
m/z (a.m.u.)